ISOLATION, IDENTIFICATION, ANALYSIS OF PHEROMONE OF MALE WAX MOTH AND ITS APPLICATION AS BIOCONTROL TOOL AGAINST *Galleria mellonella* AND *Achroia grisella*

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Zoology

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**ISOLATION, IDENTIFICATION, ANALYSIS OF PHEROMONE OF MALE WAX MOTH AND ITS APPLICATION AS BIOCONTROL TOOL AGAINST *Galleria mellonella* AND *Achroia grisella*"** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **DR LOVLEEN** of working as **PROFESSOR** in the **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.

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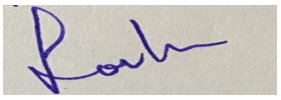
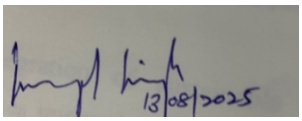
CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**ISOLATION, IDENTIFICATION, ANALYSIS OF PHEROMONE OF MALE WAX MOTH AND ITS APPLICATION AS BIOCONTROL TOOL AGAINST *Galleria mellonella* AND *Achroia grisella*"** submitted in fulfillment of the requirement for the award of degree of Doctor of Philosophy (Ph.D.) in **ZOOLOGY/SCHOOL OF BIOENGINEERING AND BIOSCIENCES,** is a research work carried out by **MANPREET KAUR SAINI, 41900558**, 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.





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ABSTRACT

Wax moths exhibit a parasitic relationship with honey bee colonies, leading to substantial economic and ecological impacts. The larvae of these moth species infiltrate beehives, where they destroy honeybee combs by consuming these combs and making tunnels near the midrib of the combs. Wax moths are the major pest of honey bee colonies especially during the lean period, although it is active from March-November in Punjab. This feeding behaviour results in direct damage to the structural integrity of the combs and hive, weakening the colony's ability to defend against other threats and reducing honey storage capacity. Furthermore, wax moth infestations exacerbate stress on bee colonies already burdened by factors such as pesticide exposure, pathogens, and habitat loss. The cumulative effects of these stressors can lead to colony collapse, diminished honey production, and reduced pollination services, thereby threatening agricultural productivity and ecosystem stability.

In the face of these challenges, the integration of sustainable and environmentally friendly pest control measures becomes imperative. This requires a deeper understanding of the intricate ecology of wax moths and the development of innovative strategies to safeguard beekeeping and stored hive products industries. By delving into the chemical intricacies of their communication, we aspire to unlock biocontrol tools that offer a beacon of hope for beekeepers and stakeholders in the apiculture industry. This study explores the isolation, identification, and analysis of male wax moth pheromones, offering a potential control tool that holds promise for mitigating the impact of wax moths on honey bee colonies and stored beehive products. Through these endeavours, the study aims to address the challenges posed by *Galleria mellonella* Linnaeus and *Achroia grisella* Fabricius, fostering resilience in apicultural practices and the storage of bee-related products.

The primary objective of the study was to isolate and identify the specific pheromones produced by male wax moths *Galleria mellonella* (*G. mellonella*) and *Achroia grisella* (*A. grisella*) to address the pressing problem. The identification of these pheromones is a substantial development in the field of biocontrol. The chemical structure and composition of pheromones of androconial glands were investigated using

a combination of analytical techniques, such as Stereomicroscopy, Scanning Electron Microscopy (SEM), and Gas Chromatograph Mass Spectrometry (GC-MS). Stereomicroscopy and SEM analysis revealed the existence of an oval, bulb-shaped pheromonal gland on the mesowing of the chosen species. Dimensional analysis of the androconial gland showed that in male, *G. mellonella*, it measured 1.33 μm in length and 4.74 μm in width, while in male, *A. grisella*, these morphometric measurements were 1.23 μm and 2.33 μm, respectively.

The study delves into the chemical ecology of wax moths through qualitative and quantitative analysis of specific pheromones. The compounds quantified in male wax moth *G. mellonella* were aldehydes namely nonanal (5.218 parts per million (ppm), 6.182 min RT, 1359472 area), undecanal (7.162 ppm, 12.251 min RT, 10873560 area), heptadecane (0.203 ppm,18.005 min RT, area 3799665), heneiocosane (0.267 ppm, 22.118 min RT, area 749917) and alcohols namely 1-nonanol (1.181ppm, 6.937 min RT, area 954113). During the present study novel compounds have been isolated heptadecane and heneiocosane, are the newly reported compounds in male, *G. mellonella.* The compound cis-9-hexadecenal has not been previously reported in the literature. Aldehydes namely undecanal (12.302 min RT) and cis-9-Hexadecenal (20.393 min RT) have been identified as volatile compounds of *A. grisella.* Cis-9-hexadecenal has not previously been reported from *A. grisella* in scientific literature.

The chemical composition of the identified pheromones was analyzed to determine their specificity and efficacy in attracting female moths. Laboratory trials were conducted to evaluate the potential of these pheromones as biocontrol agents. The behavioural bioassay conducted on female *G. mellonella* revealed significant differences in behavioural responses to different pheromone treatments in a bioassay chamber. The treatments included an untreated control, hexane (solvent) as control, an extracted blend (15 ppm), and synthetic blends at different concentrations (15 ppm, 10 ppm, 5 ppm, 1 ppm, and 0.5 ppm). To determine the threshold of the minimum concentration of synthetic and extracted blend which elicits a response by female wax moth adults. Behavioural parameters such as upward flight, flight to 10 cm arena where the blend was placed, ovipositor display, closest approach to filter paper, and orientation time were recorded. In the control treatment with no pheromone stimulus, the moths exhibited no response across all measured parameters.

For the extracted blend (15 ppm) from *G. mellonella* and the highest concentration of the synthetic blend (15 ppm), all females observed exhibiting consistent and intense responses, including upward flight, flight to 10 cm arena, ovipositor display, closet approach to the filter paper, and extended orientation time. The responses decreased with lower concentrations of the synthetic blend, with the weakest response observed at 1 ppm and no response at 0.5 ppm. ANOVA results indicated that pheromone dose had a highly significant impact on all behavioural responses. The highest mean responses were observed with the extracted blend at 15 ppm and synthetic blend at 15 ppm, which were not significantly different from each other. Lower concentrations of the synthetic blend showed progressively reduced responses.

The behavioural responses of female *A. grisella* moths to various pheromone treatments within a bioassay chamber were also analyzed similarly. The treatments included an untreated control, hexane (solvent) as control, an extracted pheromone blend (10.7 ppm), and synthetic pheromone blends at concentrations of 10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, and 0.1 ppm. Behavioural parameters such as upward flight, flight to 10 cm within the arena, ovipositor display, closest approach to filter paper, and orientation time were meticulously observed. In the control treatment, where no pheromone stimulus was given, the moths exhibited no response across all measured parameters. The extracted blend (10.7 ppm) and the highest concentration of the synthetic blend (10.7 ppm) elicited the most consistent and intense responses.

All female *A. grisella* moths observed exhibited behaviour such as upward flight, flight to 10 cm arena, ovipositor display, closest approach to the filter paper, and extended orientation time. As the concentration of the synthetic blend decreased, the intensity of the observed behaviour also decreased, with the weakest response at 0.2 ppm and no response at 0.1 ppm. ANOVA results indicated that pheromone dose had a highly significant impact on all behavioural responses. The highest mean responses were observed with the extracted blend (10.7 ppm) and synthetic blend at 10.7 ppm, which were not significantly different from each other. Lower concentrations of the synthetic blend showed progressively reduced responses. The ANOVA tests, characterized by large F-values and small p-values, confirmed the strong effect of pheromone treatments on moth behaviour.

The identified pheromone compounds, the extracted and synthetic pheromone crucial for mating behaviour, analyzed using gas chromatography-mass spectrometry were applied in field trials to disrupt the mating patterns of *G. mellonella* and *A. grisella*, resulting in a significant reduction in their population. The blends can effectively attract female *G. mellonella* and *A. grisella* at appropriate dosages, with the dosage being a critical factor in determining trapping success. In *G. mellonella,* at a concentration of 40 ppm, the synthetic blend captured an average of 14.67 moths, whereas the extracted blend captured an average of 13.33 moths. This suggests the initial level of effectiveness. The trapping efficiency was the highest between 50 ppm and 70 ppm, with the largest average catches occurring at 60 ppm (synthetic blend: 27.67 moths, extracted blend: 26.00 moths). After reaching a concentration of 70 ppm, the level of attractiveness decreased. At the maximal dosage of 100 ppm, only 10.33 and 9.00 moths were trapped with synthetic and extracted blends, respectively. The mean trap catch for the synthetic blend was (9.92 moths) in comparison to the extracted blend (9.28 moths).

In the case of *A. grisella,* below a concentration of 40 ppm, moths did not exhibit any trapping behaviour, indicating that these concentrations are ineffective. The extracted blend captured a mean of 3.00 moths at 40 ppm, while the synthetic blend trapped a mean of 4.67 moths, demonstrating the minimum concentration of pheromone blend which elicited a response by female wax moths in the field conditions. The most effective trapping was observed at concentrations of 50 to 70 ppm, with the highest average captures recorded at 60 ppm for synthetic blends (20.00 moths) and extracted blend (18.33 moths). At concentrations beyond 70 ppm, the level of appeal decreased. The most concentrated dose, 100 ppm, only captured 5.67 moths with the synthetic blend and 4.33 moths with the extracted blend. The mean trapping for the synthetic blend was 6.59 moths and the extracted blend was 5.57 moths. The dosage had a greater impact on determining the success of trapping for both species, compared to the type of blend used. Both *G. mellonella* and *A. grisella* demonstrated their highest trapping effectiveness within the concentration range of 50 ppm to 70 ppm, regardless of whether synthetic or extracted blends were used. Attractiveness diminished when doses exceeded 70 ppm, potentially due to oversaturation or repellent properties.

These findings provide valuable insights into managing wax moth infestations through pheromone-based strategies. Male wax moth pheromones can be effectively utilized as a tool, offering a sustainable and eco-friendly alternative to chemical pesticides. Analytical techniques were employed to identify and quantify pheromones in male wax moths, with key compounds including undecanal and nonanal in *G. mellonella*, and undecanal and cis-9-hexadecenal in *A. grisella*. Heptadecane and heneiocosane, are the newly reported compounds in male, *G. mellonella.* The compound cis- 9- hexadecenal has not been previously reported in the literature.

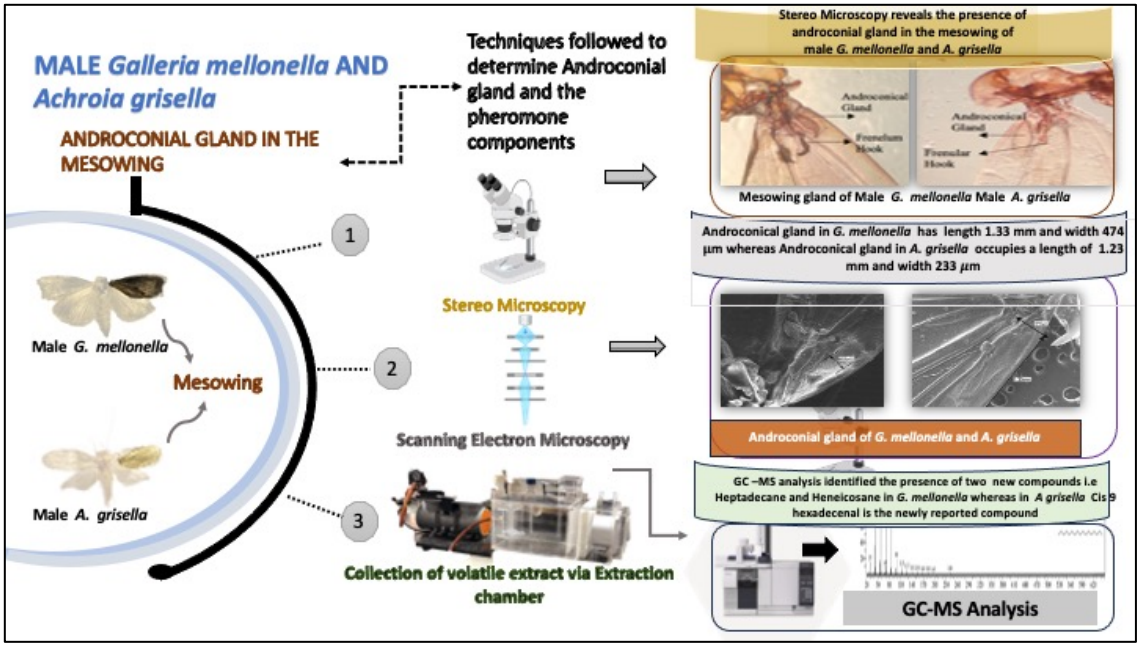
The behavioural bioassay results provide valuable insights into pheromone-induced behaviour in these moth species. The research demonstrates that optimal concentrations of both extracted and synthetic pheromone blends elicit strong behavioural responses in female moths. These responses include upward flight, flight to 10 cm arena, ovipositor display, and closet approach to pheromone sources and orientation time. The field trials confirmed the efficacy of these pheromone blends in disrupting mating patterns and reducing moth populations, particularly at concentrations between 50 ppm and 70 ppm. These findings suggest that pheromone blends of optimal dose are effective in eliciting strong behavioural responses in female *G. mellonella* and *A. grisella,* demonstrating the potential for developing pheromone-based biocontrol strategies.

Pheromone-based pest management is frequently incorporated into more comprehensive integrated pest management (IPM) strategies, which also encompass cultural, biological, and other environmentally sustainable approaches. By employing this integrated strategy, efficacy is optimized while dependence on chemical pesticides is reduced, thereby supporting the sustainability of beekeeping, enhancing agricultural productivity, and contributing to food security.

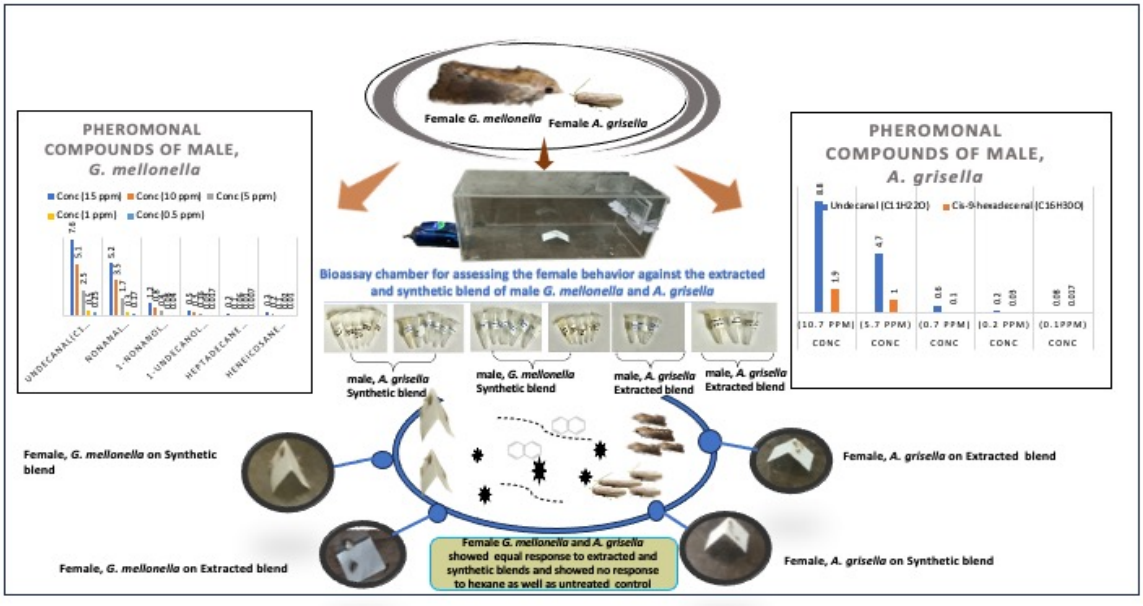
Keywords- *Achroia grisella,* Androconial gland*, Galleria mellonella*, Lepidoptera, Pheromone

GRAPHICAL ABSTRACT

Objective 1: **Isolate and catalogue the volatile organic compounds from adult wax moth, *G. mellonella* and *A. grisella***

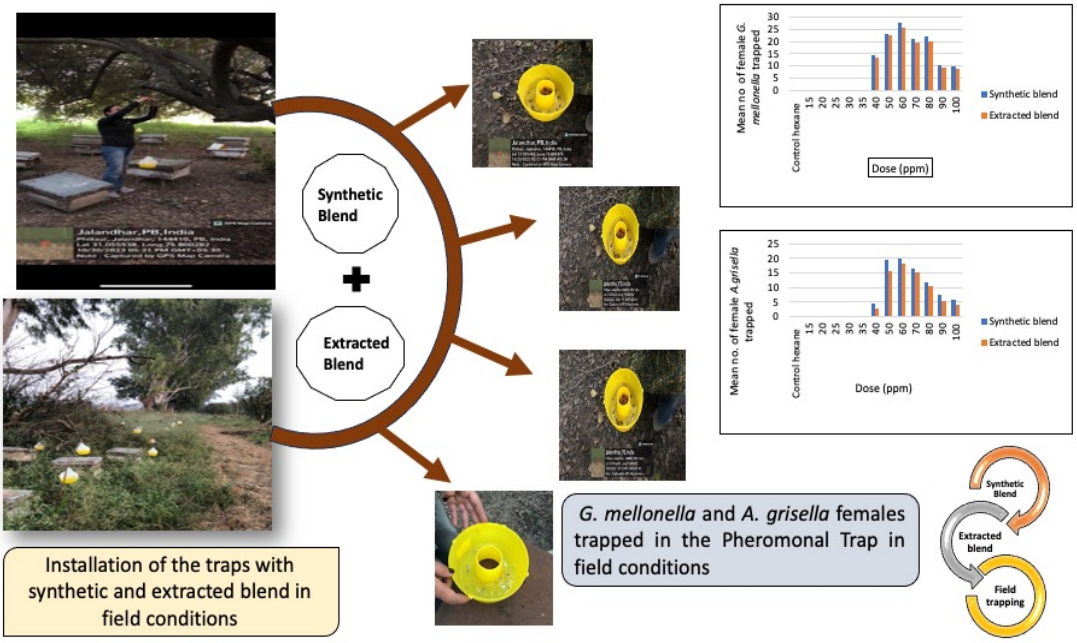


Objective 2 To elucidate the attractiveness of volatile organic compounds blend to female wax moths



To evaluate the efficiency of extracted pheromone blend in trapping female wax moths in the apiary

Objective 3



Installation of the traps with synthetic and extracted blend in field conditions

G. mellonella and *A. grisella* females trapped in the Pheromonal Trap in field conditions

Conclusion

Stereomicroscopy and SEM analysis was employed to examine the existence of an oval, bulb-shaped pheromonal gland on the mesowing of the chosen species. Dimensional analysis of the androconial gland revealed that in male, *G. mellonella*, it measured 1.33 µm in length and 4.74 µm in width, while in *A. grisella*, the morphometric range was 1.23 µm in length 2.33 µm in width. The GC-MS analysis identified two previously unreported compounds in male *G. mellonella*, including Heptadecane and Heneicosane, while cis-9-Hexadecenal, was a novel compound found in male *A. grisella*.

The mean behavioural responses of female *A. grisella* and *G. mellonella* under different treatments, each associated with specific pheromone doses or control conditions. The results of this study suggest that female moth behaviour is influenced by the dosage. Synthetic blend and extracted blend 15 ppm treatment in *G. mellonella* and 10.7 ppm in *A. grisella* led to more robust behavioural reactions, with increased activity observed in parameters like up flight and orientation time, etc. than other treatments.

The analysis conducted on trap catch in relation to blend, dose, and their interaction in female *A. grisella* and *G. mellonella* revealed significant insights. The Tukey HSD test for blend did not find significant pairwise differences between the two blend levels, indicating that blend variation had a limited effect on trap catch. Both blend levels belonged to the same homogeneous group, suggesting that the blend factor does not have a significant effect on trap catch.

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Difficult roads often lead to beautiful destinations. During this journey, special people come across and make this journey memorable. It would not have been possible to write this doctoral thesis without the help and support of the kind people around me, only some of whom it is possible to give particular mention here.

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constantly supportive and encouraging. My brothers, **Er Gagandeep Singh** and **Er Manjot Singh** were pillars of strength throughout my Ph.D. years. This dream would have not been realised without their moral and financial support, to all I am indebted.

I am short of words to express my deepest gratitude to those who stood by me at every walk of my life, for the constant cooperation, selfless love, care and pain they took to make me achieve this goal.

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Manpreet Kaur Saini

ABBREVIATIONS

G. mellonella	*Gallaria mellonella*
A. grisella	*Achroia grisella*
A. cerana	*Apis cerana*
A. mellifera	*Apis mellifera*
A. dorsata	*Apis dorsata*
A. florea	*Apis florea*
B. thuringiensis	*Bacillus thuringiensis*
WM	Wax Moth
LWM	Lesser Wax Moth
GWM	Greater Wax Moth
GC-MS	Gas chromatography- Mass Spectrometry
SEM	Scanning Electron Microscopy
CIF, LPU-	Central Instrumental Facility, Lovely Professional University
ppm	Parts Per Million
TIC	Total Ion Chromatograph
cm	Centimetre
PDB	Paradichlorobenzene
PBPs	Pheromone-Binding Proteins
VOCs	Volatile Organic Compounds
NIST	National Institute of Standards and Technology
SPME	Solid-Phase Microextraction
IPM	Integrated Pest Management
NMR	Nuclear Magnetic Resonance
mm	Millimetre (s)
i.e	That is (id est)
KOH	Potassium Hydroxide
RT	Retention Time
BOD Incubator	Biological Oxygen Demand Incubator

GLC	Gas Liquid Chromatography
EDS detector	Energy Dispersive Detector
min	Minute
m	Meter
Conc	Concentration
EB	Extracted Blend
SB	Synthetic Blend
MS	Mass Spectroscopy
DCSE	Direct-Contact Sorptive Extraction
PDB	Paradichlorobenzene
CV	Coefficient of Variation
µm	Micrometre
µl	Microlitre
m/z	Mass to Charge Ratio
SE	Standard Error
CCD	Colony Collapse Order
AICP	All India Coordinated Project (AICP)
GBIF	Global Biodiversity Information Facility
ANOVA	Analysis of Variance
C9: OH	Nonanol
C11: OH	1-Undecanal
ICAR	Indian Council of Agricultural Research
US	United States
€	Euro (Euro currency symbol)
e.g.	For Example (exempli gratia)
US dollars	USD (United States Dollar)
FAO	Food and Agriculture Organization
km²	Square Kilometre(s)
UP	Uttar Pradesh
dl	Decilitre
USD	United States Dollar

kg	Kilogram
cm	Centimetre
mg	Millilitre
ICAR	Indian Council of Agricultural Research
kHz	Kilohertz
GCM	Gas Chromatography-Mass Spectrometry
G-protein	G protein
API	Application Programming Interface
GPS	Global Positioning System
ML	Machine Learning
PPE	Personal Protective Equipment
FDA	Food and Drug Administration
CDC	Centres for Disease Control and Prevention
BC	Before Christ
AD	Anno Domini
Py	Pyralidae (family)
Gal	Galleriinae (subfamily)
GBIF	Global Biodiversity Information Facility
°C	Degree Celsius
%	Percentage
ZSI	Zoological Survey of India
R+M	Radius + Median
Govt	Government
IAPV	Israeli Acute Paralysis Virus
BQCV	Black Queen Cell Virus
PM	Post Meridiem
SIM	Selected Ion Monitoring
CRD	Completely Randomized Design
Tukey's HSD	Tukey's Honestly Significant Difference

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CHAPTER 1

INTRODUCTION

Apiculture is a lucrative commercial business that directly and indirectly enhances the income of small-scale farmers and contributes to the overall economy of the nation (Hou et al., 2024). Globally, the production of honey varied by continent, with a total of 1.7 million kilograms produced. Asia has been reported to be the world's largest producer of natural honey, with an estimated production of 859 thousand tons. Europe has approximately 383 thousand tons, Africa has approximately 151 thousand tons, and Oceania has approximately 32 thousand tons, following America, which has approximately 345 thousand tons (Sokhai & Mardy, 2024).

Currently, Punjab is one of the leading states in the country in beekeeping, producing 20,000 metric tons of honey which constitute 14.1 per cent of the apiary honey production of the country. The average production of honey in Punjab is about 40 kg per colony (https://www.indiastat.com/data/agriculture).

Honey bees have long been serving as a source of sustenance, economic security, and ecological well-being for humans. They can serve as valuable bioindicators for identifying and monitoring alterations in the quality of agricultural landscapes over both geographical and temporal dimensions. They generate or gather a diverse range of goods that provide advantages to humans (Ambaw et al., 2020).

The assortment of goods encompasses honey, beeswax, pollen, royal jelly, and propolis within the hive (Bruneau, 2017). The primary importance of honey bees is their crucial role in pollination, particularly for crops, despite the fact that they can be effectively manipulated to produce substantial amounts of these commodities (Castellanos-Potenciano *et al*., 2024). Their pollination services are crucial for the reproduction of several plant species, including many crops that are fundamental to agricultural economics (Prodanovic et al., 2024).

Beekeeping and the storage of hive-related goods are crucial components of agriculture, as they contribute significantly to pollination and the apicultural business (Prendergast et al., 2021). In India, the apicultural sector experiences annual losses that are systematic and attributed to detrimental insect pests. The total losses caused by insects are estimated to be 26% (Samanta et al., 2024). Agricultural productivity and ecosystem integrity can be significantly impacted by the loss of honey bees as a result of pest-related mortality. Nevertheless, beekeepers have been motivated to improve

their technology and apparatus in order to increase the bee population and reduce the overexploitation of current bee species, a trend that is alarming.

1.1 Beekeeping Status

The scientific beekeeping tradition in India is not particularly primordial, despite its long history and the presence of references to it in ancient Vedic and Bodhi scripts. In India, beekeeping has long been practised in an unorganized manner. However, it was organized and developed to improve the financial situation of rural residents under the direction of the All India Khadi and Village Industries Board. *Apis dorsata* Fabricius (*A. dorsata*), *Apis cerana* Fabricius (*A. cerena*), and *Apis florea* Fabricius (*A. florea*) are the three native species of honey bees found in India; foreign species, such as *Apis mellifera* Linnaeus (*A. mellifera*), were brought in 1962 for commercial apiculture (Horo & Singh, 2023). Out of these traditional honey bee species of the world, *viz* two non-domesticated wild species are *A. dorsata* and *A. florea* while two domesticated species are *A. cerena* and *A. mellifera* (Painkra, 2023).

While beekeeping in Punjab has a long history, the late 19th and early 20th centuries saw a major upsurge in activity (Gupta, 2014). This was the inception of contemporary beekeeping practices in the region. At the end of the nineteenth century, Indian traditional beekeeping began to be guided by scientific ideas. States like Punjab, Jammu & Kashmir, Himachal Pradesh, Haryana, Uttar Pradesh, Bihar and West Bengal concentrate most of the *A. mellifera* beekeeping (Thakur, 2016). By pollinating the wide spectrum of edible fruits and cereal crops, *A. mellifera* is rather beneficial in the agricultural industry (Appalasamy et al., 2023).

The initial endeavours in India to maintain *A. cerana* F. bees in movable frame hives to improve manoeuvrability were made in Bengal in 1880 and Punjab and Kullu Valley in 1883-84, but they were not successful (Chauhan et al., 2021; Manzoor, 2021). During 1911-17, Newton initiated beekeeping training in South India and instructed numerous rural residents. Additionally, he developed a hive for *A. cerana*, which is now known as the Newton hive, that was specifically designed to withstand the climatic conditions of India (Abrol, 2013). Intensive beekeeping activities were initiated in Travancore in 1917 and in Mysore in 1925 (Aswini, 2013). Beekeeping in rural India

received a boost from the Royal Commission on Agriculture's (1928) recommendation to cultivate cottage industries (Dalwai, 2021). Subsequently, beekeeping was implemented in Madras in 1931, Punjab in 1933, Coorg in 1934, and Uttar Pradesh in 1938 (Sivaram, 2012).

The All-India Beekeepers Association was established by the beekeepers of India in 1938-39. The Indian Council of Agricultural Research (ICAR) subsequently established the first Beekeeping Research Station in the Punjab in 1945 and a second station in Coimbatore, Tamil Nadu, six years later (Narang & Kumar, 2022). ICAR has been providing financial support for a variety of research initiatives on beekeeping since 1950. ICAR initiated the All India Coordinated Project (AICP) on Honey Bees Research and Training in 1980. Currently, the project has 16 project centres across the country, with an administrative centre located at the Haryana Agricultural University Campus in Hisar, Haryana state (Painkra, 2023).

1.2 Beekeeping Industry Challenges

Wild bees are of immense importance as essential natural pollinators on a global scale. Murthy et al. (2024) have described approximately 20,000 bee species worldwide. They are efficient pollinators due to their reliance on weather conditions, range of floral preferences, flight periods, and species diversity and abundance (Abrol, 2013). Furthermore, the scope and characteristics of pollination services offered by wild bees vary depending on the geographic location, kind of landscape, climate conditions, and floral morphology. The reduction of bees can be ascribed to a confluence of variables, including habitat loss, habitat modification, habitat fragmentation, pesticide utilization, climate change, and the introduction of pests and diseases (Ganie et al., 2024).

Honey bee colonies attract approximately 40 predators, including humans, owing to their ideal habitat. These adversaries pose a threat to the survival of bees since they can harm the colony and inflict significant damage to the combs and hive products (Phiri et al., 2022). Scientists have shown a worldwide decrease in bee species annually during the 1990s, as seen by data from the Global Biodiversity Information Facility (GBIF). Between the years 2006 and 2015, there was a decrease of 25 percent in the number of bee species compared to the year 1990 (Zattara & Aizen, 2021).

Pesticide and mite injury to bee colonies was the most prevalent issue among beekeepers, affecting 79.17% of them. The percentage of honey production per hive that fell below 20 kg was 41.67, while the percentage that fell between 20 and 40 kg was 57.29 (Singh et al., 2021).

According to Milum (1940) and Singh (1962), the following 10 moth pests have been identified as enemies: *Galleria mellonella*, also known as the greater wax moth, the *Achroia grisella* (F.), lesser wax moth, The *Plodia interpunctella* Hubner, Indian meal moth. The species is known as the Mediterranean flour moth, scientifically named *Ephestia kuhniella* Zeller. The fig moth, *Ephestia cautella* Walker, is the fifth species. The dried fruit moths are known as *Vitula serratilinella* Ragonot and *Vitula edmansi* Packard. The codling moth, *Carpocapsa pomonella* (Linnaeus), *Aphomia sociella* (Linnaeus), the bumble bee moth, and the species known as *Acherontia styx* (Westwood) is commonly referred to as the Death head moth.

The intricate balance of the hive and the overall health of honey bee populations are profoundly affected by these two wax moth species: the Greater wax moth (*Galleria mellonella*) and the Lesser wax moth (*Achroia grisella*) (Sarwar, 2016) (Table 1.1). Beehives, crucial for the well-being and efficiency of honeybee colonies, are susceptible to wax moths, posing a threat. These seemingly inconspicuous moths are important pests that provide substantial issues for both beekeepers and the stored product industry. Wax moths attack the complex structures of beeswax combs, causing severe damage and jeopardizing the essential structure of honey bee colonies (Mucsi, 2020). The larva inflicts damage to the combs and hive products in both active colonies and during storage. The damage caused is catastrophic, especially in vulnerable colonies, where the combs can rapidly deteriorate into a tangled mess of webs and debris.

Concurrently, the preservation of gathered bee products, such as beeswax, becomes a conflict zone due to the negative influence of wax moth larvae, resulting in financial losses and reduced product quality.

1.2.1 The Biology of The Greater Wax Moth

Gallaria mellonella (*G. mellonella*) adult moths are pale brown to grey, usually about 15 to 20 mm long. The grey wings are often mottled and appear as 'roof' or 'boat' shaped when folded over the body. The bees vigorously resist females who have mated and try to enter a hive during the early evening. However, the bees cease performing this about two hours after dusk (Ali et al., 1973). Eggs can be deposited in the vicinity of apiary structures, particularly in areas with honey or comb residue, as well as in stored combs and active beehives (Singh et al., 2019).

Typically, female moths deposit between 300 and 600 eggs in small crevices in the hive material or in clusters on the comb (Vijayakumar et al., 2019; Wojda et al., 2020). The pinkish-white, almost spherical eggs have a diameter of 0.5 mm. When the temperature is between 29°C to 35°C, the eggs hatch in 3 to 5 days (Warren and Huddleston, 1962). At 18°C, hatching begins approximately 30 days following egg laying and is delayed at lower temperatures (Nganso et al., 2024).

The larvae hatch at 1–3 mm in length and 0.12–0.15 mm width (Paddock, 1918; Smith, 1965). Late instar larvae are 25–30 mm long and 5–7 mm wide before pupation. Six thoracic legs and many prolegs on the third to sixth abdominal segments make up the polipod (eruciform) larva. The larvae have cream-coloured, sclerotized skin, which darkens with each succeeding moult. There are no sub-apical teeth in the head, but there are three fully formed apical teeth (Smith, 1965).

The pupa has an average length of 12–20 mm and a diameter of 5–7 mm. The pupa belongs to the obtect type, and during ecdysis, a fluid is produced that causes all of its extremities to stick to the body. Pupae that are female are typically lengthier than those that are male. Upon emerging from their pupae in a hive, adult bees depart from the hive and extend their wings. Shortly after nightfall, they take flight towards trees to engage in mating (Birah et al., 2008). From the time the eggs are laid until the adult emerges from the pupa, it usually takes a minimum of one month at the most favourable temperature of 35°C. However, in temperate areas, this period is extended, often resulting in the pest only being able to complete one generation every year (Bhatnagar et al., 2020).

1.2.2 Biology of The Lesser Wax Moth

Achroia grisella (*A. grisella*) is a moth that is smaller in size than *G. mellonella*. Its slender body, which is approximately 13 mm in length, is silver-grey to dull-yellow in colour. Their colour varies from silver-grey to beige, and their yellow head stands out. Greenfield and Coffelt (1983) found that males congregate in groups in or near the beehive at night when they are prepared to reproduce and attract females by emitting ultrasonic signals for 6–10 hours each night. After detecting these courtship calls, receptive females typically pursue emitting males and select their partners based on the characteristics of their songs (Jang and Greenfield, 1998; Limousin and Greenfield, 2009).

Adults hide in trees and bushes near hives during the day. Female adults usually lay eggs near food sources in sheltered nooks at night. It is estimated 250–300 eggs are laid by *A. grisella* throughout its brief life (Smith, 1965). The eggs are round and creamy white. The duration of an egg's incubation is contingent upon its temperature, which ranges from 5 to 22 days. Egg hatching time varies, with warmer temperatures accelerating development across all life stages. Hatching normally takes 5-8 days for eggs.

Lesser wax moth larvae are typically white with a brownish head. They are typically solitary, whereas greater wax moth larvae frequently congregate in large numbers. Larval development occurs at 29° to 32°C for an average of six to seven weeks but can take anywhere from one to five months (Smith, 1965). There are seven moults for the larvae. Mature larvae measure about 20 mm in length, with the last two instars representing the majority of the larva's growth. The larval stage is the only phase of the life stage that consumes food. Honey bee larvae and pupae, pollen, and honey are the primary items that larvae consume from combs. Larvae favour pollen and brood comb over virgin and/or honeycomb (Greenfield and Coffelt, 1983).

Pupae are approximately 11 mm in length and exhibit a yellow-tan colour. Cocoons are white and are secured in position by webbing. Frass and other debris frequently obscure cocoons, rendering them challenging to identify. The average time to adult emergence is approximately 37 days, although pupae can take up to two months to mature (Egelie et al., 2022). At a temperature of 25°C, females have an average

lifespan of 7 days, while males have an average lifespan of 10 to 14 days (Greenfield and Coffelt, 1983).

Table 1.1: Comparison of the key features and differences between *G. mellonella* and *A. grisella*

Stage	**Greater Wax Moth (*Galleria mellonella*)**	**Lesser Wax Moth (*Achroia grisella*)**	**References**
Egg			
Appearance	Small, spherical shaped, initially white turning pale yellow or brownish	Small, oval shaped, initially white turning yellowish	(Smith, 1965; Kwadha et al., 2017)
Size	0.44 ± 0.04 (length) × 0.36 ± 0.02 (breadth) mm	0.41 ± 0.02 (length) × 0.31 ± 0.01(breadth) mm	(Ellis, et al., 2013)
Location	The female lays about 175 to 355 eggs in cluster in the cracks and crevices of colony and on comb surfaces	Female lays 200-300 eggs in clusters or singly in cracks, crevices, and on comb surfaces	(Desai et al., 2019)
Hatching Time	Hatching commences 3–5 days after oviposition at 29–35°C and can last up to 35 days at 18°C	They hatch within 3-5 days under favourable conditions	(Smith,1965; Williams, 1997)
Larvae			
Appearance	Creamy white body with a dark brown head; older larvae may	White to light grey body with a dark head capsule	(Smith, 1965; Sharma et al., 2015)

	develop a pinkish or yellowish tint		
Size	Can grow up to 25-28 mm in length	Can grow up to 20 mm in length	(Smith, 1965)
Behaviour	The most destructive stage; larvae feed on wax, honey, pollen, and bee brood, leaving behind silk threads and frass (excrement)	Feed on pollen, honey, wax, and bee brood; less harmful than the larvae of the Greater wax moth	(Williams, 1997)
Development Time	Larval stage lasts about 1-2 months depending on environmental conditions	Larval stage lasts about 1-1.5 months depending on environmental conditions	(Ellis, et al., 2013)
Pupae			
Appearance	Enclosed in a silken cocoon, white or light brown in colour; cocoons are usually found in protected areas within the hive, such as wooden surfaces or within the comb	Enclosed in a silken cocoon, white or light brown in colour; cocoons are usually found in protected areas within the hive	(Williams, 1997)
Size	About 15-20 mm in length	About 10-15 mm in length	(Williams, 1997)
Pupal Stage Duration	Pupation lasts about 6-7 weeks at 29 to 30^{o}C temperature, duration	Pupation lasts about 6-7 weeks, affected by temperature and humidity	(Ellis, et al., 2013)

	gets longer in cooler temperatures		
Adult			
Size	Body length: 15 to 20 mm (length) with average wingspan forewing ranges from 18 to 23 mm and hindwing ranges from 8 to 15 mm	Body length: 10 to 13 mm long, average wingspan forewing ranges from 8 to 13 mm and hindwing ranges from 5 to 9 mm	(Williams, 1997)
Colour	Forewings are greyish-brown with darker markings; hindwings are lighter, almost whitish or pale grey and lightly fringed	Large fringes on the hind wings and oval-shaped forewings	(Williams, 1997)
Wings	Forewings are elongated and narrow with a pointed tip, having a wavy pattern and a more uniform coloration; hindwings have a fringe of fine hairs along the edges. The termen of the forewing is concave, and the cu of the hindwing is purportedly four-branched. The labial palp is long,	The hindwings are more translucent and have a fringe of tiny hairs, while the forewings are shorter, rounder, and contain a variety of dots and blotches. Forewing termens are convex, whereas male hindwing termens are concave; hindwing cusps appear to be three-branched; male labial palps are transversely incurved and pincer-like; and labial	(Ellis, et al., 2013)

	approximately the same length as the eye's diameter	palps are noticeable but brief, not longer than the diameter of the eye	
Body	Reddish colour, heavy-bodied, cylindrical covered with fine scales, appearing smooth	Small, silver-bodied with a prominent yellow head, Shorter, more robust body with a broader abdomen, covered with scales but appearing less smooth due to more pronounced markings	(Williams, 1997)
Antennae	Thread-like (filiform) and long, proportionate to body length	Thread-like (filiform) and slightly shorter than those of the Greater Wax Moth	(Smith, 1965; Williams, 1997)
Behaviour	Adults are more active at night and are strong fliers; tend to be more destructive to beehives due to larger size and higher reproductive rate	Adults are nocturnal but weaker fliers; tend to cause less damage compared to the Greater Wax Moth	(Smith, 1965)
Life Span	Female have a life span of nearly 12 days while male have 21 days	The males lived nearly twice as long as the females, with an average of 13.03±0.51 and 7.46±0.29 days, respectively	(Williams, 1997; Mahgoub et al., 2015)

1.3 Beekeeping in Crisis

Wax moths are the pervasive pest of honey bees (Hand et al., 1987). The pests persistently assault the stability of beeswax combs and devour various substances within the hive, such as wax, discarded honey bee pupal skin, remnants of larval bees, pollen, honey bee excrement found on the brood cell walls, honey bee cocoon silk, and create clusters of web by infiltrating the central vein of the comb (Dweck et al., 2010). The larva is a voracious eater and poses a significant threat to beekeepers. It causes the entire colony to disappear since it feeds on wax combs, honey, pollen, cast-off larval bee skin, and brood. As a result, the whole wax comb is covered with a mountain of frass and detritus (Kundungal et al., 2024).

The prevalence of wax moths in the bee colonies often leads to many problems like colony collapse disorder, colony dwindling and absconding caused by honey bee pests and pathogens. As these pests infiltrate the sanctuaries of beehives, beekeepers find themselves locked in an ongoing battle to preserve the vitality of their colonies and safeguard the cherished products of their labour (Figure 1.1). This contributes to the colony mortality and substantial loss to the comb (Kulhanek et al., 2021).

Moreover, they also decline in size of migratory bee swarms (Williams, 1997; Xu et al., 2023). The beekeepers suffer significant financial losses as a result of the damage (Kapil and Sihag, 1991; Almadani and Hiware, 2020) and is attributed to its high reproduction potential, swift growth stages, and numerous individuals (Warren and Huddleston, 1962; Shimanuki, 1981; Turker et al., 1993).

1.3.1 Silent Invaders: Nocturnal Pests in Stored Apicultural Products

Beyond the realms of beehives, wax moths extend their influence into the storage facilities that house harvested honey, beeswax, and beekeeping equipment. *G. mellonella* and *A. grisella*, driven by an insatiable appetite, infest stored agricultural products, leaving behind contamination, spoilage, and economic losses. The larvae of wax moths create menace by consuming beeswax, pollen, and honey residues, wreaking havoc on stored products, and resulting in economic losses and a diminished quality of honey and beeswax.

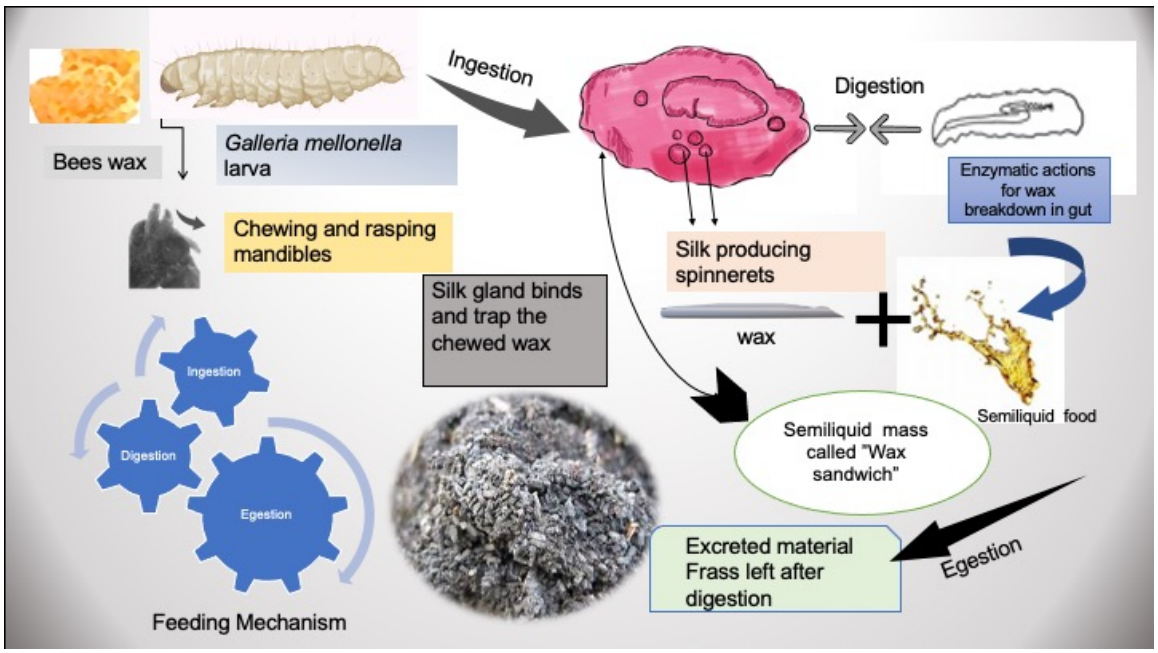


Figure 1.1: *Galleria mellonella* larvae's intricate eating habits within honey bee colonies are depicted in this illustration. The larvae demonstrate their ability to consume beeswax by utilizing their mandibles, which are equipped with chewing and rasping capabilities. The wax that is consumed is enzymatically decomposed in the gastrointestinal tract. Subsequently, the larvae generate silk from their silk glands, which ensnares and immobilizes the masticated wax. This procedure leads to the creation of a partially liquid mass referred to as a "wax sandwich." The graphic depicts the excretion process, specifically emphasizing the residual material (frass) (Copyright Filed)

As nocturnal creatures, wax moths undertake clandestine flights under the cover of darkness, complicating prevention and control efforts (Hamida, 1999). They undergo complete metamorphosis stages and are considered as true wax moths (Colter, 1994). Their life cycle, characterized by an egg-larva-pupa-adult progression, demands nuanced strategies for effective management (Ellis et al., 2013). The closely related species; the lesser wax moth is less devastating than the greater wax moth and not so common (Ellis et al., 2013).

They almost invade in all the continents except Antarctica (Kwadha et al., 2017). The pest is highly adapted to live in bee hives (Ellis and Munn, 2005) causing damage to beeswax comb that comprises an integral component of the honey bee nest (Berry and Delaplane, 2001; Swamy et al., 2005; Hamby, 2007; Abou El-Ela, 2014). The destructive pest infests in folds of *A. Mellifera* and *A. cerana* colonies during rainy seasons and dearth period (Marston, 1975).

The infestation of *G. mellonella* commonly called as wax moth or web worm or honeycomb moth in *A. mellifera* colonies was recorded from February till July 2020 (Figure 1.2). During the colder months of February and March, no infestation was

recorded. The infestation was first observed in the month of April which increased in May-June. In the month of July, infestation rate was maximum. The percentage infestation of wax moths varied from 35% in the month of April to 80% in the month of July (Singha et al., 2023).

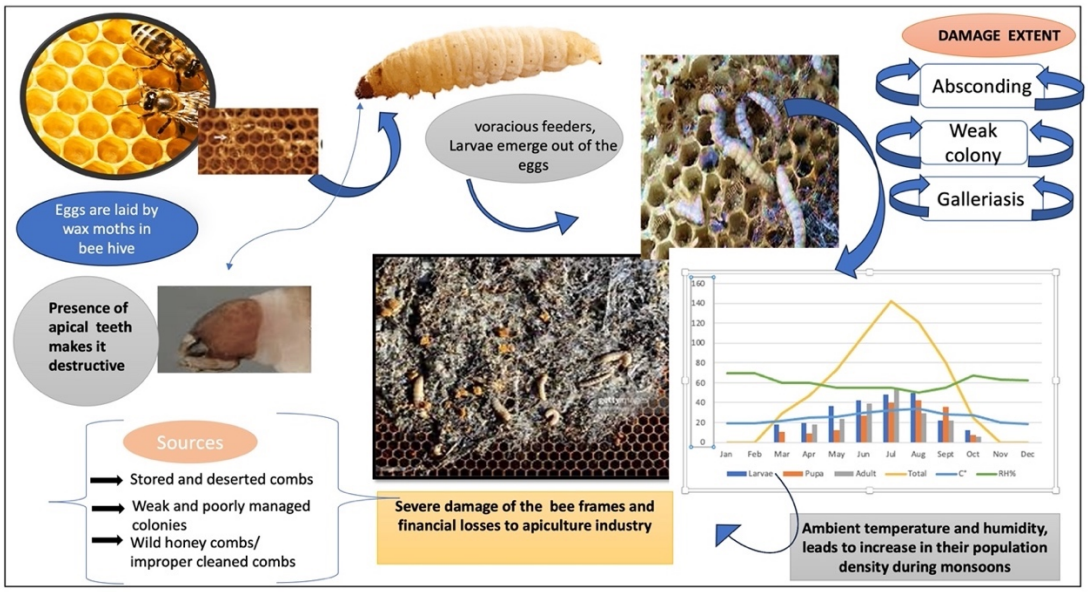


Figure 1.2: The diagram depicts the life cycle of wax moths and their significant detrimental effects on honeycombs found in bee colonies. The statement emphasizes the process by which eggs produced by the moths transform into ravenous larvae that inflict substantial harm by consuming the honeycomb. The diagram illustrates the causes of infestation, including stored and abandoned combs, inadequately maintained colonies, and incorrectly sanitized combs. The repercussions of infection are illustrated, encompassing absconding, debilitation of the colony, and the manifestation of galleria disease (galleriasis). Moreover, the graph illustrates the correlation between ambient temperature, humidity, and the population density of wax moths, specifically in the monsoon season. (Copyright Protected: L-148934/2024)

The impact of these silent invaders on the quality and marketability of bee products poses a multifaceted challenge for honey producers and stakeholders in the stored product industry (Figure 1.3). Adding to the complexity of combating these pests is their nocturnal behaviour (Pasho et al., 2021). The night becomes their ally as they embark on flight patterns drawn toward sources of light, eluding traditional prevention and control methods.

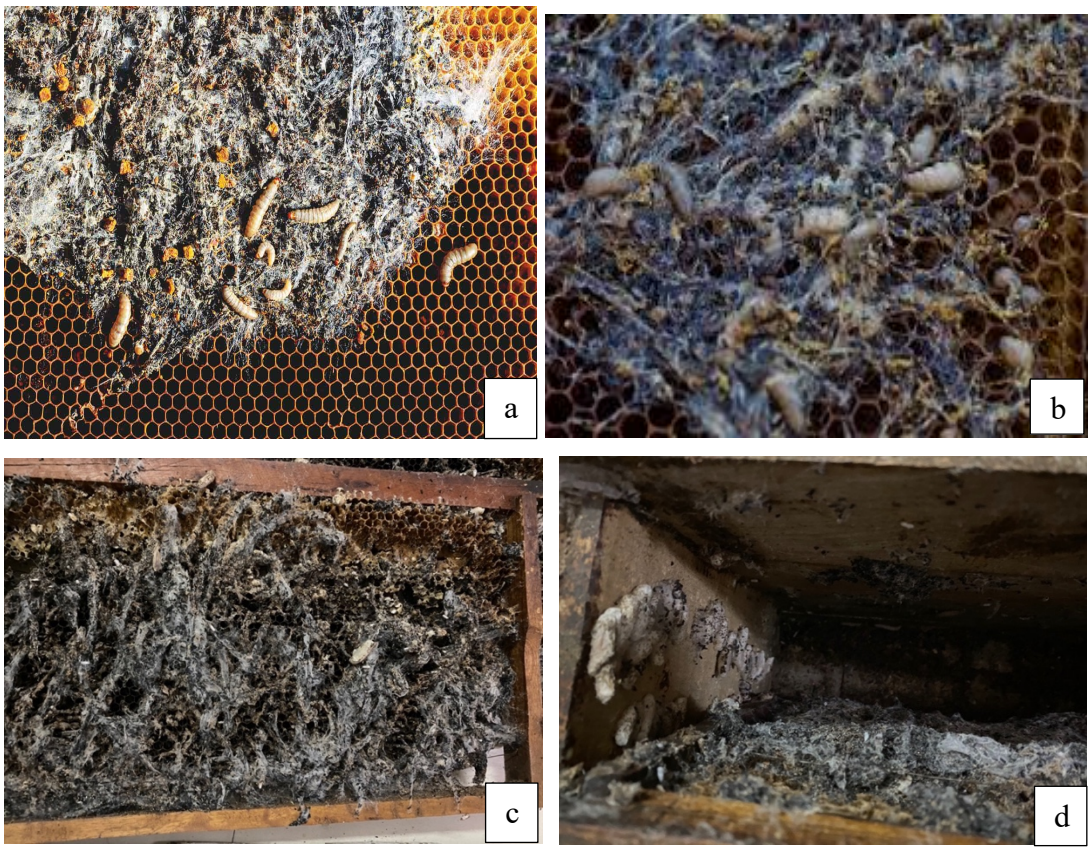


Figure 1.3: Progressive Stages of Wax Moth Infestation in Honeybee Colonies (a) Initial Infestation: Wax moth larvae beginning to spread across the honeycomb, with early signs of damage and webbing visible (b) Intermediate Stage: Extensive larval activity resulting in significant damage and dense webbing within the honeycomb structure (c) Advanced Infestation: Severe destruction of the comb, with extensive webbing and larval debris covering the frames, indicating heavy infestation (d) Severe Damage: The hive box shows advanced structural damage and contamination by wax moth larvae and their by-products, leading to compromised hive health and potential colony collapse

1.4 Navigating the Research Landscape

Empirically data reveals that these pests contribute to the decline of the feral and wild honeybee population. They usually take advantage of the colonies weakened by pesticide exposure, bee diseases and pathogens (Newton, 1917; Gulati and Kaushik, 2004). The strong and active bee colonies are less prone to infestation by the wax moths where the worker bees effectively control the wax moth larvae and adults (Romel et al., 1992). The honey bee colonies with a low population density of adults and high starvation rate are at high risk of attack by wax moths (Shimanuki et al., 1980). Elevated infestation rate could lead to colonies absconding (Owayss and Abd-Elgayed, 2007; Tsegaye et al., 2014). As the battleground between honeybees and wax moths

intensifies, a deeper understanding of the ecological nuances of these pests becomes imperative. Moreover, the moths have developed resistance to certain chemical pesticides, rendering conventional strategies less effective. This evolution in resistance underscores the need for innovative and sustainable approaches to navigate the challenges posed by *G. mellonella* and *A. grisella.*

1.5 Control Strategies

Apiary management and the management of apparatus and honey bee products away from the colony are the two primary categories into which wax moth control strategies are divided. Apiary management strategies are designed to ensure the health of bee populations and implement general sanitation protocols, including the elimination of wax and debris from hive boards (Sawadogo et al, 2025; Sharma et al, 2024). The management of honey bee products and apparatus entails the freezing of combs at a specific temperature, the improvement of hive structures, the rotation of combs, and the management of pollen. These strategies help prevent wax moth infestation and maintain healthy honey bee colonies (Catania & Vallone, 2020; Kankare et al., 2022).

Williams (1997) specifies that off-colony product management encompasses a variety of constraints, including biological, chemical, physical, and cultural. To address the threat posed by the greater wax moth and the lesser wax moth, these measures have been implemented. The pest, however, continues to be a significant threat to the beekeepers (Jyothi & Reddy, 1992; Fraser, 1997; Abrol & Kakroo, 1998; Garg, 1998).

A number of investigations have highlighted the possibility for the development of a pheromone-based monitoring and control system to combat wax moths (Finn, 1977; Flint and Merkle, 1983; Romel, 1991). In spite of the potential of these methods, commercial pheromone based control products are not yet available for routine use in apiaries or bee product storage facilities due to environmental variability and limitations in field efficacy (Kaur & Singh, 2023; Zhang et al., 2021).

According to Mohanraj et al. (2024), the commercial viability and reliability of pheromone-based control systems are anticipated to be enhanced in the near future by ongoing advancements in pheromone chemistry, trap design, and behavioral bioassays.

1.6 Shift Towards Sustainable and Eco-Friendly Pest Control Methods

In recent years, a pivotal shift has taken place in agricultural practices, marked by a transition away from conventional pest control methods reliant on chemical interventions. This evolution is driven by a growing awareness of the environmental impacts associated with synthetic pesticides, concerns about the development of pesticide-resistant pests, and a broader commitment to sustainable agricultural practices. The emerging paradigm embraces integrated, eco-friendly approaches that not only effectively manage pest populations but also prioritize environmental stewardship and long-term ecological sustainability.

1.6.1 Integrated Pest Management (IPM)

Integrated Pest Management (IPM) is a comprehensive approach that minimizes the need for chemical pesticides by incorporating a variety of pest control methods. The approach encompasses biological, cultural, and mechanical control methods alongside the judicious use of chemical interventions when necessary. IPM entails the implementation of preventive measures, the monitoring of pest populations, and the utilization of natural predators and parasites to manage pests. This approach endeavours to preserve environmental conservation while simultaneously implementing insect control measures.

1.6.2 Pheromones as Potential Tool for Biocontrol

Pheromones have been effectively employed in the biomonitoring of pest emergence patterns and population numbers, as well as in the assessment of insect resistance (McNeil, 1991), annihilated entrapment, and communication disruption (McLaughlin and Heath, 1989). It can identify low-density populations, allowing for the assessment of species, and physiology and determining the need for control actions. It reduces the adult population as well as pests in the next generation and is a popular method for controlling pest populations. Pair formation could be exploited to directly control the reproducing individuals (Romel, 1991). The females are successfully restricted from

laying eggs, and population size can be controlled. These pheromone-baited traps are employed to identify exotic invaders, determine whether pest levels are high enough to necessitate intervention and arrange the timing of the application of conventional insecticides or other control measures (Klassen, 2008).

They are appropriate stimuli for insects' powerful chemosensory systems, which enable them to sense and communicate with other members of their species. Lepidopteran sex pheromones are typically composed of two to seven components, which are either non-cyclic (in the case of females) or heterocyclic (often, in the case of males), and contain functional groups such as acetates, alcohols, or aldehydes (Baker & Heath, 2005; Howse et al., 2013). Pheromone signals, emitted by insects to impact the behaviour or physiology of individuals of the same species, are crucial for several animal species (Matthews et al., 2010). They begin and govern a range of crucial functions, such as the allocation of tasks in eusocial animals, as well as mating, reproduction, gathering, and alerting (Wyatt, 2017). They are predominantly composed of a combination of compounds that are synthesized in specialized androconical glands located on their forewings (Roller et al., 1968).

The specialized sensory neurons of the olfactory system, located on the antennae, detect these volatile pheromone molecules (Hansson and Stensmyr, 2011). The pheromone molecules then spread throughout the interior of the sensilla, which are specifically designed to detect the pheromone, via tiny openings in the outer layer. It is hypothesized that these fat-soluble chemicals are transported to the chemosensory membranes by pheromone-binding proteins (PBPs) that have a wide range of specificity. This transfer occurs through the watery sensillum fluid after the compounds have been absorbed. The pheromone, or its PBP-complex, then binds with a receptor protein specific to the pheromone, which then turns the chemical signal into an electrochemical signal. Specific sensory neurons, receptors, and PBPs distinguish the components of multi-component pheromone compounds (Blomquist and Vogt, 2021).

The application of insect pheromones has been facilitated by the apicultural industry's distinctive operational characteristics. The majority of these applications employ synthetic reproductions of pheromones that facilitate either attraction or aggregation. The pheromone is emitted by compounds that are formulated in protective matrices or reservoirs over the course of weeks or months (Carde and Millar, 2009).

The commercial implementation of pheromone trapping systems is a feasible enterprise, particularly if a company manufactures a line of beekeeping products, due to the absence of existing controls against wax moths and market acceptance. The application of insect pheromones has been facilitated by the apicultural industry's distinctive operational characteristics. The majority of these applications employ synthetic reproductions of pheromones that facilitate either attraction or aggregation. The pheromone is emitted by compounds that are formulated in protective matrices or reservoirs over the course of weeks or months (Carde and Millar, 2009). The commercial implementation of pheromone trapping systems is a feasible enterprise, particularly if a company manufactures a line of beekeeping products, due to the absence of existing controls against wax moths and market acceptance..

Recent research has been conducted on the utilization of pheromone traps as part of a pest suppression program for the management of insects (Finn, 1977; Flint and Merkle, 1983) and have shown great potential as biocontrol agents in the field of insect control. In most cases, sex pheromones attract mates and have been extensively used in integrated pest management, especially with lepidopteran pests (Witzgall et al., 2010). The practical application of the sex pheromones of numerous economically significant lepidopteran insect species extends to control programs that are intended to monitor or suppress nuisance populations (Heath et al., 1983; McDonough, 1983). Utilizing pheromones as a means of pest control signifies a focused and ecologically sustainable methodology, presenting numerous benefits in comparison to conventional chemical pesticides.

The benefits of pheromones over conventional controls include their low maintenance requirements, their cost efficiency, their lack of toxicity, and their ability to be used in both storage and field environments (Scott, 1984). The comparatively uniform habitat and confined areas of stored product pests make them well-suited for IPM programs, which include pheromones (Fraser, 1997). The creation of a pheromone-based capturing system would have a global commercial impact, providing substantial advantages to beekeepers in both developed and less economically developed countries. Strategic pheromone trapping improves field and storage conditions, requiring active space, temperature effects, trap placement, and secure storage (Figure 1.4). Regular inspections and control measures are recommended which

provide information about the population density of pest which in turn helps to determine the optimal control strategy and has received considerable attention to control and monitor the invasion of wax moths in the honey bee colony (Van Emden and Van Emden, 1991).

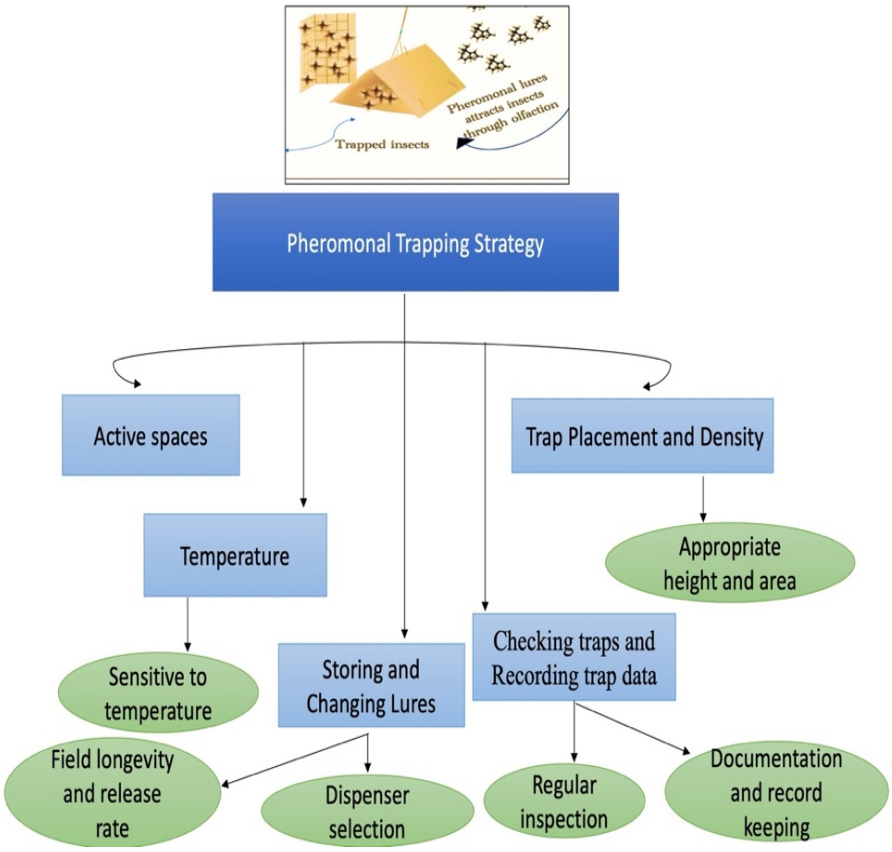


Figure 1.4: Flow chart represents pheromone trap strategy, an approach to pest management allowing targeted interventions

1.6.2.1 Isolation & identification of pheromones

Typically, pheromonal compounds are extracted from insects using solvent extraction, solid-phase microextraction (SPME), or dynamic aerosol collection with adsorbents such as Porapak Q or activated charcoal. Under natural or induced conditions, these methodologies are effective in the collection of volatile organic compounds released by insects (Millar & Haynes, 1998; Leal, 2013).
Gas Chromatography-Mass Spectrometry (GC-MS) is the primary method used to separate and identify individual chemical constituents in the chemical analysis of these

volatile compounds. In order to verify the biological activity of compounds, GC is employed to ascertain which compounds elicit physiological responses in insect antennae (Witzgall et al., 2010).

Synthetic analogues are generated after the identification of the pheromonal components, and their behavioral significance is verified through olfactometer assays, field entrapment experiments, or mating disruption studies (Symonds & Elgar, 2008; Saveer et al., 2023). The chemical and behavioral substantiation of a compound's function as a pheromone can be assured by this integrative approach. Lepidoptera, Coleoptera, and Hymenoptera are among the insect orders that have implemented these methodologies extensively (Toth et al., 1992; Zhu et al., 2001).

1.6.2.2 Pheromonal Profile of The Greater Wax Moth

The male *G. mellonella* produces a strong aromatic sex pheromone with aldehydes as the main component, especially nonanal and undecanal, which are enticing to female *G. mellonella* (Flint and Merkle, 1983). Other minor aldehyde components include decanal, hexanal, and heptanal. Fatty acids (nonane and undecane), carboxylic acids (nonanoic acid and undecanoic acid), and alcohols (1-undecanol, 1-nonanol, and 6,10,14 trimethylpentacanol-2) (Svensson et al 2014) are the other constituents. The male pheromones of the wax moth were previously identified as a blend of nonanal and undecanal.

A third minor alkane component (5,11-dimethylpentacosane) was identified in male-emitted volatiles (Svensson et al., 2014). Female greater wax moth (GWM), *Galleria mellonella* Linnaeus responded to different binary blends of undecanal and nonanal in the percent ratio of 95:5, 90:10, 85:15, 80:20, 60:40, 50:50, 30:70, 20:80, and 10:90 (Sangramsinh et al., 2014). The pheromonal volatiles from calling males of *G. mellonella* in six regions of Russia exhibited different compositions of pheromones. The major components of the volatiles constitute nonanal and undecanal. The ratio of the components varies in different regions (Lebedeva et al., 2002).

The potent and pleasant scent of *G. mellonella* has been isolated and characterized as a combination of n-nonanal and n-undecanal, with the former

compound being the most abundant. The volatiles collected and examined from newly emerging virgin males (Leyrer and Monroe, 1973) showed a ratio of undecanal to nonanal (C9:AL) of 3:7. Two aldehydes, undecanal and nonanal, are the primary components of the sex pheromone generated by males. These aldehydes are attractive in a 1:1 ratio (Flint and Merkle, 1983). The aldehydes, primary alcohols, and nonane and undecane fatty acids were identified and quantified through chemical analyses of pheromone-gland extracts and volatiles released by male *G. mellonella* (Romel, 1991).

Finn (1977) documented a 28% capture rate in field cage bioassays that employed a 1:1 formulated blend. Traps baited with a 3:7 formulated blend were used to recapture less than 5% of GWM females released into the greenhouse and 1% of those released into apiaries in trials conducted by (Flint and Merkle, 1983).

Manufacturers of synthetic pheromone stimuli persist in marketing products without pertinent volatile output data, despite the recommendations of (Butler and McDonough, 1981; Heath and Tumlinson, 1986). This supposition has been supported by recent endeavours to create a trapping system that is appropriate for the surveillance and suppression of GWM in beekeeping storage facilities and apiaries.

1.6.2.3 Pheromonal Profile of The Lesser Wax Moth

The two odour compounds are chemically identified as a combination of undecanal and cis-11-octadecanal (Dahm et al., 1971). These two compounds play a significant role in pair forming with conspecific females, along with sexual communication and acoustic wing fanning by the males. During the scotophase, the male *A. grisella* has been observed to remain stationary and fan its wings continuously in the upper parts of the plexiglass cage (Kunike, 1930).

1.7 Male Behaviour Associated with Pheromonal Release

In Lepidoptera, sexual communication depends on female produced sex attractants but in the exceptional case of the wax moth, it is the male moth that produces the pheromone (Lofstedt et al., 2016). The female moth exhibits characteristic behaviour and elicits attraction response to the scent produced by the male *G. mellonella*. The

scent has specific characteristics in combination with the burst of ultrasonic signalling which can attract conspecific females over the long distances (Greenfield and Coffelt, 1983). It is emitted from the specialized forewing glands that triggers the attraction of females and can be perceived by the human nose (Roller et al., 1968). The fluttering of wings appeared to be linked to pheromone release in a male moth. The release of pheromones follows a similar movement to the release of sound.

The male moth releases pheromones from the pair of glands located ventrobasally on the forewing to attract the female *G. mellonella*. Males do not continuously fan their wings while calling, as *A. grisella* does by extending the wings about 45 degrees from the body (Flint and Merkle, 1983). The pheromonal release aids in searching for a mate (Finn, 1967). After sunset, male wax moths begin to initiate sound impulses. They are near or in contact with other wax moths inside the bee hives. Male courtship pheromones combine with acoustic and usually ultrasonic signals for attraction. Acoustic signals can play a role in both reproductive isolation and mate choice (Figure 1.5).

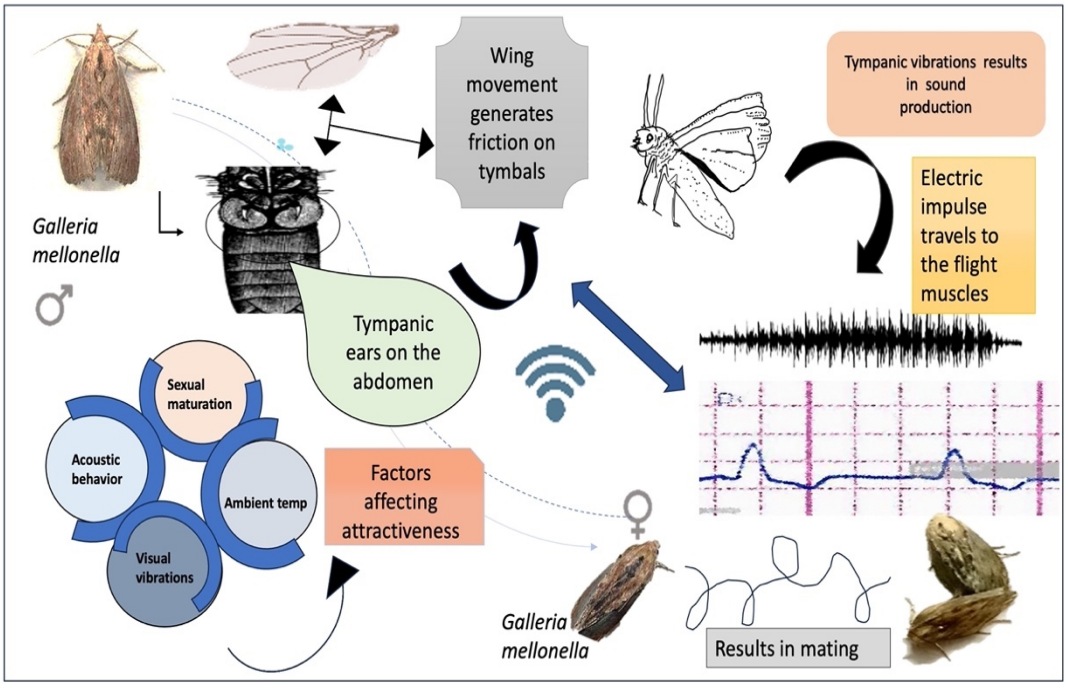


Figure 1.5: Diagram depicting the mating behaviour of *Galleria mellonella*, emphasizing the role of tympanic vibrations and wing movement in sound production, the influence of factors affecting attractiveness, and the process leading to successful mating. The intricate nature of coupling cues and signals in this species is illustrated through the interactions between acoustic behaviour, environmental factors, and sexual maturation (Copyright no-L-138067/2023)

In addition, males use pheromones to attract females and dissipate short pulses of sound at a frequency of 75 kHz, which can play a role in reproductive pair selection (Finn, 1977). They produce acoustic signals using structures found on their wings (Spangler et al., 1984; Spangler, 1988b). The tymbal covering the forewing insertions on each tegula produces the sound. A membranous bag-like structure creating an air chamber is attached to the underside of the tegulae. The tegulae are elevated when the sound is being produced. The tymbals expand frontally during the wing upstroke and buckle inward during the downstroke (Spangler et al., 1984).

1.8 Male Searching and Female Signaling

Females are not known to produce any sex pheromones. Although they cannot locate the sound source, females react by fanning their wings (Spangler, 1988b). Males release pheromones in response to female wing movements, which aid in attracting partners before mating (Leyrer & Monroe, 1973; Spangler et al., 1984; Spangler, 1985, 1986, 1987, 1988b; Jones et al., 2002).

Attracted females approach, calling males from behind and passing along with them, prompting the male to make rapid circular movements to locate the female (ca. 2 to 5 cm in diameter). Their bodies were oriented in the same way, with the male dorsally overlaid on the female. With their heads looking in different directions, the males swung to the side and arranged themselves in a tail-to-tail configuration (Flint & Merkle, 1983) (Figure 1.6).

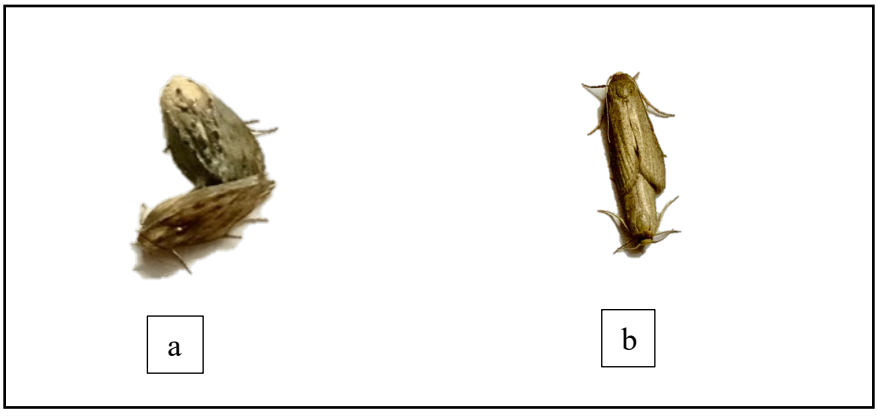


Figure 1.6: Copulation typically occurs in the tail-to-tail position involving the insertion of the male intromittent part into the female genital tract. The male seizes the female by her genital orifice, facilitating fertilization. a) Mating in *G. mellonella* b) Mating in *A. grisella*

1.9 Economic Importance of The Greater Wax Moth and Lesser Wax Moth

The larvae of wax moths are known for their destructive feeding behaviour, which renders them one of the most significant pests that affect honeybee products. In addition to pollen, the larvae consume wax, honey etc. They leave masses of webs on the frame and create tunnels in the comb (Aarifie et al., 2024). The larvae cause damage by constructing silk-lined tunnels that extend through the hexagonal cell walls and over the comb surface (Nganso et al., 2024). Larvae create tunnels and borings on the cell caps, which lead to the release of honey through openings (Kondrateva et al., 2020). A phenomenon known as galleriasis occurs when the silken filaments entangle emerging bees, resulting in their death from starvation (Whitcomb, 1942; Kundungal et al., 2024). Wax moth larvae infestations on a large scale frequently result in the absconding of colonies, colony loss, and a decrease in the size of migratory bee populations.

Potential vectors of pathogens have been identified in both the adult and larval stages of the wax moth. For example, it has been found that the faeces granules of the larvae contain *Paenibacillus* larvae spores. Furthermore, recent research has shown Israeli acute paralysis virus (IAPV) and black queen cell virus (BQCV) in the larvae, pupal skins of honeybees that are cast off, and their progeny (Mutinelli, 2011; Tantillo et al*.,* 2015; Tsevegmid et al., 2016). A comprehensive evaluation of the global economic impact of wax moths is still lacking. The wax moth is a factitious host that is susceptible to a variety of bioagents for reproduction, despite its status as a significant parasite of honeybees (Mansour et al., 2010). Therefore, it is highly valuable. The significance of wax moths in biological research and control methods is underscored by the ability to mass maintain a variety of entomopathogenic nematodes and larval parasitoids (Vashisth et al*.*, 2013).

1.10 Advancements in Technology and Research and Educational Outreach and Adoption

Ongoing research and technological advancements contribute to the development of new, sustainable pest control methods. This includes the use of precision agriculture,

remote sensing technologies, and data analytics to optimize pest management strategies with minimal environmental impact. Collaborations between scientists, farmers, and policymakers play a crucial role in driving innovation and promoting the adoption of sustainable pest control practices. Knowledge-sharing platforms and initiatives facilitate the dissemination of best practices.

Programs for education and public outreach help farmers, stakeholders, and the general public understand the advantages of using sustainable pest management techniques. Training programs equip farmers with the knowledge and skills needed to implement eco-friendly practices. Government incentives and support for the adoption of sustainable practices, including financial incentives and certification programs, encourage farmers to transition towards eco-friendly pest control methods.

CHAPTER 2

REVIEW OF LITERATURE

The term "wax moth" is a generic term that denotes a variety of moth species that invade, attack, and damage honeybee colonies and hive products (Paddock, 1930; Williams, 1997; Ellis et al., 2013). These moths are also known as the wax miller, the bee moth, or the webworm (Paddock, 1918; Ellis et al., 2013). It includes *Galleria mellonella* Linnaeus, *Achroia grisella* Fabricius (Shimanuki, 1967; Williams, 1997; Ellis et al., 2013; Chantawannakul et al., 2016), *Plodia interpunctella* Hubner (Williams, 1997), *Aphomia sociella* Linnaeus (Williams, 1997), and *Anagasta kuehniella* Zeller (Shimanuki, 1967). The most prevalent and pernicious species is the greater wax moth, *Galleria mellonella*, which is recognized for its larger size and substantial impact on bee colonies by destructive honeycombs. *Achroia grisella*, the lesser wax moth, is smaller in size but it inflicts significant damage by infesting bee colonies and consuming wax, honey, pollen, and bee brood (Ellis et al., 2013).

They are classified in the kingdom Animalia, phylum Arthropoda, class Insecta, order Lepidoptera and belong to the family Pyralidae. This specific Pyralidae family, also known as snout moths, are a family of moths. Their unique palpi distinguish them from other moths. *Galleria* and *Achroia* are the two primary genera within this family that are pertinent to beekeeping. Earlier the pest was classified by Fabricius as *Galleria cereana* and *Galleria obliquella* by Walker (Paddock, 1918) it was later reclassified by Linnaeus and named *G. mellonella* (Harding et al., 2013). The identification of these moths and the implementation of effective control measures to safeguard bee colonies are facilitated by an understanding of their taxonomy (Chang & Hsieh, 1992).

2.1 History and Distribution

The history and distribution of *G. mellonella* are reviewed by Paddock, (1918). The earliest reference to this was possibly made by Virgil, who lived from 70-19 B.C. Aristotle, a philosopher, from 384-322 B.C., later referenced the bee moth in his writings (Warren & Huddleston, 1962). In the 1st century A.D., Columella, a Roman writer specializing in agricultural topics, documented the bee moth as a threat to honey bees. In Holland, Swammerdam (1637-1680) used the term "bee wolf" to refer to a species of the beemoth (Paddock, 1918). Reaniur, a French scholar who lived from

(1685-1757), documented the destructive impact caused by the beemoth (Paddock,1913).

Linnaeus, a Swedish naturalist who lived from 1707 to 1778, documented the existence of this nuisance of wax moths among the beekeepers in Sweden in his book "Systema Naturae" while stating the introduction of *G. mellonella* into Sweden from Germany in 1750 recorded the ravages of this species in the honey bee colonies. The introduction of the beemoth into America took place around the start of the nineteenth century. The exact date of the introduction of this pest into Texas remains unknown (Paddock, 1918).

The wax moth incidence and its ravages were recorded by some of the ancient Greek and Roman philosophers (Ramachandran & Mahadevan, 1951). Paddock (1930) reported that the first record of *G. mellonella* was from Euphrates Valley. This was more prevalent in plains than at higher altitudes. The distribution of the pest was limited mainly due to its inability to tolerate prolonged subfreezing temperatures. According to Fletcher (1978), the wax moth seems to have been originally an inhabitant of the Euro-Asiatic continent and Africa north of the Sahara, but spread throughout the world by human agency.

The greater wax moth has perhaps evolved in southern Asia along with honey bees because of which the destructive activities of the wax moth are most severe in tropics and subtropics (Morse & Meighen, 1987). All four major species of honey bees occur in India and the wax moth is widely distributed in all the States (Fletcher, 1915; Newton, 1917; Garg, 1998; El-Gohary et al., 2018; Sohail et al., 2020). Vijayakumar et al. (2019) conducted a study on the prevalence of larger wax moth infestation in both plain and hilly regions of Karnataka state between 2015 and 2017.

According to Nagaraja & Rajagopal (2019), *G. mellonella* moth infestation is prevalent in both higher and lower elevations in India consistently throughout the year. The pest has a worldwide distribution (Figure 2.1) and there is no country in the world wherein honey bee colonies are free from the attack by the greater wax (Paddock, 1930; Winston et al., 1981; Williams, 1997; Mishra et al., 2022; Flint and Merkle, 2023).

However, its growth has particularly increased in temperate, sub-tropical, and tropical climates. The number of wax moths is strongly influenced by weather conditions, as any changes in weather lead to fluctuations in their population (Ben,

1999). As poikilotherms, insects are greatly influenced by temperature, which is the reason the wax moth is less common in colder places (Charriere & Imdorf, 1999).

Kapil and Sihag (1983) assert that the GWM's cosmopolitan distribution is solely restricted by its vulnerability to cold temperatures. Despite the fact that wax moth infestations in robust, active colonies are effectively managed by worker bees, queenless colonies (Paddock, 1913) and those that have been compromised by exposure to pesticides, disease (Romel et al., 1992), or the presence of parasitic mites may experience substantial losses.

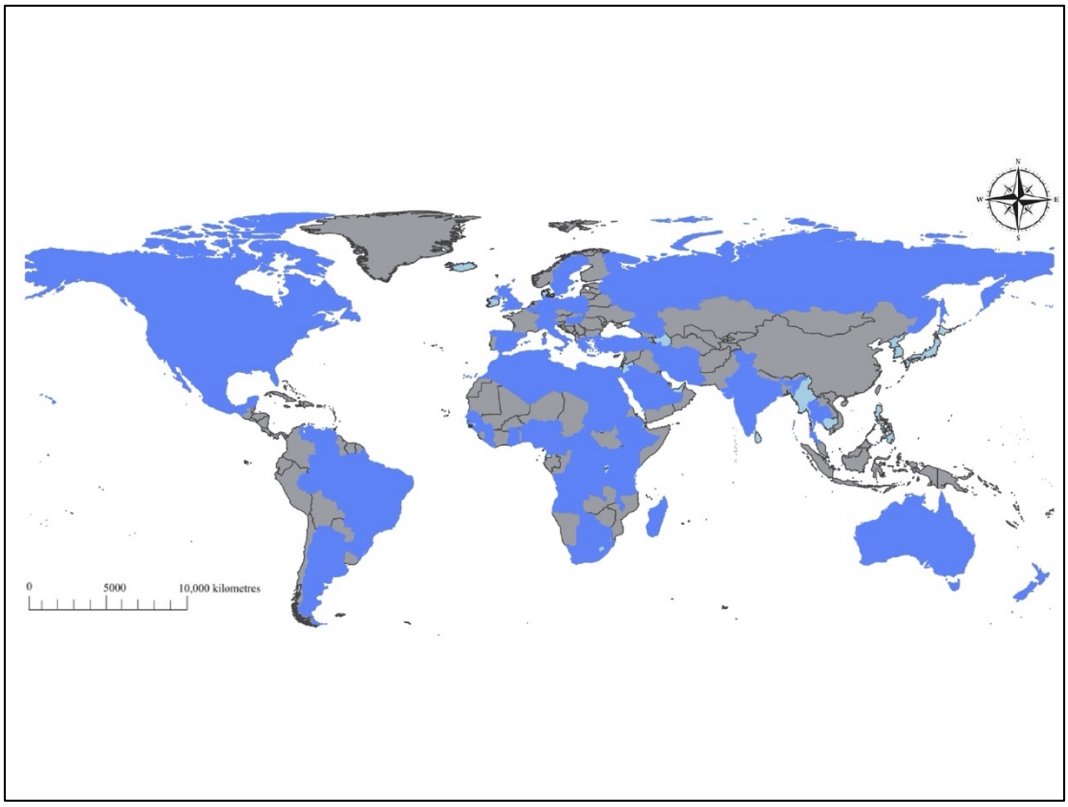


Figure 2.1*:* Global Distribution of Wax Moths: This map illustrates the presence (blue) and absence (gray) of wax moth populations across various regions of the World. The highlighted areas in black indicate regions where wax moths are known to be present, while the gray areas show regions where wax moths are absent or have not been reported (Kwadha et al., 2017)

2.2 Life Cycle of Wax moths

The wax moths are the holometabolous insect, and undergoes four distinctive developmental stages: egg, larva, pupa, and adult (Fasasi & Malaka, 2006; Swamy, 2008).

2.2.1. Factors Affecting Life Cycle

The wax moths' life cycle is impacted by a variety of environmental, biotic, and abiotic factors. Key environmental parameters include temperature, humidity, food availability, light, and ventilation. Interspecific interactions encompass predators, parasitoids, and competition with other species. Intraspecific factors involve competition for food, diet quality, cannibalism, population density, genetic variability, reproductive tactics, and behavioural adaptations (Charriere & Imdorf, 1999; Gulati & Kaushik, 2004).

a) Intraspecific Drivers: Intraspecific factors such as competition for food (Williams, 1997), diet quality (Krams et al., 2015), and cannibalism of early instars by later ones significantly affect survival rates. High population density, genetic variability, reproductive strategies, larval competition, disease transmission, and behavioural adaptations also impact wax moth populations. Resource competition slows growth and increases mortality in densely populated areas (Pinniger & Harmon, 1999). Genetic diversity enhances adaptability and survival, but reproductive output influences population growth. Intense larval competition for food and space elevates mortality rates, while dense populations facilitate faster disease transmission, causing population declines (Sarwar, 2016).

b) Abiotic Factors: Abiotic factors, notably relative humidity and temperature, are crucial throughout the life cycle. The optimal temperature range for development is between 29°C and 33°C (Paddock, 1918; Williams, 1997; Kumar & Khan, 2018). Humidity levels between 60% and 80% are critical for proper egg hatching and larval growth. Low humidity can desiccate larvae, while high humidity promotes mould growth. A combination of 20°C and 25°C temperatures with respective relative

humidity levels extends developmental times and reduces fecundity. The optimal temperature for all developmental phases is 30°C (Hanumanthaswamy et al., 2013).

c) Food and Light Availability: Food availability, particularly beeswax, honey, and pollen, is essential for larval development. Insufficient food results in stunted growth and higher mortality rates (Kumar et al., 2010). Light levels and ventilation also influence development. Optimal laboratory conditions with adequate temperature and food availability enhance population growth and reduce mortality rates due to the absence of predators (Mahgoub et al., 2020).

c) Interspecific Factors: Interspecific factors include interactions with honeybees, parasitoids (Paddock, 1918), and hive beetles. Natural predators such as avian species, chiropterans, coleopterans, and hymenopterans play a vital role in regulating wax moth populations by consuming their larval and adult stages, thus mitigating their negative impact on bee colonies (Fawzy et al., 2017). Parasitic wasps and nematodes specifically target wax moth larvae. Competition with other insects and fungi for beeswax, honey, and pollen limits feeding and breeding sites, thereby controlling wax moth populations. The internal dynamics of bee colonies also significantly affect wax moth prevalence. Robust bee colonies can resist wax moth infestations by destroying their eggs and larvae, thereby interrupting their life cycle (Smith et al., 2013; Hristov et al., 2020). Human activities, such as effective hive management, chemical treatments, and biological control agents, can enhance predation, parasitism, and competition, thus protecting bee colonies from wax moth infestations (Tucker, 1978).

2.2.1.1 Biology of *Galleria mellonella*

Eggs: They are oval in shape, white when they are first deposited, and cream or pale pink as they age. They are reticulate and incredibly rough, made up of interconnecting polygons (heptagons, squares, pentagons, and hexagons) (Figure 2.2). The carinae encircling the primary cells are uniformly wide, and they are only very faintly visible across the entire surface (Ellis et al., 2013). The micropylar area is encircled by

microstructure elements that are concentrically arranged, resembling rounded flower petals (Ellis, Graham, and Mortensen 2013).

Egg laying commences shortly after the emergence of adults and mating (Paddock 1918). Oviposition starts around approximately 24 hours after the emergence and persists for four consecutive nights (Nielsen and Brister,1977). Oviposition typically occurs at night, specifically between 19:00 and 03:00 (Hosamani et al., 2017). The mated female moth enters the colony to oviposit and deposits masses of eggs in the crack between the hive bodies (Nielsen & Brister, 1977; Hosamani et al., 2017; Padimi et al., 2023). A female wax moth starts laying eggs immediately after mating and continues for approximately 5 days. The female lays the eggs in batches, in dark out of way places. Eggs are deposited in batches of 50 to 150 (Kwadha et al., 2017), or as reported by Desai et al. (2019), even from 175 to 355. The eggs are glued together and are placed in weak colonies during the dark time (Burges, 1978). The egg parameters provided by various authors are consistent: between 0.44 and 0.47 mm in length and 0.29 and 0.39 mm in width (Swamy 2008; Ellis, Graham and Mortensen 2013; Hosamani et al. 2017; Kwadha et al. 2017; Desai et al. 2019).

The larva is visible as a dark ring approximately four days prior to eclosion. The formed larva is evident through the thin chorion twelve hours prior to hatching (Paddock 1918). The hatching starts from 3 to 5 days of oviposition when the temperature is between 29-35ºC, and up to 35 days at 18ºC (Smith, 1965; Williams, 1997). The egg stage lasts for about 9 to 10 days. The survival range of eggs varies from 85 to 100 percent (El-Sawaf, 1950; Pastagia & Patel, 2007).

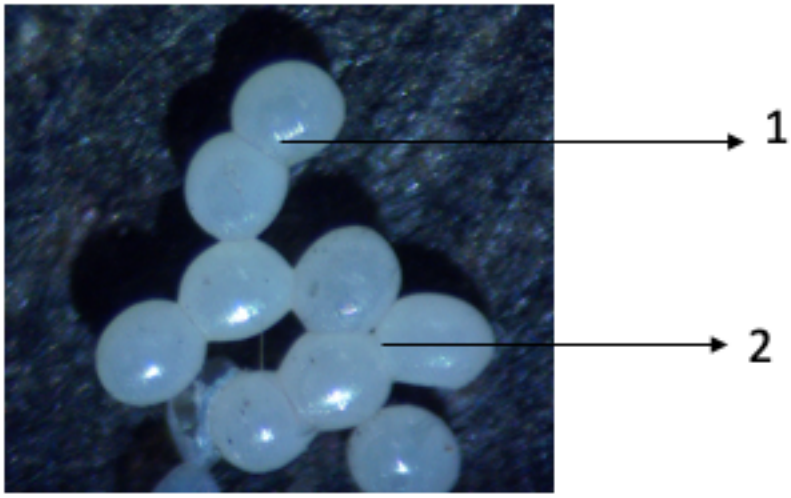


Figure 2.2: Morphology of Eggs of Greater Wax Moth under stereo microscope at 40X magnification 1) eggs are oval in shape and whitish in colour 2) eggs are glued together

Larvae: They have a small, somewhat pointed, reddish head with four stemmata on each side, and they are creamy white with gray to dark gray patterns. The spiracle has a uniformly thick yellowish peritreme (Figure 2.3) (Ellis et al., 2013). The size of a fully developed larva is typically 2.2 cm (Sharma et al., 2011) and the length of the tunnel it creates can be more than 15 cm in the comb by construction of feeding tunnel through it (Swamy et al., 2005). The larvae period is between 22 to 60 days (Jyothi & Reddy, 1992; Khanbash & Oshan, 1997). It undergoes 6 to 8 moults (Nielsen & Brister, 1977). The optimal temperature for the larval development of this moth is 29–33°C on average (Warren and Huddleston 1962; Nielsen and Brister 1979; Williams 1997). The total duration of the larval stage is approximately 45 days, as the average duration of each consecutive larval instar L1-L7 is 4.08, 5.72, 5.28, 6.96, 6.76, 7.64, and 8.40 days, respectively (Pastagia and Patel 2007; Swamy 2008; Hosamani et al. 2017; Desai et al. 2019). In warmer temperatures it can take only 20 days for the larvae to grow, but in cooler conditions it can take upwards of 5 months. The most intensive growth occurs in the final two larval instars (Ellis, Graham, and Mortensen 2013).

The first larval instar (L1) is white, slender, and extremely short (mean length 1.27 mm) immediately following eclosion (Hosamani et al. 2017). It undergoes a transformation from a light gray to a greyish white hue during its subsequent growth. Subsequently, it experiences a noticeable increase in body thickness, culminating in a massive and stocky appearance by the conclusion of its development (Fasasi and

Malaka 2006; Ellis et al., 2013; Desai et al. 2019; Kwadha et al., 2017). The majority of the first-instar larva's body surface is devoid of pigment, with the exception of the head, which is the most strongly sclerotized region of the body, due to its very weak sclerotization. In the later larval instars, the protarsus and claws of the ventral prolegs, as well as the tergites of the pronotum and abdominal segment X, are also well sclerotized. These structures progressively darken after each moult, acquiring a range of shades from light to dark brown (Ellis et al., 2013). A bright ecdysial line is visible along the middle of the dorsal side of the fully colored final stage larva, particularly well-marked on the prothorax (Kwadha et al., 2017).

Larva moults 4 to 6 times in its life. Larval period is between 22 to 60 days (Jyothi & Reddy, 1992; Khanbash & Oshan, 1997). During the beginning of pupation, the pupa appears white or yellow in colour, but over time and as it develops, it undergoes a slow transformation to brown and eventually to dark brown (Kwadha et al. 2017).

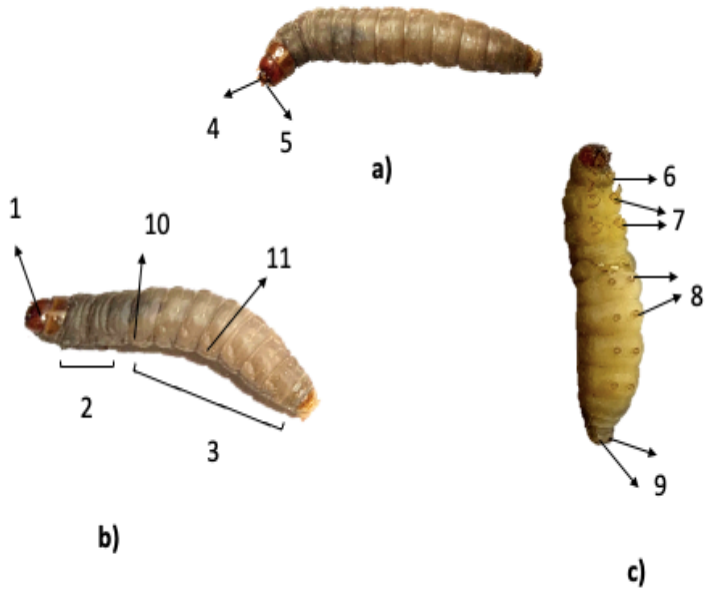


Figure 2.3: Morphology of Greater Wax Moth Larvae under stereo microscope at 20X magnification: a) Dorsal view b) Lateral view c) Ventral view. 1-head, 2- thorax, 3-abdomen, 4-antennae, 5-mouthparts, 6-prothoracic spiracle, 7-claws, 8- pair of prolegs,9-anal proleg,10-11 abdominal segment spiracle

Pupae: The pupa undergoes metamorphosis and is enclosed in a durable silk cocoon, either inside or outside of the hive. This stage involves the transformation of the juvenile larvae to the adult wax moth. Initially, the newly formed pupa inside the cocoon is white to yellow, transitioning to dark brown at the end of pupation (Figure 2.4). The pupal stage can develop and hatch within 3-8 days in warm conditions, but this period extends to two months in cooler climates (Ellis et al., 2013).

According to Mahapatra et al. (2023), the duration of the pupal period ranges from 6.5 to 8.00 days. The eclosion, or emergence of adult moths from the pupae, typically occurs during the night hours (Jyothi & Reddy, 1992; Yadav & Kaushik, 2017). The length of the pupa for female Greater wax moths is approximately 15.83 mm with a width of 4.17 mm, whereas the male pupae have a length of about 11.86 mm and a width of 3.17 mm (Desai et al., 2019).

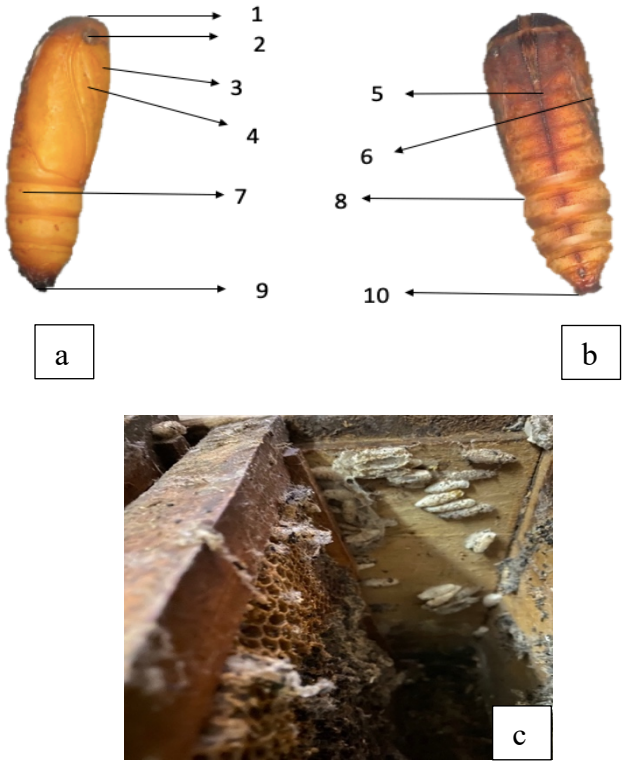


Figure 2.4: Stereomicroscope image of pupa of *G. mellonella* a) Represents ventral view of male pupa at 18X magnification b) Dorsal view of pupa at 20X magnification c) represents photographic image of cocoon formation in the hive box. 1-anterior region or head, 2 -compound eye, 3-labial palps, 4-antennae, 5-ecdysial line, 6-forewing, 7-spiracle, 8-abdominal segments, 9-anal area, 10-posterior region

Adults: Adult female moths, larger at about 20 mm, are grey to purple-brown with dark forewing marks (Ellis et al., 2013) (Figure 2.5). Males attract females with pheromones and ultrasound signals, and females lay eggs near apiaries post-mating (Jacobson, 2012). Adults live 7 to 30 days, with males living 21–30 days and females 8–15 days (Paddock, 1918; El-Sawaf, 1950). Adult wax moths exhibit distinct reproductive behaviours, with males searching for females. Males attract females through a combination of chemical pheromones and ultrasound signals. After eclosion, females exhibit a characteristic calling posture and remain close to the site of emergence (Jacobson, 2012). Copulation can occur on trees or foliage adjacent to apiaries, and only females return to the hives to lay eggs (Nielsen & Brister, 1977). Adult moths do not feed on wax combs during their lifespan (Charriere & Imdorf, 1999; Mahgoub et al., 2020) .

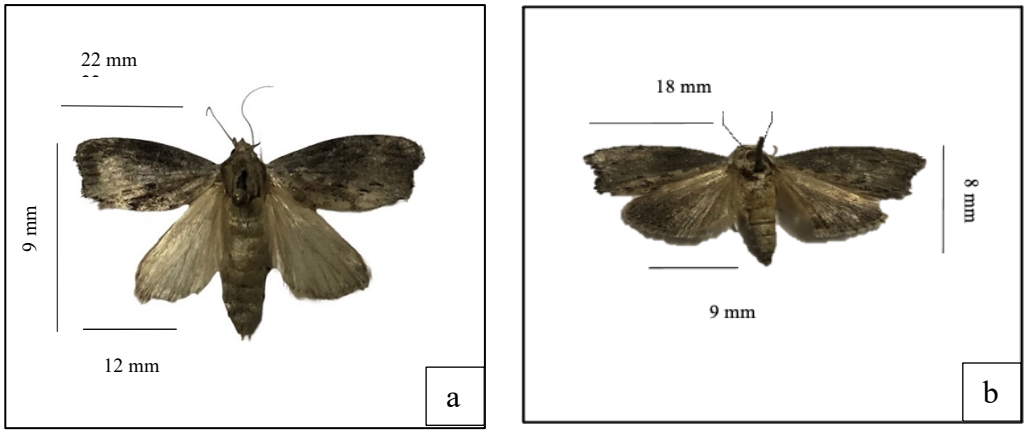


Figure 2.5: The body length of the female *G. mellonella* measures 9 mm, with a forewing span of 22 mm and hindwing span of 12 mm. b) Similarly, the male *G. mellonella* has a body length of 8 mm, with a forewing span of 18 mm and hindwing span of 9 mm at 20X magnification

2.3 Biology of *Achroia grisella*

Eggs: The eggs exhibit a creamy and uniform white coloration (Egelie et al., 2022), taking on a spherical shape and require a damp atmosphere to hatch. They are covered with a thin, waxy coating that helps protect them from drying out (Figure 2.6). The Reticulation is limited to anterior end, and the carinae surrounding primary cells conspicuously broader around outer margins of cells (Ellis et al., 2013). The eggs are about 0.41 ± 0.02 × 0.31 ± 0.01 mm (Williams, 1997). The duration of egg incubation

varies, with higher temperatures accelerating the development of all life stages. The incubation period for eggs is usually between five and eight days (Mahgoub et al., 2020).

Lesser wax moths generally deposit their eggs in sheltered cracks close to a food supply, such as honey bee combs or stored honey bee goods after mating. Eggs are typically laid near a source of nourishment to guarantee that the larvae can obtain nutrients once they hatch (Renwick & Chew, 1994). Eggs are laid for about five days (Kumar & Khan, 2018). Females exhibit specific behaviour during egg-laying, preferring to deposit eggs in crevices and cracks of combs. They select these locations for protection and proximity to food sources for the larvae (Mahgoub et al., 2015). Oviposition occurs at night hours in the hives (Egelie et al., 2022). Typically, 10–20 eggs are placed in clusters when the eggs are laid (Ellis et al., 2013). Each female moth can deposit between 50 to 150 eggs, depending on the temperature (Ellis et al., 2013).

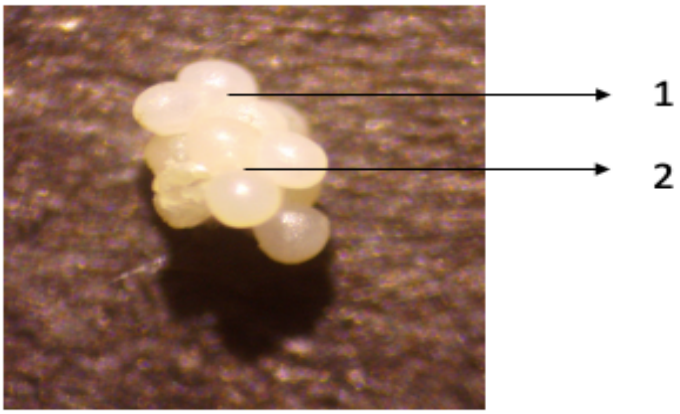


Figure 2.6: Morphology of Eggs of Lesser Wax Moth under a stereo microscope at 30X magnification 1) eggs are creamy in colour 2) they are glued together

Larvae: The larvae possess slender, pale-white bodies with a brown head and pronotal shield (Ellis et al., 2013) (Figure 2.7). The stemmata is absent in lesser wax moths. The spiracle possesses black peritreme on the caudal margin (Ellis et al., 2013). The majority of larval development occurs during the final two instars, resulting in adult larvae that measure roughly 20 mm in length (Egelie et al., 2022).

The larval development period typically ranges from one to five months, with an average duration of six to seven weeks at a temperature of 29° to 32°C (Egelie et al., 2022). The second instar also had a developmental period of six days. The head capsule

girth was 1.64±0.42 mm, whereas the head capsule width measured 0.52±0.13 mm in the third instar. The duration of the developing period was eight days. However, the fourth larval instar had a developmental span of 8 days. The circumference of the capsule was measured to be 2.71±0.36 mm, while the width of the head capsule was found to be 0.79±0.11 mm. Finally, the fifth larval stage had a head capsule width of 0.99±0.07 mm and a capsule girth of 3.27±0.31 mm, as reported by Morse and Nowogrodzki in 1990. According to Mahgoub et al. (2015), the larval duration varied significantly between males and females. Males had an average larval period of 29.84±0.27 days, while females had an average larval period of 31.42±0.33 days. These observations were made at a temperature of 31±2°C and a relative humidity of 66.28%. The larvae experience seven moults. The majority of larval growth occurs during the final two instars, resulting in mature larvae that are roughly 20 mm in length (Sharma et al., 2011). Larvae grow by moulting, shedding their skin as they outgrow it. They typically moult four times before entering the pupal stage (Egelie et al., 2022).

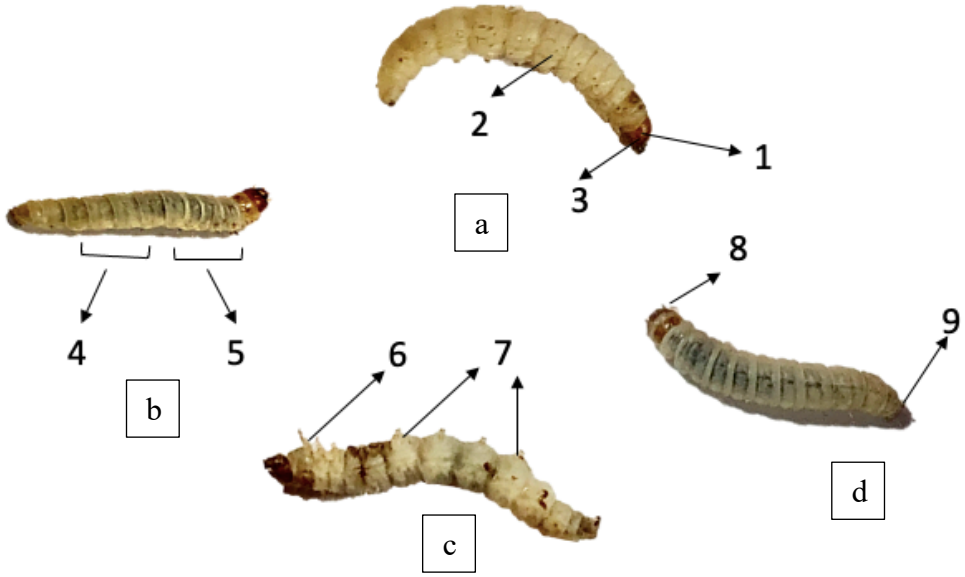


Figure 2.7: Stereomicroscopic images of *A. grisella* larvae a) Lateral view b) Dorsal view c) Ventral view d) Dorsal view. 1-head without stemmata, 2-spiracles, 3-mouth parts, 4-abdomen 5-thorax, 6- claws,7- pair of prolegs, 8-antennae 9-anal prolegs, in 18X magnification

Pupa: Fully developed larvae will undergo pupation within the honey bee hive and encase themselves in durable silk cocoons before entering the pupal stage (Figure 2.8). The pupae are approximately 11 mm long, exhibit a yellow-tan tint, and change from

whitish-yellow to dark brown as they mature (Ellis et al., 2013). Cocoons are characterized by their white colouration and are secured in position by webbing (Egelie et al., 2022). Recognition of cocoons can be challenging due to their frequent covering with frass and other debris.

The maturation process of pupae can last up to two months, while the usual duration for adult emergence is approximately 37 days (Ellis et al., 2013). The pupa hatches within 3-8 days in optimally warm conditions or several months in cold weather. The eclosion, or emergence of adult moths from the pupae, typically occurs during the night hours (Jyothi & Reddy, 1992; Yadav & Kaushik, 2017). Pupae are inactive and do not move (Ellis et al., 2013). They are sensitive to temperature and humidity and can be killed by extreme conditions (Egelie et al., 2022). The length of the pupa for female Greater wax moths is approximately 15.83 mm with a width of 4.17 mm, whereas the male pupae have a length of about 11.86 mm and a width of 3.17 mm (Desai et al., 2019).

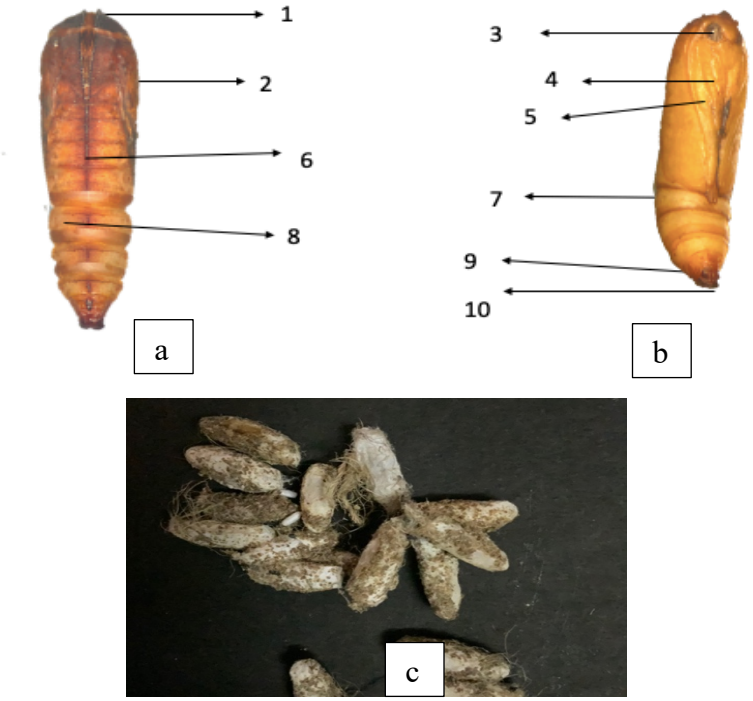


Figure 2.8: Stereomicroscope image of pupa of *A. grisella*, showing the formation of basic body structures at an advanced stage of development, with visible formation of wings and other adult features a) Represents ventral view of male pupa at 18X magnification b) Dorsal view of pupa at 20X magnification (c) photographic image of clusters of cocoons where larvae have spun silk to create a protective casing for their pupal stage. 1-anterior region or head, 2- forewing 3-compound eye, 4-labial palps, 5-antennae, 6-ecdysial line, 7- abdominal segments, 8-spiracle, 9-anal area, 10-posterior region

Adults: Adult wax moths exhibit sexual dimorphism, with females being larger than males. They are typically silver-gray to beige with a distinctive yellow head, measuring around 1/2 inch in body length and wingspan (Figure 2.9). Adults are nocturnal and do not feed. Mating occurs within hives, where males use ultrasonic signals to attract females. Female adults live approximately 7 days, while males live around 13 days (Ellis et al., 2013; Mahgoub et al., 2015). Throughout the day, adult individuals conceal themselves inside the foliage of trees and shrubs in close proximity to beehives (Egelie et al., 2022). Mating usually takes place in honey bee hives, with males enticing females to mating locations through the use of ultrasonic signals. Females mate in the evening, with males performing a courtship dance to attract them. Adult moths do not feed and are active at night, with most mating and oviposition occurring during this time.

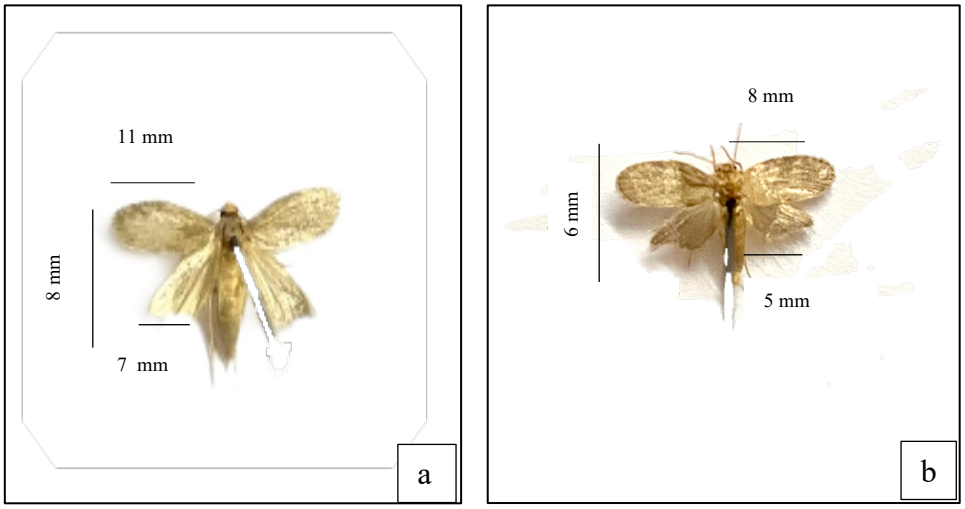
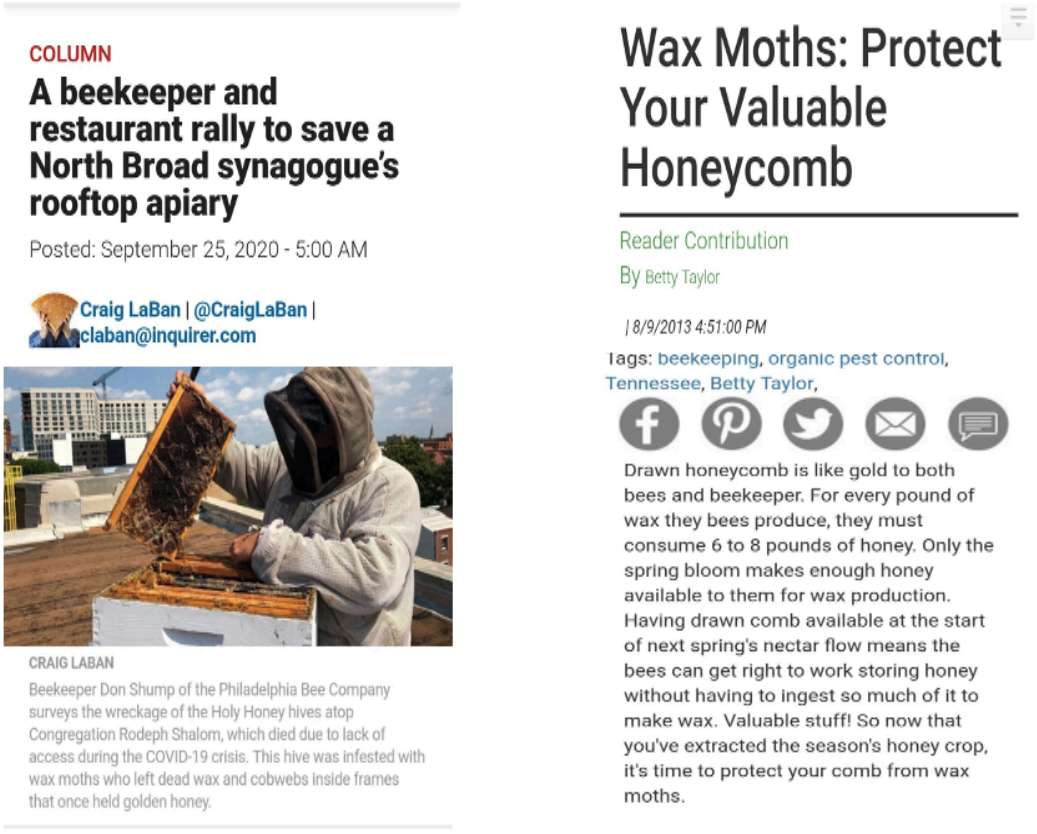


Figure 2.9:The body length of a female *A. grisella* measures 8 mm, with a forewing span of 11mm and hindwing span of 7 mm b) Similarly, the body length of a male *A. grisella* measures 6 mm with a forewing span of 8 mm and hindwing span of 5 mm at 15X magnification

2.4 Wax Moth Infestation Dynamics and Economic Impact on Apiculture

The Greater wax moth (*G. mellonella*) and The Lesser wax moth (*A. grisella*) are highly significant and economically detrimental pests of honey bees, with a long history of infestation (Burges, 1978; Ritter & Akratanakul, 2006; Chang & Metz, 2021). They inflict economic losses in India, amounting to 60-70 per cent of the beekeeper's income every year (Figure 2.10) (Hosamani et al., 2017). They are the parasites of social bees

(Kalinova et al., 2009; Kindl et al., 2011). Asian honeybee colonies are likely to be vulnerable to infestation by wax moths (Adlakha & Sharma, 1975; Hepburn & Hepburn, 2011; Almadani & Hiware, 2020).



COLUMN

A beekeeper and restaurant rally to save a North Broad synagogue's rooftop apiary

Posted: September 25, 2020 - 5:00 AM

Craig LaBan | @CraigLaBan | claban@inquirer.com

CRAIG LABAN

Beekeeper Don Shump of the Philadelphia Bee Company surveys the wreckage of the Holy Honey hives atop Congregation Rodeph Shalom, which died due to lack of access during the COVID-19 crisis. This hive was infested with wax moths who left dead wax and cobwebs inside frames that once held golden honey.

Wax Moths: Protect Your Valuable Honeycomb

Reader Contribution

By Betty Taylor

| 8/9/2013 4:51:00 PM

Tags: beekeeping, organic pest control, Tennessee, Betty Taylor,

Drawn honeycomb is like gold to both bees and beekeeper. For every pound of wax they bees produce, they must consume 6 to 8 pounds of honey. Only the spring bloom makes enough honey available to them for wax production. Having drawn comb available at the start of next spring's nectar flow means the bees can get right to work storing honey without having to ingest so much of it to make wax. Valuable stuff! So now that you've extracted the season's honey crop, it's time to protect your comb from wax moths.

Figure 2.10: Articles highlighting the importance of beekeeping and the protection of honeycombs from wax moths. The first article discusses a beekeeper's effort to save a rooftop apiary in North Broad synagogue, while the second article provides tips on protecting valuable honeycombs from wax moth infestations, emphasizing the need for proper storage and management to maintain honey production and hive health

The activity of *G. mellonella* was observed more pronounced in the rainy season and dearth periods in various parts of India. Ramachandran & Mahadevan, 1951; Adlakha & Sharma, 1975; Sharma, et al., 2015; Kishan et al., 2017; Singha et al., 2023 reported the wax moth infestation in *A. dorsata* from May to July in Haryana.

Several overlapping generations in a year depending on the temperature, availability of food, and uninterrupted breeding of wax moths in the hives were observed. But in storage, 70 per cent of wax moth larvae and 30 per cent of pupae hibernated in cold winter months (Kapil & Sihag, 1983; Sihag, 1991; Ahmad et al., 1994).

The wax moths posed a significant issue during the monsoon (dearth period) in Punjab, as a significant number of colonies absconded as a result of infestation in *A. mellifera* apiaries (Brar et al.,1985). The greater wax moth infestation commenced in June and progressively increased until it reached a climax in September. Subsequently, it decreased to a minimum in November.

Shylesha (1987) conducted a study in Bangalore and observed wax moth in *A. cerana* consistently throughout the year. The highest number of larvae found in an infested hive was 336.36. In Dharwad, Karnataka, the egg laying of *G. mellonella* was observed from March to August. In July, an average of 312 eggs were found in three bee colonies. The larval stage of the pest was found consistently throughout the year, with a peak population occurring from May to August, which aligns with the period when flowers are scarce in the area. In July-August in Bangalore, there was a significant occurrence of *G. mellonella* infestation in *A. dorsata* colonies, which led to the bees abandoning their colonies. The discarded combs, which have a high prevalence of pests, serve as a source of infestation for newly established bee colonies.

The highest level of infestation by this insect was recorded in South India during the period of reduced flower availability. The pest overwintered in larval (about 70%) and pupal (approximately 30%) stages within stored combs. Throughout several seasons, the tropical climate may have facilitated the coexistence of multiple generations. Controlling the wax moth in hive bees is of significant economic relevance (Turker et al., 1993).

Abrol and Kakroo (1998) demonstrated that the highest level of infestation occurred between the months of August to October in apiaries of *A. mellifera* in Punjab. The population dynamic of the greater wax moth, *G. mellonella* L., were influenced by the specific bee species. Wax moth larvae, pupae, and adults were observed in *A. mellifera* colonies during the period from May to December.

According to Swamy (2008), the number of wax moth larvae in *A. cerana* exhibited fluctuations throughout the year. The incidence of wax moth infestation in bee colonies in Bangalore was 90.68 in June 1996, 199.33 in April 1997, and decreased to 49.55 in March 1998.

According to Sharma and Gupta (2014), the highest infestation of *G. mellonella* in *A. cerana* combs occurred in June, reaching 59.33 per cent in Bangladesh (Dhaka).

In August 2002, a notable occurrence of *G. mellonella* in *A. mellifera* colonies was observed in Himachal Pradesh, India.

The pest was most widespread in South India at a period of low flower availability, which happened to coincide with weak bee colonies. Due to the overlapping generations within a single year, the pest infestation spread across several geographical locations throughout the entire season. The population of *G. mellonella* was sustained by infected combs and weak colonies (Viraktamath et al., 2005).

In a study conducted by Swamy (2008), it was discovered that there was a higher occurrence of infection by the larger wax moth *G. mellonella* in both robust and feeble *A. cerana* colonies. Higher prevalence of wax moth infestation was seen in the less robust colonies, with the peak infestation rate occurring between October and February. In August, the infestation was seen to have diminished. In addition, there was a significant prevalence of wax moth infestation in robust colonies in March, whereas the lowest percentage of infestation was seen in December and September.

Varshneya et al. (2008) examined the occurrence of the greater wax moth (*G. mellonella* L.) in European honey bee colonies (*A. mellifera* L.) across the seasons and discovered that the greater wax moth infestation in bee colonies began in July, namely during the early rainy season. The larval infestation exhibited a progressive increase from January to September, culminating in its peak in September. During the larval stage, they consume the wax from the honeycombs, resulting in significant destruction. The presence of wax moths has resulted in a decline in honey bee colonies, leading to a reduction in honey production.

Kumari and Jha (2013) documented the presence of pest infestation in both the bee hive and stored combs. The largest area affected by infestation was seen in September, while the smallest area was recorded in June (3.42 cm^2, 4.41 cm^2) for the years 2011 and 2012, respectively. The possible cause could be attributed to the elevated temperature and humidity levels. Worker bees exhibited self-defence activities, resulting in frames that were completely covered with bees experiencing the lowest infection rates.

Raghunandan and Basavarajappa (2014) found that colonies with a small population were more vulnerable to infestation by *G. mellonella*. This infestation was most common in the semi-arid zone during the summer, affecting 30.80% of colonies,

followed by the rainy season, affecting 23.40 per cent of colonies. In the Malnad region of Mysore, Karnataka, the infestation rates were 11.00 percent and 6.60 per cent during the summer and winter seasons, respectively.

Kebede et al. (2015) examined how common wax moths were in modern hive colonies in four villages located in Kafta Humera, Ethiopia. The study was conducted between April 28 and May 30, 2009. The study categorizes the degree of infestation into three groups: light, moderate, and critically damaged colonies, with infestation rates of 11.4, 15.3, and 0.65 per cent respectively. The overall prevalence of wax moth larvae in modern bee hives is 27.4 per cent.

Sohali et al. (2017) examined the fluctuation in the population of *G. mellonella* larvae in honey bee hives located in the Sargodha district of Punjab, Pakistan, throughout several seasons. The peak moth population was observed during the period of low availability in the region, which extended from May to November. In terms of wax moth larvae abundance, August had the highest number with an average of 14.8±3.9 larvae per hive.

Lalita et al. (2018) provided evidence of monthly fluctuations in the wax moth population across the years 2016 and 2017. The seasonal occurrence of *G. mellonella* began in April, with the maximum population of wax moths seen in July. Subsequently, the population decreased until March. The lowest number of larger wax moth larvae, pupae, and adults was seen in March for all frame strengths in both years of the research. No larval, pupal, or adult population of *G. mellonella* was observed in the colony with the highest frame strength from February to March.

The highest occurrence of wax moth (2.6 per cent) was recorded in *Apis cerana* colonies in April, when the temperature was elevated (21.45°C) and the average relative humidity (44.50 per cent) and rainfall (25.60 mm) were low. The incidence of wax moth in *A. cerana* colonies showed a positive link (which was not statistically significant) with temperature, colony strength, and brood area (Negi et al., 2019).

The present study examined the seasonal occurrence of *G. mellonella* on *A. mellifera* colonies in the terai agro-ecological zone of West Bengal, India. The highest occurrence of larger wax moths was seen between June and August, with the highest number of individuals per hive recorded in July (13.83 ± 0.68). The highest proportion of beehives with combs infested by moths was seen in July, with a percentage of 88.75

± 2.95 per cent. The occurrence of wax moths was strongly positively correlated with several climatic variables, including maximum and minimum temperature and minimum relative humidity (Singha et al., 2023).

2.5 Strategies to Combat Against Wax Moths

A multifaceted approach is necessary for the efficient control of wax moths. In the management of wax moth infestations, preventive measures, mechanical and physical controls, biological control, chemical control, and pheromone-based strategies all come into action. The most sustainable and comprehensive solution is Integrated Pest Management (IPM), which combines a variety of methods to maintain the health of bee colonies and minimize the environmental impact while controlling wax moth populations.

2.5.1 Physical Control Against Wax Moths

Extreme temperature exposure of the comb is one of the physical restrictions. Wax moths can be killed or their development stopped by cold or heat treatments, although heat levels that are sufficient to eradicate wax moths can also cause damage to the comb. Based on enough and precise information, cooling is an appealing and completely safe strategy (Burges, 1978). All developmental stages of *G. mellonella* (with a little food) were subjected to various low temperatures in tiny containers in a comprehensive study, the organisms quickly acclimated to the experimental conditions (Burges, 1978).

To mitigate the risk of cold susceptibility being intensified by shock, the insects were subjected to a moderate and favourable temperature for one day before and after cold exposure, so order to minimize the impact of rapid temperature fluctuations. Large larvae and pupae were equally resistant to cold temperatures, making them the least vulnerable stages of development (Burges, 1978).

No differences were seen in the eggs at different stages of development. The temperature is -17 degrees was considered lethal. At a temperature of 15°C, all of the larger larvae were able to live for a duration of 8 weeks, with minimal or no observable growth in size (Jyothi & Reddy, 1992, 1996; Mohamed & Hasan, 1998).

In practical terms, this means allowing additional time for the material to stack and for temperature variations to stabilize. Overnight exposure is sufficient for domestic deep freezers. It is advisable to adjust the temperature of home refrigerators to a low setting of 2°C for a duration of 10 days, and then to a high setting of 5°C for a period of 3 weeks. If a substantial quantity of material is stored in commercial deep freezers and cold stores, the cooling process may be prolonged due to the freezer's capacity. When stacking, it is imperative to ensure there are adequate air spaces to promote the circulation of cold air. Cooling is an optimal method for keeping comb honey, except for freezing some types of honey that may harden. While *G. mellonella* is considered a nuisance, its significance is limited due to the cool environment (Ritter et al., 1992).

The *A. grisella*, commonly known as the smaller wax moth, has a widespread distribution in temperate regions. It may possess greater resilience compared to *G. mellonella*, as well as certain other moth species like *Ephestia kuehniella*, which sometimes infests beehives (Solomon & Adamson, 1955). In aggregations, GWM larvae generate significant amounts of metabolic heat, with temperatures up to 25ºC above ambient levels (Williams, 1997). Because of this, wax moths can withstand harsh winter weather even when their equipment is kept in cold storage spaces (Burges, 1978).

Thermal energy capable of exterminating wax moth can be employed for vacant beehives and structures. The temperature range at which comb honey degrades is between 46°C and 49°C (Cantwell & Smith, 1970). In other studies, the entire life cycle of the larger wax moth was eliminated by subjecting it to a temperature of 60°C and a relative humidity of 50% for a duration of 24 hours, or by exposing it to temperatures ranging from 46-49°C for a period of 80 minutes (Cantwell & Lehnert, 1968).

Moderate exposure to gamma radiation can effectively kill all stages of the larger wax moth. While this treatment could be a practical option for comb honey, as long as it doesn't affect the quality of the honey, the high cost of the necessary equipment would likely prevent beekeepers from adopting it. Reduced exposure of pupae renders them sexually sterile, enabling the release of infertile males to compete with wild ones, resulting in the production of infertile eggs.

Implementing such a program is not feasible due to the need for its execution across a whole region and the high cost associated with breeding pupae. Additionally, a comprehensive control program would need to be implemented beforehand to significantly lower the wax moth numbers.

2.5.2 Chemical Techniques for Managing *Galleria mellonella*

Chemical pesticides may be applied to unoccupied beehives or apiary structures, as well as to stored honeycombs. Fumigant gases are the only suitable option, as they have the ability to enter into small spaces in structures and within honeycomb, and can be totally eliminated afterwards by airing. Contact insecticides are not as suitable due to their inability to penetrate and the potential for leaving behind toxic residues.

Ethylene dibromide can be dispensed from a higher position within a room or underneath sheets (Lehnert & Shimanuki, 1967). Carbon dioxide can operate as a fumigant by efficiently displacing almost all of the air (Tremblay, 1978). It is necessary to continuously monitor and refill the concentration as required, using securely contained buildings like metal container trucks. The larva is the most durable phase. The larvae were maintained in eight commercial-scale tests with a concentration of 98% or higher of carbon dioxide. After being subjected to carbon dioxide for 10-12 hours at temperatures ranging from 23°C to 40°C, this treatment caused the mortality of more than 93% of the larvae.

To ensure the preservation of combs during their storage period, immerse the combs in one of the provided contact insecticide solutions for one minute, followed by drying. The composition includes the following percentages of chemicals: cidial 0.008%, endosulfan 0.002%, endosulfan with fenitrothion 0.001%, toxaphene 0.004%, and trichlorphon 0.008%. Due to its slow evaporation rate, it is necessary to vaporize it, for example, by placing it in a porcelain bowl on an electric hot plate. The boiling point of this substance is 132°C. All developmental stages of *G. mellonella* were eradicated during a 24-hour period at a concentration of 0.02 ml/litre (equal to 32 mg/litre) (Ali et al., 1973).

Hydrogen cyanide and methyl bromide are the most suitable options for this restriction, and possibly phosphine and ethylene oxide as well. Their dangerous

qualities are exemplified by the current permissible levels in the atmosphere, which are 50 parts per million (ppm) for ethylene oxide, 15 ppm for methyl bromide, 10 ppm for hydrogen cyanide, and 0.3 ppm for phosphine. Only individuals who have undergone appropriate training (Bell, 1974) should handle these products.

Phostoxin pellets, which release phosphine gas when exposed to moisture, can also be used in a confined area or under coverings. Longer exposure durations are required in comparison to methyl bromide. According to Bell (1974), eggs are most resistant when they are newly laid, compared to any other point in their life cycle.

The resistant stage of *G. mellonella* occurs during the first 4-7 days of a 12-day egg cycle at a temperature of 25°C. The phosphine concentration is 0.1 mg/L at a temperature of 25°C. The duration of potential exposure leading to death is 10 days. Based on the research conducted by Bell and Glanville in 1970, the levels of phosphine exposure are adequate for *G. mellonella*, except for the exposure at 25°C, which needs to be prolonged to 6 days. Following exposure, it is crucial to ensure that there is a thorough and widespread aeration (Burges, 1978).

Insecticidal fumigants, including sulphur, hydrogen cyanide, naphthalene, paradichlorobenzene (PDB) crystals, methyl bromide, and ethylene oxide, have been employed to manage both the adult and larval stages of the parasite. The use of numerous fumigants, including ethylene oxide, has been discontinued due to concerns regarding toxicity and persistent breakdown products that could contaminate future honey crops. Beekeepers continue to employ PDB crystals; however, they are ineffective at temperatures less than 21ºC and are incapable of eliminating the embryonic stage of the moth (wax moth development persists at 18ºC). This product is not permitted to be applied to comb honey, and the treated equipment must be ventilated prior to its subsequent field use (Scott, 1984 ; Colter, 1994).

The fumigants consist of carbon disulphide, hydrogen cyanide, methyl bromide, phosphine, ethylene dibromide, ethylene oxide combined with an inert gas, and carbon dioxide. Carbon disulphide will be excluded due to its flammability, and hydrogen cyanide will be excluded due to its severe toxicity and hazardous nature during use (Charriere & Imdorf, 1999; Gulati & Kaushik, 2004; Wolfgang Ritter & Akratanakul, 2006).

A chemical control approach was implemented using a treatment containing Aluminium phosphide tablets. Honeycomb samples prepared this way were preserved for two months. Hermetic storage and aluminium phosphide kept the comb fresh for two months, the best treatment. The untreated control group (61.00) had more emerging moths than other treatments, but it was much more than salt-treated comb in opened containers. The therapies had little effect on wax and slum gum weight. Hermetic storage reduces wax moth in honeycomb better than aluminium phosphide after honey extraction. Because treated honeycombs may include aluminium phosphide residue (Babarinde et al., 2013).

A study examined chemical control methods for *G. mellonella* L. and *A. grisella* F. The chemical compounds formic acid and para-dichlorobenzene (PDB) were used. Formic acid reduced infestations the least and was less efficient against both pests. Compared to formic acid, para-dichlorobenzene was more effective at controlling both pests (Telles et al., 2020).

Furthermore, these substances pose a threat to both honeybee colonies and other species, and are currently encountering significant resistance in numerous nations that engage in beekeeping (Charriere & Imdorf, 1999; Ritter et al., 1992).

2.5.3 Biological Control Approaches for Wax Moth Management

The potential efficacy of *Bacillus thuringiensis* in controlling wax moths was recognized in the early 1960s in multiple countries (Johansen, 1962; Burges, 1978; Marwaha, 2023). Commercially available powders and stabilized solutions include both bacterial spores and bipyramidal crystals of poisonous protein, which are formed simultaneously with the spores.

When moth larvae consume little amounts of these substances, the crystals act as a toxin, while the spores begin to grow and reproduce, leading to deadly illnesses (Burges et al., 1976). Therefore, the components can be likened as toxins that affect the stomach of larvae. Both the crystals and the bacteria are non-toxic to bees (Bailey, 1971) and humans (Heimpel, 1974).

Vandenburg and Shimanuki, (1990) developed a variety of methods for the application of *B. thuringiensis* spores, such as machine and manual sprayers, dips,

aerosols, impregnation in foundation, and fogging devices. Each technique necessitates the manipulation of a limited number of frames. CERTM is a successful method for controlling the larval phases of the insect; however, its application is labour-intensive (Scott, 1984) and too expensive for small-scale beekeeping operations (Vandenberg and Shimanuki, 1990), particularly when used as a preventive precaution.

The most effective technique of applying suspensions in the hive is by using comb foundation that has been impregnated during milling. This is because commercial powders and suspensions may be easily mixed with water. Factory impregnation can be accomplished by using an undiluted suspension to lubricate the wax mill. The spores and crystals that are compressed into the foundation contribute to approximately 1% of the total weight of the beeswax. Studies have shown that to prevent the deterioration of spores and crystals in the foundation while they are stored before being used in hives, it is essential to refrain from using Teepol and soap as wetting agents in the mill lubricant (Johansen, 1962; Burges & Bailey, 1968; Burges, 1978).

Additionally, sheets of foundation should be dried before storage and kept in dry conditions (Burges & Bailey, 1968). Triton X-100 can be used as a viable substitute lubricant. When impregnated foundation is used in the hive, some spores are released into the initial batch of honey (Burges & Bailey, 1968). These spores do not harm the honey and do not affect bees or humans when the honey, or a food product containing the honey, is consumed.

A precautionary measure has been implemented to establish a maximum threshold of 1% w/w for bacterial solids in wax. This is to prevent an excessive release of spores into hone (Burges & Bailey, 1968). However, it is not anticipated that any issues would arise at greater concentrations. Moth larvae are most vulnerable while they are young. while the larvae hatch from eggs, they consume a small amount of food that is deadly to them, but does not cause any obvious harm to the comb.

The crystals quickly immobilize the larval mouthparts and digestive system, effectively avoiding any comb damage caused by larvae of any age that come into contact with the comb. The comb provides complete protection against *G. mellonella* for one year, after which the level of protection gradually decreases. However, considering that the comb can be used for up to 10 seasons, this restricted level of protection may not be cost-effective (Burges, 1978).

The lesser wax moth *A. grisella*, although a less major pest of comb, has comparatively better resistance to *B. thuringiensis* and cannot be effectively managed. Other sporadic moth pests that infest honeycomb have comparable vulnerability to *G. mellonella* (Burges & Bailey, 1968). It is likely that applying *B. thuringiensis* to the comb might fully restore protection against *G. mellonella* after the initial year in the hive. However, this could potentially result in a higher transmission of spores to honey, or worker bees may eradicate the pathogens by licking. According to Millar (1965), the bacteria would not harm the bees. Thorough investigation into methods of enhancing control has revealed multiple factors contributing to the decrease in toxicity over time. One factor contributing to inadequate protection throughout different sections of the comb is the variety in levels of protection. The origins of this variety can be traced back to various stages of production and usage of impregnated foundation.

The abundance of impregnated spores and crystals varies randomly across different regions of the sheet foundation. The primary source of this phenomenon is the deformation of the initial thick wax sheet as it passes through the mill, leading to the build-up of the lubricant that holds the spores and crystals. Although an extensive search was conducted to find more potent strains of *B. thuringiensis* bacteria, none of the 360 strains discovered exhibited greater efficacy against *G. mellonella* than the serotype V strain employed in the author's original research endeavour (Burges & Bailey, 1968). Some recently identified strains, which are ineffective against *G. mellonella*, have exhibited enhanced efficacy against agricultural pests. Consequently, these strains have taken the place of serotype V, which is no longer used in industrial *B. thuringiensis* products (Raun & Jackson, 1966; Phillips, 1968).

2.5.4 Alternative Biological Approaches Against *Galleria mellonella*

The nucleopolyhedrosis virus of *G. mellonella* is enclosed within protein-based inclusion bodies that are similar in size to bacterial spores. These might be integrated into the foundation in a similar manner as *B. thuringiensis*. While the virus is deadly to wax moth larvae, it would still face the same challenges as bacteria. In practical terms, the virus is expected to have reduced effectiveness due to three factors: its slow action in killing larvae, resulting in a delayed ability to stop damage; its limited ability to

survive, leading to a quicker deterioration of its protective effect; and the higher cost associated with its production, as it can only be produced in insect larvae or cell cultures. While it has not yet been filed for safety registration, it is highly likely that it would be considered completely safe for both humans and bees.

An ideal control organism would be one that is passed through eggs from one generation of pests to the next generation. This specific variant of the virus is generally not effectively transmitted using this particular approach. Transmission of viruses to *G. mellonella* (Martignoni & Iwai, 1986) through eggs may be possible, although it is unlikely that these viruses would be considered safe.

G. mellonella is susceptible to various parasitic Hymenoptera species. However, implementing effective biological control using these parasitic insects would be challenging due to the widespread distribution of apiaries, the diverse breeding grounds of wax moths, and the high expense associated with rearing the parasitic insects in wax moth larvae (Burges, 1978).

The entomopathogenic fungus, *Metarrhizium anisopliae* (Mets.) Sar., the larval parasitoid, *Apanteles galleriae* Wilk, were tested against *G. mellonella* and *A. grisella.* Ten days following the fungal treatment, the quantities of *G. mellonella* and *A. grisella* were, respectively, decreased to 12.0 and 4.3, indicating a 64.0 and 50.0% reduction in infestation. Following the administration of the fungus, results were observed 20, 30, 40, and 50 days later, as all *A. grisella* and *G. mellonella* larvae and pupae were destroyed.

Nevertheless, only a small percentage of *G. mellonella* and *A. grisella* larvae and pupae (11.3 and 6.4 individuals, respectively) died 60 days after treatments. Results for *A. grisella* were obtained 20, 30, 40, 50, 60, and 70 days following application, whereas for *G. mellonella* and *A. grisella*, the reduction in wax moth infestation ranged from 54.0 to 67.3% and 55.6 to 68.4%, respectively (Telles et al., 2020).

2.6 Efficacy of Pheromones in Wax Moth Control

Pheromones are essential for the improvement of Integrated Pest Management (IPM) strategies by influencing insect behavior, particularly in the capture of pests during their adult stages. Mass trapping, which entails the deployment of numerous pheromone-baited traps to reduce pest populations, and mating disruption, which involves the interference of synthetic pheromones with the insects' ability to locate mates, are among the techniques that have been extensively adopted. Pheromone-based traps are utilized in both methods to effectively monitor and control insect populations. These traps are a valuable instrument in pest surveillance and management programs due to their application across a wide range of pest densities and their high sensitivity in detecting adult pest activity (Athanassiou et al., 2004).

A comprehensive comprehension of pheromone extraction and identification techniques is indispensable for the successful implementation of such strategies. Solid-phase microextraction (SPME), solvent extraction, and entrainment methods utilizing activated charcoal or Porapak Q are among the numerous standardized procedures that have been developed. These methods are succeeded by analytical techniques, including electroantennographic detection (EAD) and Gas Chromatography-Mass Spectrometry (GC-MS), which facilitate the establishment of a correlation between insect olfactory responses and chemical compounds (Millar & Haynes, 1998; El-Ghany, 2019). These tools have been extensively utilized across a variety of insect species to isolate pheromonal components and validate their behavioral effects through bioassays, thereby establishing a strong foundation for pheromone-mediated pest control (Symonds & Elgar, 2008).

Several pheromone-based control systems have been shown to be effective, particularly in the protection of enclosed or storage spaces in apiaries where wax moths pose a significant hazard.

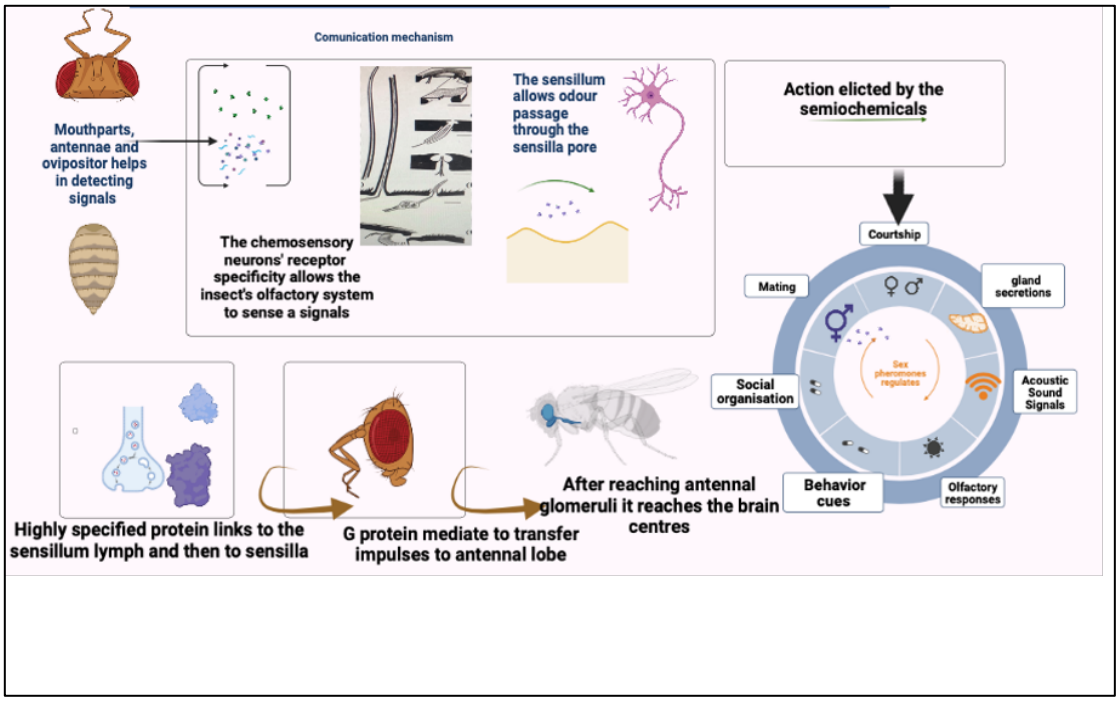


Figure 2.11: Schematic representation of the communication mechanism in insects, illustrating the process from detection of semiochemicals to elicited actions. The diagram shows how mouthparts, antennae, and other appendages detect signals, with chemosensory neurons transmitting these signals to the brain. The role of sensilla in allowing odour passage and the subsequent neural processing leading to various behavioural cues such as courtship, mating, and social organization is depicted, highlighting the complex interplay of sensory and neural pathways in insect communication (Copyright filed)

These traps are not only cost-effective and simple to deploy, but they also provide high specificity for the target species. While maintaining a high level of selectivity for the insect of interest, the optimal pheromone trap system should be affordable, sensitive, and user-friendly. These characteristics render them particularly well-suited for integrated pest control applications and small-scale apiaries (Saveer et al., 2023).

The strong odour released by the male moth can attract females over long distances (Kunike, 1930; Spangler, 1985, 1986, 1987, 1988b; Jones et al., 2002) (Figure 2.11). The males of the lesser wax moth, *A. grisella* (F.), another pest of honey bee hives, produce pheromones namely undecanal and ll-cis-octadecenal (Dahm et al. 1971). Upon the male's proximity, it emits a sex pheromone that initiates the process of mating.

Roller et al. (1968) discovered that undecanal, obtained from the wing glands of male larger wax moths, functions as the pheromone that attracts the opposite sex.

Nevertheless, Leyrer and Monroe (1973) discovered that a secondary chemical, nonanal, was also a significant constituent of the pheromone. Their findings revealed that the male moth produced a ratio of 7:3 (nonanal-undecanal).

Experimental trials using a mixture of nonanal and decanal in a ratio of 7:3 in bait traps successfully captured male moths that were searching for female moths. Nevertheless, Flint & Merkle (1983) discovered that traps containing the sex pheromone components, namely nonanal and decanal, failed to lure insects from a long distance. The findings suggest that *G. mellonella* employs auditory signals as far mating cues, whereas sex pheromones serve as close-range mating cues. These experiments unequivocally illustrate that the larger wax moth exhibits multiple behaviours that are presumably influenced by semiochemicals.

The pheromonal volatiles from six regions of Russia were studied by GC-MS. The calling males of *G. mellonella* resulted in different compositions of pheromones. Volatile compound found constitutes undecanal, hexanal, heptanal, decanal and 6,10,14 trimethylpentacanol-2 while the major component of the volatiles includes nonanal and undecanal. The ratio of the components varies in different regions (Lebedeva et al., 2002).

According to Svensson et al. (2014), various binary mixes of undecanal and nonanal in the percent ratios of 95:5, 90:10, 85:15, 80:20, 60:40, 50:50, 30:70, 20:80, and 10:90 elicited a reaction in a female moth of the Greater Wax Moth (GWM), *G. mellonella* L. Among the 30 moths seen, one to four moths exhibited various behavioural patterns, including ambulation, stationary fanning, ambulatory fanning, hovering, searching, and circling. The behavioural bioassay demonstrated that moths that were 3 to 5 days old showed the highest level of sensitivity to a mixture of undecanal and nonanal (in a ratio of 3:7) between the hours of 7 and 9 pm. Over 60% of moths had distinct behavioural tendencies related to pheromones. A funnel trap with a binary blend of undecanal and nonanal in a 3:7 ratio was employed to attract GWM in outdoor conditions.

Svensson et al. (2014) discovered a third molecule that is exclusive to males, called 5,11-dimethylpentacosane. They found that this compound enhances the effects of the aldehydes on behaviour. The male pheromones of the wax moth were previously determined to be a combination of nonanal and undecanal. These chemicals stimulate

short-range sexual behaviour in female moths of the same species, such as wing fanning. However, the blend of aldehydes was found to be ineffective in attracting females over longer distances.

In this study, female GWM responded to various binary blends of undecanal and nonanal in the following proportions: 95: 5, 90: 10, 85: 15, 80: 20, 60: 40, 50: 50, 30: 70, 20: 80, and 10: 90. The findings revealed that between one and four out of the total of 30 moths displayed various behavioural patterns, such as remaining still while fanning their wings, walking, walking while fanning their wings, hovering, searching, and moving in circles. The female insects exhibited the highest level of responsiveness to the 30:70 blend. The behavioural bioassay revealed that moths aged 3 to 5 days displayed the most pronounced reactions to the ideal binary mixture of undecanal and nonanal (in a ratio of 3:7) between 7 pm and 9 pm. Furthermore, more than 60% of the moths displayed distinct behavioural patterns related to pheromones. The funnel trap was employed to attract female GWM, with the optimal ratio of undecanal and nonanal at 3:7 (Bhopale et al., 2016).

The Dual-choice olfactometer assay examined the role of conspecific larval odours in the clustering behaviour of 3–5th instar and 8th instar larvae. The experiments demonstrated that solely the 8th instar larvae exhibited a considerable attraction towards the odours emitted by recently formed cocoons. An examination of the scents from the head space of larval samples using gas chromatography-mass spectrometry (GC-MS) showed the presence of four chemicals: nonanal, decanal, tridecane, and tetradecane. These compounds were found in both pupal and mature larval odour extracts. The role of volatile organic chemicals in the aggregation behaviour of fully developed wax moth larvae was uncovered. Additionally, it provided opportunities for the creation of a scent-based trapping system within beehives to manage wax moth larvae (Kwadha et al., 2017).

After reviewing these papers, it was noticed that only few studies were conducted on pheromones of *G. mellonella* and *A. grisella.* The wax moth has been a serious problem in apiaries and no detailed study in Punjab, India has been done on isolation, identification and analysis of pheromone of male wax moth and its application as biocontrol tool against *G. mellonella* and *A. grisella*. Therefore, to devise efficient control measures against pest species, it is imperative to identify and characterize the

sex pheromones used in their communication, which can serve as potent biocontrol tools. By investigating the intricacies of these pheromones, we aim to develop biocontrol strategies that offer innovative and sustainable solutions for beekeepers and stakeholders in the stored product industry. This research focuses on *Galleria mellonella* (the greater wax moth) and *Achroia grisella* (the lesser wax moth), both of which pose significant threats to apiculture and the integrity of stored bee-related products.

Understanding the chemical signalling mechanisms of these pests will enable the formulation of targeted interventions, potentially disrupting their mating behaviours and reducing their populations. Through these endeavours, the study seeks to address the multifaceted challenges presented by these pests, ultimately fostering greater resilience in apicultural practices and improving the storage and preservation of bee-related products. The identification and application of specific sex pheromones not only represent a novel approach to pest management but also align with the broader goals of ecological sustainability and reduced reliance on chemical pesticides.

CHAPTER 3

HYPOTHESIS

Wax moths are a significant pest that cause significant economic loss to beekeepers and honey bee colonies. They damage combs, feed on honey bee products, and often lead to colony absconding. Control measures include pesticides, which can cause long-lasting effects on bee health and honey contamination. Physical treatments, such as extreme heat and cold, can also cause damage. To overcome these issues, monitoring and trapping are essential. Pheromone extracts of male wax moths could be a safer option for trapping unwanted guests in hives. However, there is no study conducted in India on pheromonal identification and analysis of existing wax moth species. Pheromones offer advantages over conventional control methods, such as low maintenance requirements, cost efficiency, and toxicity. They can be implemented in both storage and field environments. Beekeepers in both developed and less economically developed countries can benefit from a pheromone-based trapping system, which has global commercial implications.

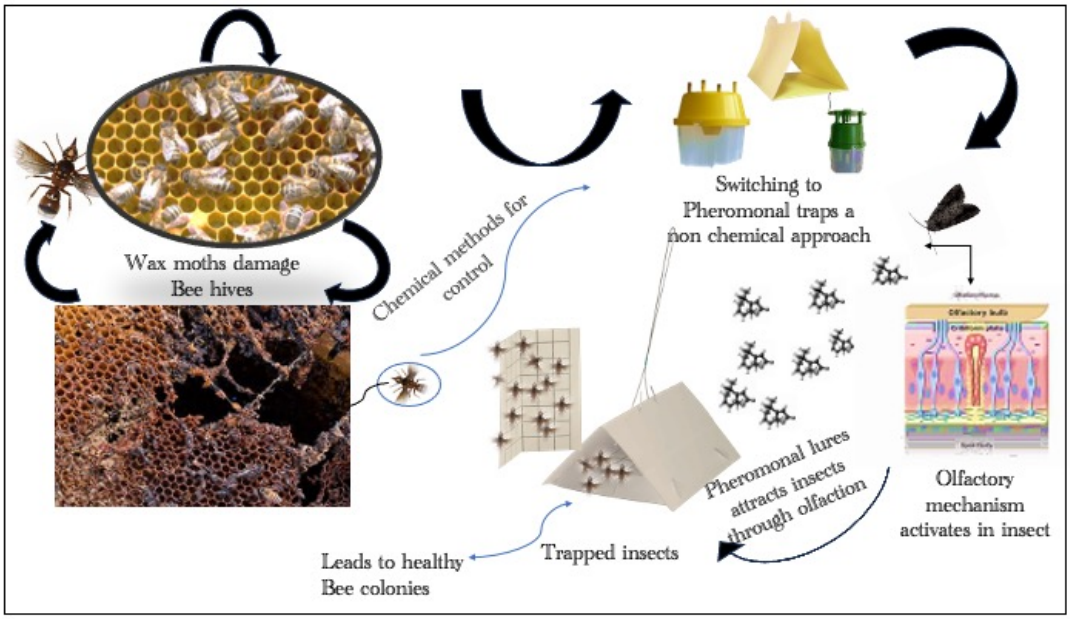


Figure 3.1: The image depicts a flowchart illustrating the transition from chemical pest control methods to non-chemical pheromone-based approaches for protecting Bee hives. Unravelling the potential of pheromone traps in combating wax moths infestations. The sequence starts with pest damage to bee colonies and highlights the use of chemical methods for insect control. It then shows a shift to pheromone traps as a non-chemical alternative. The pheromones lure insects into traps by activating their olfactory mechanisms. This method results in trapped insects and ultimately leads to healthy bee colonies. (copyright filed)

CHAPTER 4

OBJECTIVES OF THE RESEARCH

The brief objectives of the present research work are:

- To isolate and catalogue volatile organic compounds from adult male wax moth, *G. mellonella* and *A. grisella.*
- To elucidate the attractiveness of volatile organic compounds blend to female wax moths.
- To evaluate the efficiency of extracted pheromone blend in trapping female wax moths in the apiary.

CHAPTER 5

MATERIAL AND METHODS

Galleria mellonella (*G. mellonella*) and *Achroia grisella* (*A. grisella*) infestations stress bee colonies, resulting in reduced colony growth, decreased honey production, and reduced pollination. This threatens agricultural output and environmental stability. To develop eco-friendly control of wax moths, this research work delves into the isolation and identification of male wax moth pheromones from *G. mellonella* and *A. grisella*.

SEM and stereo-microscopy were used to detect pheromone glands. GC-MS was used to analyse pheromones qualitatively and quantitatively. In a controlled laboratory setting, female moths were tested for pheromone reactions using behavioural bioassays. Field studies assessed the efficacy of these pheromones in natural settings. This research intends to reduce chemical pesticide use and promote sustainable agriculture by revealing alternative pest management options.

5.1 Collection and Rearing of Test Insects

5.1.1 Collection of Test Insects

The stock of wax moth larvae (*Galleria mellonella* and *Achroia grisella)* was collected from three distinct locations one in the Jalandhar district and two in the Ludhiana district of Punjab, India, to ensure a diverse genetic pool for the study. The first collection site was Sangeeta Bee Farm, situated at the coordinates 31°17'39.588" N and 75°32'34.98" E in Jalandhar. The second and third collection sites were both located in Doraha, with Big Bee Agro at 30°47'53.52" N and 76°1'51.6" E, and Tiwana Bee Farm at 30°16'56.928" N and 76°37'33.78" E (Figure 5.1). The collection was done based on previous work done on the insect and based on the visual morphological characteristics (Williams, 1997; Ellis et al., 2013; Kwadha, 2017) (Figure 5.2). The collected larvae were transported to the laboratory in sterile containers to maintain their integrity and prevent cross-contamination. Upon arrival, the larvae were carefully examined for any signs of disease or parasites to ensure the health and viability of the stock. The healthy larvae were then transferred to a dedicated rearing facility within the laboratory, where they were subjected to a rigorous process of acclimation to the controlled environment.

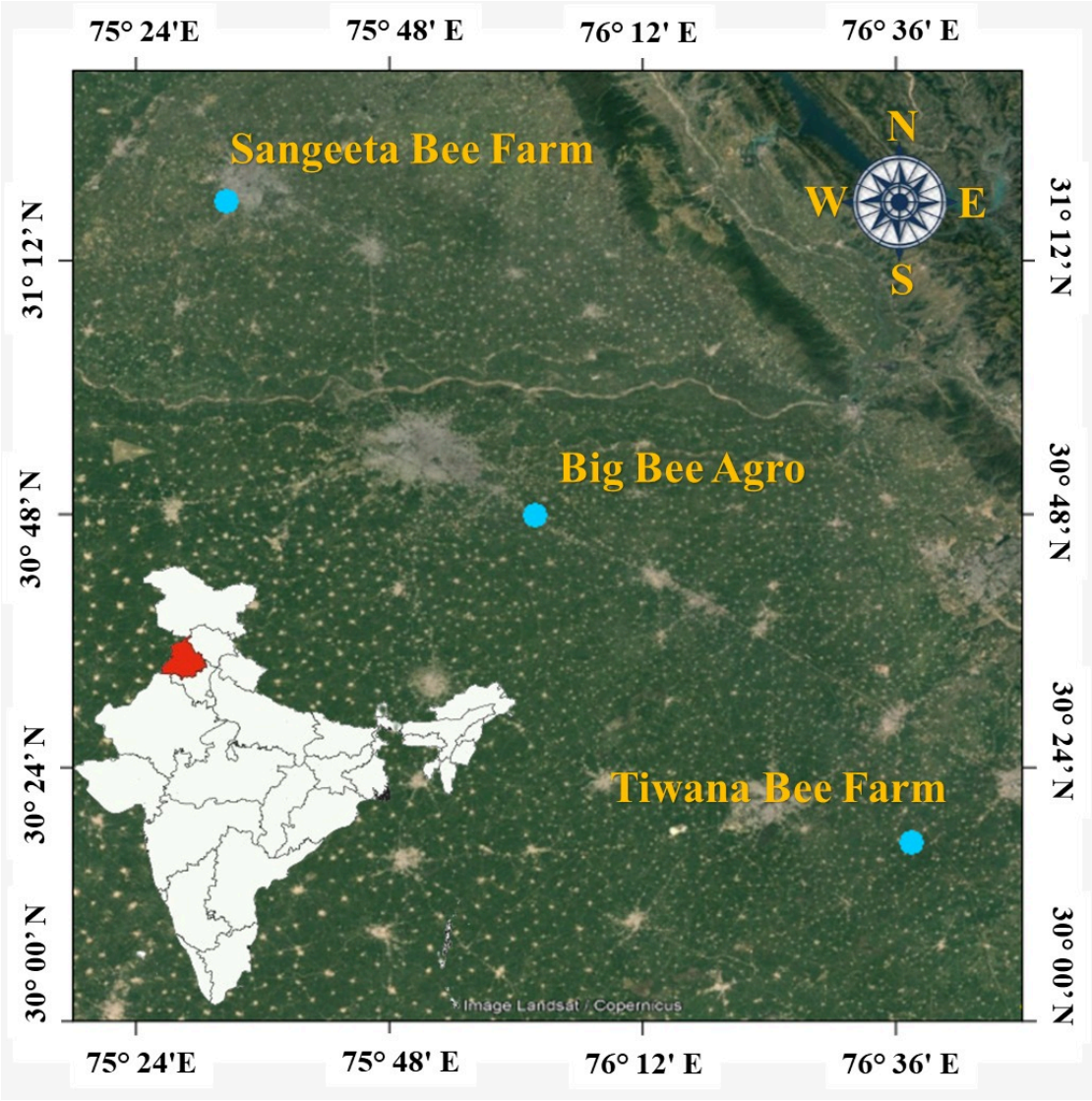


Figure 5.1: Geographical representation of the different bee farm sites for the collection of test insects (Sangeeta Bee Farm, Big Bee Agro Farm and Tiwana Bee Farm) with specific locations

5.1.1.1 Test Organism Description

The wax moths (*Galleria mellonella* and *Achroia grisella*), holometabolous insects, undergoes four distinct developmental stages: egg, larva, pupa, and adult (Fasasi & Malaka, 2006; Swamy, 2008).

5.1.1.2 Mass Culturing and Maintenance

The rearing and maintenance of the wax moth larvae were carried out under strictly controlled laboratory conditions to ensure consistency and reproducibility of the experiments. The temperature was maintained at a range of 28–31°C (Lebedeva et al., 2002) which is optimal for the growth and development of the larvae. The humidity level was set at 60 per cent, which mimics the natural environment of the larvae and prevents desiccation (Figure 5.3). These conditions were achieved and maintained using a BOD (Biochemical Oxygen Demand) incubator, a specialized piece of equipment designed for precise control of temperature and humidity.

Figure 5.2: Collections of wax moth larvae from different apiaries: a) Sangeeta Bee Farm b) Tiwana Bee Farm c) and d) Big Bee Agro

To eliminate any potential contamination from the combs used for rearing, they were frozen at a temperature of -15°C before use. This process effectively kills any existing infestation of wax moths or other pests that may have been present in the combs. The frozen combs were then thawed and sterilized using ultraviolet light to further ensure the cleanliness and safety of the rearing environment.

The adult wax moths selected for the laboratory experiments were aged between 2 and 7 days. This age range was chosen to ensure that the moths were sexually mature and capable of mating, while still being young enough to exhibit robust behaviour and physiology. The adult moths were held in glass chambers in the dark at a temperature of 28-31°C, which is consistent with the rearing conditions of the larvae (Romel et al., 1992). This temperature range is known to be optimal for the survival and reproduction of the wax moth species under study.

By carefully selecting the collection sites, maintaining strict control over the rearing conditions, and carefully selecting the age of the adult moths, the stock of wax moth larvae used in the laboratory experiments was of high quality, consistent, and representative of the natural population.

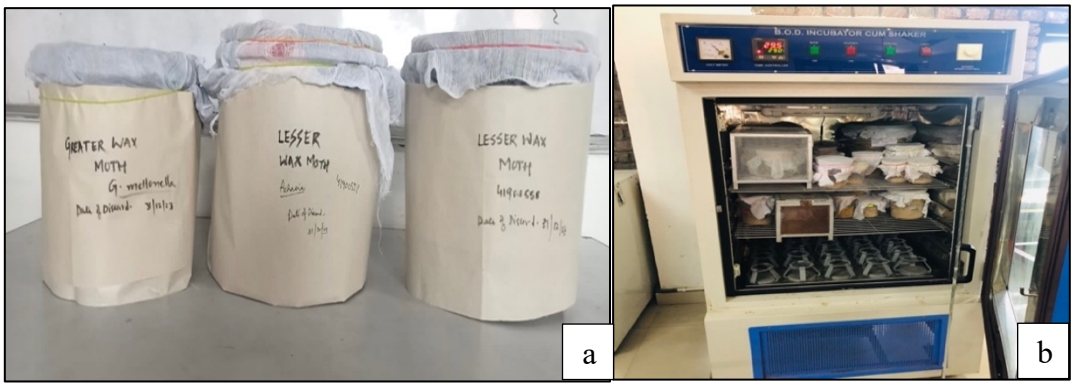


Figure 5.3: Rearing of wax moths a) Culture of *G. mellonella* and *A. grisella* in the containers b) Cultures kept in BOD Incubator in Laboratory conditions at 28°C

5.1.1.2.1 Identification of Test Insects

5.1.1.2.1.1 Morphological identification of the Adult *Galleria mellonella*

Sexual Dimorphism and Physical Characteristics: Wax moths show well-defined sexual dimorphism. The female moths are generally larger and heavier, with a length of about 20 mm, compared to male moths. The female moth has an outer margin, while

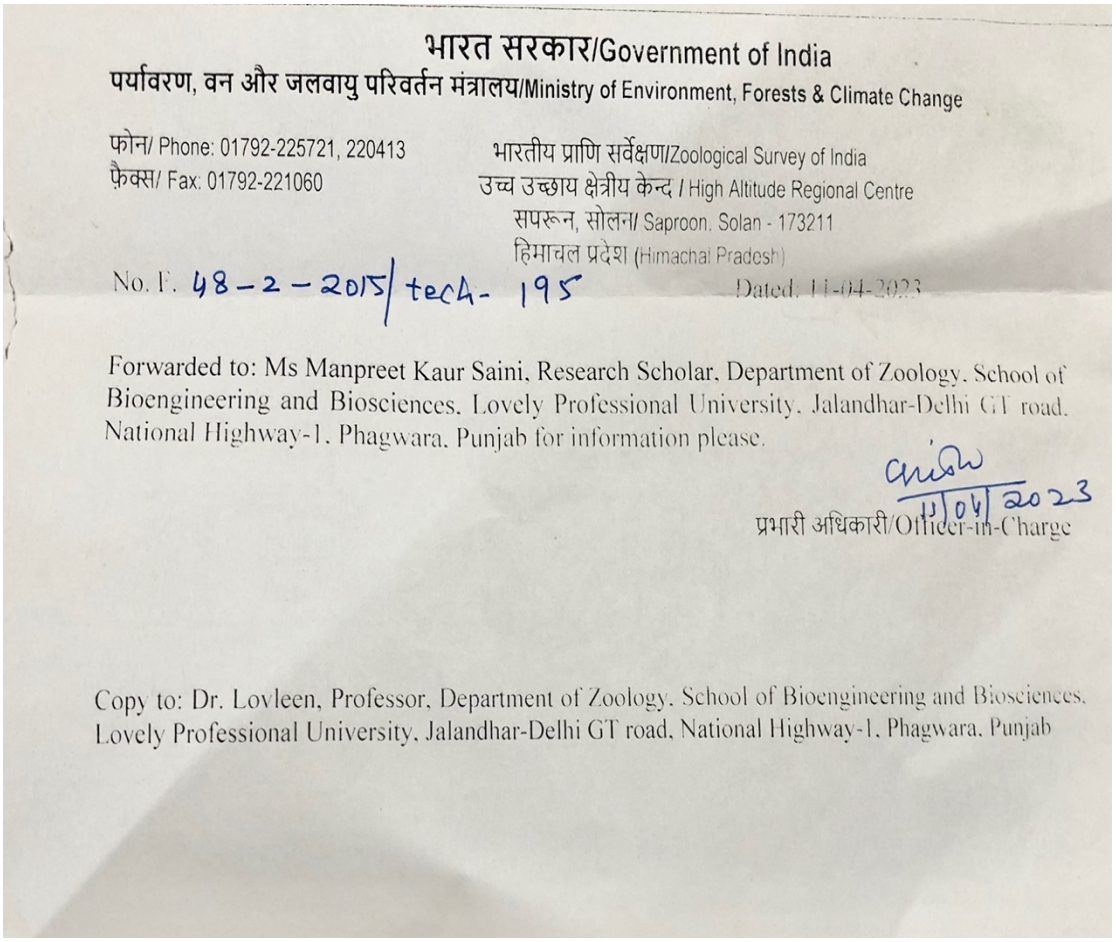
males have a semi-lunar notch on their forewings (Williams, 1997). Adult moths are grey to purple-brown in colour with dark marks and lead tips on their forewings. The forewing breadth varies from 5 to 7 mm, and they have pale brown or yellow wings (Ellis et al., 2013). The wingspan is 1 to 1.25 inches (Chang & Hsieh, 1992). The forewing has a concave termen, while the hindwing's Cu looks to be divided into four branches. In addition, the labial palp is elongated, almost the same length as the longest leg spur, and extends outward (Ellis et al., 2013).

5.1.1.2.1.2 Morphological identification of the Adult *Achroia grisella*

Sexual Dimorphism and Physical Characteristics: The adults of the lesser wax moth exhibit a spectrum of coloring, ranging from silver-grey to beige, and possess a distinctively conspicuous yellow head (Ellis et al., 2013). They measure roughly 1/2 inch in length and possess thin bodies. Their wingspan measures around 1/2 inch in width (Egelie et al., 2022). In general, males are smaller compared to females. The breadth of the forewing is less than 5 mm. The termen of the forewing is convex, while the hindwing of the male has a concave termen. The Cu of the hindwing is apparently 3-branched, and the labial palps are conspicuous though short, not exceeding the diameter of the eye. The labial palps of males are transversely incurved and pincer-like (Ellis et al., 2013).

5.1.1.2.1.3 Identification Report by Zoological Survey of India, Solan

The insects (wax moths) collected were killed by using 10 per cent formic acid and mounted properly and preserved for further taxonomic study. The specimens were sent to the Zoological Survey of India, Solan for identification. This results in the identification of two lepidopteran species *viz a viz Galleria mellonella* (Greater wax moth) and *Achroia grisella* (Lesser wax moth) with reference no 48-2-2015/tech -195 (Figure 5.4).



भारत सरकार/Government of India
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय/Ministry of Environment, Forests & Climate Change

फोन/ Phone: 01792-225721, 220413
फ़ैक्स/ Fax: 01792-221060

भारतीय प्राणि सर्वेक्षण/Zoological Survey of India
उच्च उच्छाय क्षेत्रीय केन्द्र / High Altitude Regional Centre
सपरून, सोलन/ Saproon, Solan - 173211
हिमाचल प्रदेश (Himachal Pradesh)

No. F. 48-2-2015/ tech- 195 Dated: 11-04-2023

Forwarded to: Ms Manpreet Kaur Saini, Research Scholar, Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Jalandhar-Delhi GT road, National Highway-1, Phagwara, Punjab for information please.

11/04/2023
प्रभारी अधिकारी/Officer-in-Charge

Copy to: Dr. Lovleen, Professor, Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Jalandhar-Delhi GT road, National Highway-1, Phagwara, Punjab

Figure 5.4: Identification report of species of wax moth i.e. *G. mellonella* and *A. grisella* by Zoological Survey of India, Solan

These parameters were implemented to execute the present investigation and are briefly described below:

To isolate and catalogue volatile organic compounds from adult male wax moths, *G. mellonella* and *A. grisella*- The adult moths of *G. mellonella* and *A. grisella* were raised on old honey bee combs in a BOD incubator at 28-31°C and 60 per cent humidity. First, second, and third-generation adults were collected. The cocoons were dissected and males and females separated after 5-7 days of pupation. 2-7-day-old males of each species (10, 15, and 20) were collected from 2-5 PM. Effluvium was collected using an activated charcoal disk for 30, 60, and 90 minutes. 3 replicates of 6 extractions were done on adult, male *G. mellonella* and *A. grisella*. The combination was quantified in a volumetric flask for analysis. The eluents were stored at -30°C until usage. Based on

GC-MS chemicals, quantitative analysis was done. The Procurement of chemicals and comparison to the sample was done for GC-MS quantification (Lebedeva et al., 2002).

To elucidate the attractiveness of volatile organic compounds blend to female wax moths- Female adult *G. mellonella* and *A. grisella* moths were used in the experiment. The set of 5 *G. mellonella* adult females was exposed to all treatment extracts (synthetic and extracted), as were the *A. grisella* adult females. The behavioural bioassay showed that 3–5-day-old moths responded best to the undecanal: nonanal (3:7) blend (Sangramsinh et al., 2014). An enclosed glass chamber with an inlet for the placement of the extract and release of wax moth adults was taken. Extract quantity was standardized that could generate a response in female moths released in the glass chamber. The odour stimulus was created by using a pipette to place the test solution onto a small piece of Whatman No. 1 filter paper measuring 5x5 cm. A fresh filter paper was substituted, and a new stimulus was introduced for each individual test. Before each trial, air was evacuated from the chamber to avoid contamination. Washing and cleaning chamber surfaces with ethanol prevented pheromone contamination between treatments. Connecting the exhaust tube to a wall-mounted excurrent fan flushed air from the glass chamber (Lebedeva et al., 2002).

To evaluate the efficiency of the extracted pheromone blend in trapping female wax moths in the apiary- The attractive fraction concentrations were utilized for evaluation in the field settings. The synthetic and extracted pheromones were applied to assess their impact on trapping effectiveness. The dose range from 15 ppm to 100 ppm (extracted and synthetic) pheromones was used in the field along with the control (hexane 15 ppm) for *G. mellonella* and *A. grisella* (hexane 10.7 ppm) species. The experiment included three replications in each of the three bee farms *i.e.* Alwaz Honey Bee Farm, Krishna Bee Farm and Vicky Bee Farm, Phillour, Jalandhar. The female Moths captured in the traps were killed, counted, and discarded after 2 days. Pheromone traps were installed 50 cm above the ground near the box. All traps were rerandomized. Treatments were triplicated.

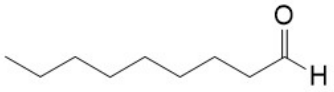
5.2. Test Compounds for experiments

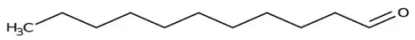
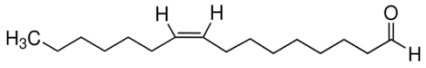
5.2.1 Insect Sex Pheromones

Sex pheromones are aliphatic, chemical signalling has the advantage of being efficient at very low intensities. Moths use unique pheromonal bouquets or pheromone components with varying vapour pressures (due to the number or location of double bond compounds, chain length, or functional groups) and exhibit more variability. The pheromones elicit different types of responses in insects: immediate behavioural responses, physiological changes leading to behavioural responses etc (Benelli et al., 2019).

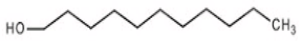
The nonanal and undecanal components of the sex attractant pheromone of the greater wax moth showed increased sensitivity and a greater number of responding acceptors in the dosage response curves when diluted serially (Dickens et al., 1986). The male scent of this species was previously identified as a combination of undecanal and nonanal (Svensson et al., 2014). Quantification experiments were conducted on the pheromone-gland extracts and volatiles that are emitted by male *G. mellonella* (L.), also referred to as the larger wax moth. The extracts contained the following mean percentages of aldehydes and alcohols: 19.0 per cent undecanal, 3.9 per cent nonanal, 48.3 per cent 1-undecanol, and 28.8 per cent 1-nonanol (Romel et al., 1992).

5.2.1.1 Aldehydes

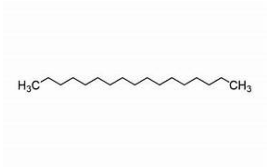
Compound	Molecular structure
Nonanal	

Undecanal	
Cis- 9- hexadecenal	

5.2.1.2 Alcohols

1- undecanol	
1-nonanol	

5.2.1.3 Alkanes

Heptadecane	
Heneiocosane	

5.2.2: Chemicals used: Chemicals tested for GC-MS Quantitative were obtained from Merck (India) Ltd. and diluted in GC-grade Dichloromethane at CIF, Lovely Professional University, Phagwara. Pheromone gland components-related chemicals were obtained from Sigma-Aldrich Chemie GmbH-Schnelldorf, Germany. Compounds were nonanal, 1-nonanol, heneiocosane, heptadecane, and undecanal and cis 9 hexadecenal with purity generally >98% by GC.

Table 5.1: Physical and Chemical Properties of Chemical Compounds

Compound	Chemical Formula	Molecular Weight (g/mol)	Boiling Point (°C)	Melting Point (°C)	Density (g/cm^3)	Appearance	Odour
Nonanal	$C_9H_{18}O$	142.24	190-193	-18	0.831	Clear, colourless liquid	Fatty, citrus-like
1-Nonanol	$C_9H_{20}O$	144.25	213-214	-7	0.83	Clear, colourless liquid	Mild, fatty
Heneiocosane	$C_{21}H_{44}$	296.58	366	40-42	0.78	White, waxy solid	Odourless
Heptadecane	$C_{17}H_{36}$	240.47	302	22-24	0.777	Colourless oily liquid	Mild, characteristic
Undecanal	$C_{11}H_{22}O$	170.29	229-230	-2	0.83	Clear, colourless liquid	Citrus, waxy
Cis-9-Hexadecenal	$C_{16}H_{30}O$	238.41	275-277	20-22	0.846	Pale yellow liquid	Characteristic

5.2.3: Other Chemicals Used: Hexane, Dichloro-methane, Ethyl acetate, Ethanol (sigma-Aldrich).

5.3 Protocol for Sample Preparation for Qualitative Analysis

5.3.1 Isolation of Volatiles

An enclosed acrylic chamber measuring 48.26 × 17.78 × 19.05 cm was specifically designed for the effective extraction and analysis of pheromones released by male moths. This setup provided a controlled environment conducive to studying insect communication, particularly focusing on volatile chemical signaling. The chamber featured a top-opening lid of 11.43 × 11.94 cm, allowing easy insertion and removal of moths while maintaining internal conditions. Airflow within the chamber was regulated using a small fan (13 × 13 cm) fitted with acrylic glass filters, which ensured a steady and uniform movement of air, crucial for the consistent transport of airborne pheromone molecules toward the collection site.

At one end of the chamber, a vacuum tube was installed to facilitate air flushing and create a directional airflow. An acrylic platform (10 × 7 × 10 cm) was strategically placed near this vacuum tube to hold a 100 mm diameter activated charcoal disc, which acted as the adsorbent medium for capturing the volatiles emitted by the moths. This positioning ensured that airborne compounds were efficiently carried to and concentrated on the charcoal disc. An acrylic shutter was included to create a vacuum after each trial, enhancing the recovery of residual volatiles. To ensure the purity and sterility of the environment, all glass tubes and containers used during the process were sterilized using a hot air oven and autoclave before use.

During the experimental trials, groups of 10, 15, and 20 male moths were placed inside the chamber for durations of 30, 60, and 90 minutes. The activated charcoal disc, located at the end opposite the fan, collected the volatile effluvia released by the moths during these time intervals (Lebedeva et al. 2002). This method was meticulously designed to maintain uniform experimental conditions and to optimize the capture of pheromone compounds for subsequent chemical analysis, such as gas chromatography–mass spectrometry (GC-MS). The entire setup reflects a precise and well-calibrated approach to studying pheromonal communication in moths under laboratory conditions.

5.3.2 Extraction Factors

For extraction of effluvium, three factors were used, insect species, number of moths and exposure period. Volatile effluvium from male moths of two species *G. mellonella* and *A. grisella* was collected for 30, 60 and 90 min, and the number of moths used were 10, 15 and 20 for each species. Each set of treatments was replicated thrice. The experiment conducted with 10, 15 and 20 males effluvium produces consistent results with 3 replications for 30, 60 and 90 min, so 10 males effluvium was taken as a standard for the quantitative analysis. The time period was also selected as a factor 30 min time period was enough for the collection of volatiles.

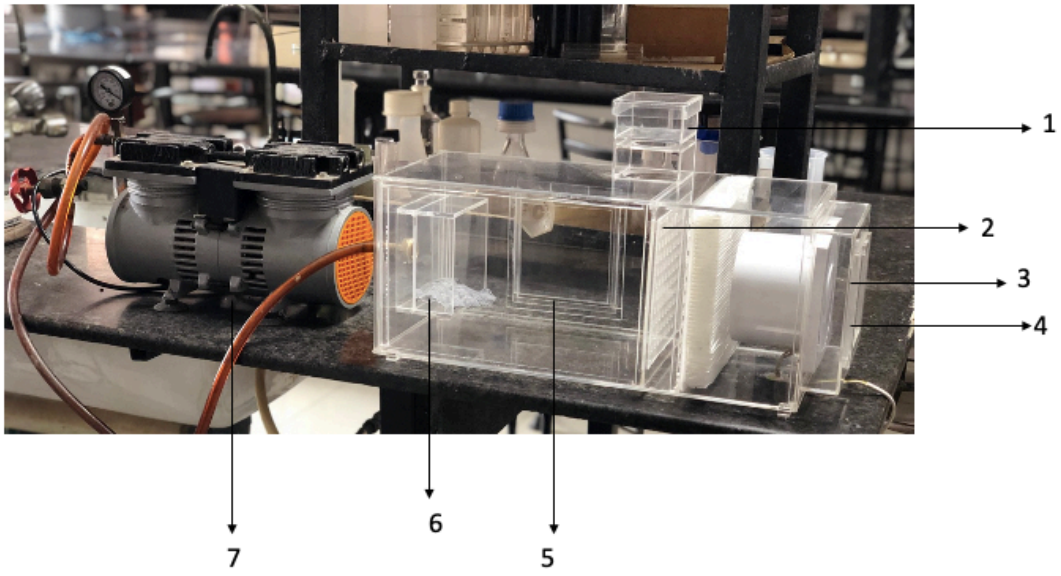


Figure 5.5: Chamber for collection of volatiles released by male *G. mellonella* and *A. grisella* 1) Inlet chamber 2) Acrylic sheets for steady flow of air 3) Shutter 4) Fan 5) Access window 6) Desk for charcoal disk 7) Vaccum pump

5.3.3 Qualitative Analysis for Identification of Pheromones

After rinsing the volatiles from the 100 mm charcoal disc (Sigma-Aldrich) with methylene chloride, the major concentration was estimated using GC-MS without solvent evaporating, allowing for the appropriate volume to be studied for minor component analysis. Subsequently, the Shimadzu TQ 8040 system was utilized to conduct GC-MS analysis, employing a capillary column with dimensions of 30 × 0.25

× 0.25m. The temperature of the injector was 240°C. The oven temperature was set at 35°C for 1 minute and then increased to 230°C at a rate of 10°C per minute. The identification of compounds was achieved using the utilization of mass spectrum libraries, as described by Lebedeva et al. in 2002.

5.3.4 Quantitative Analysis: Preparation of Sample and Standard

Calibration graphs have been generated for the samples that were processed using the specified analytic procedure, utilizing the Selected Ion Monitoring (SIM) mode. The calibration curve was generated using various amounts of nonanal, 1-nonanol, heneiocosane, heptadecane, undecanal, and cis 9 hexadecenal. For nonanal, 1-nonanol, heneiocosane, heptadecane, and undecanal 1, 3 and 5 ppm concentrations were used and 1, 2 and 3 ppm were used for cis 9 hexadecenal (Figure 5.6).

The external calibration curve method was employed to conduct the separation, isolation, and quantitative determination of the compounds. The calibration curve is generated using the standard's known concentration. The determination was made by comparing the retention time (RT) of the substance in the sample to that of the matching compound in a standard solution, which was tested under identical conditions.

The GC-MS analysis was conducted using the Shimadzu TQ 8040 system, which was equipped with a capillary column of 30×0.25×0.25m. The injector temperature was 240°C. The oven temperature was programmed from 35°C (held for 1 min.) to 230°C at 10°C min^{-1}. Compounds were identified by using mass spectral libraries. (Column:V5 (I,30 m,i.d. 0.25, film thickness 0.25um); delay; 5 min; Temperature program: 50 °C (1) 200 °C (8°C/min) 300°C (10°C/min); injector temperature: 250°C; split: 20%; injection volume: 1 µl; carrier gas: He; Flow rate: 1mL/min).

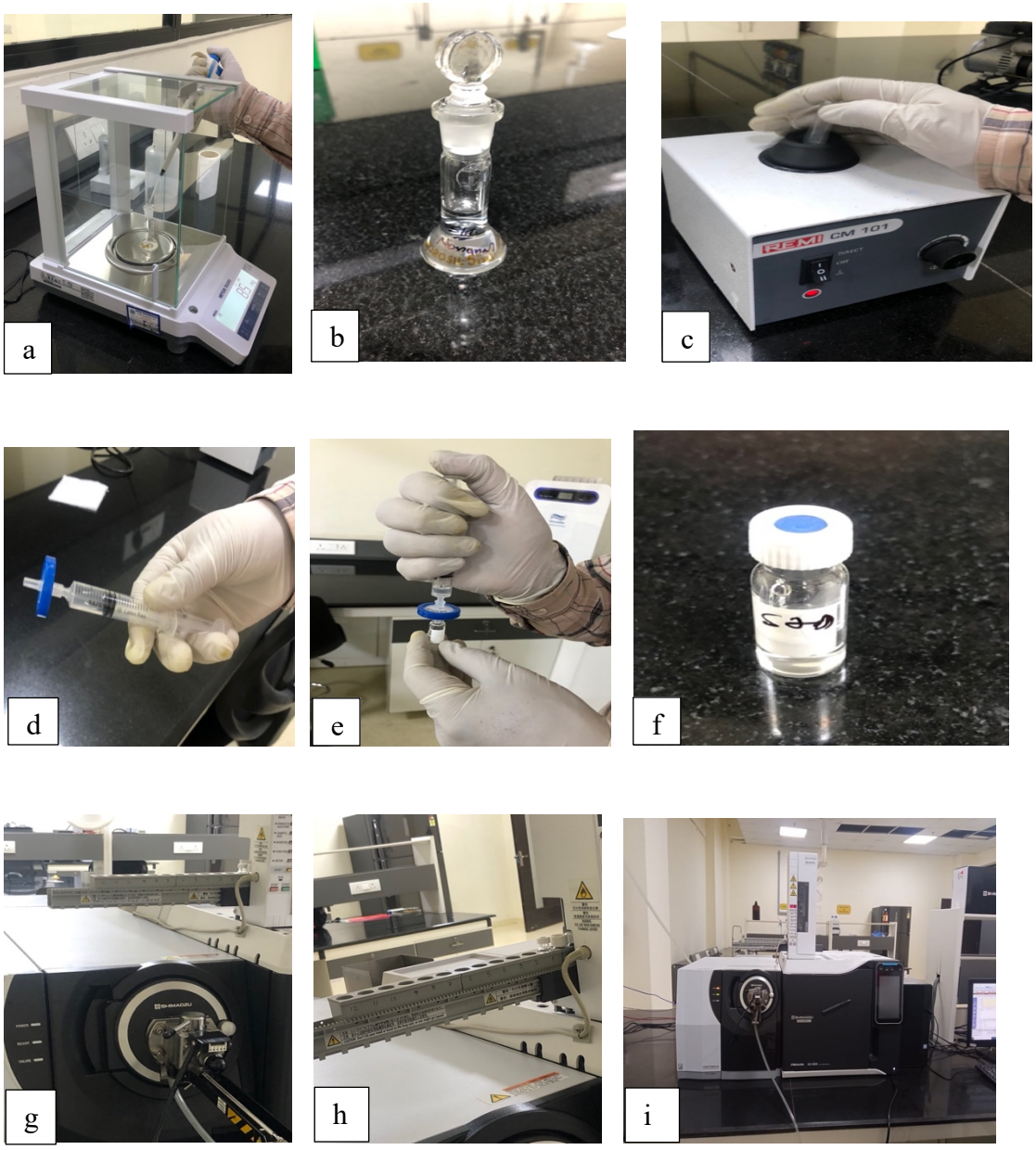


Figure 5.6: Representation of quantitative analysis procedure using Gas Chromatography a) Weighing of the sample b) Preparation of the stock solution c) Mixing uniformly via vortex device d) Filter the sample using syringe filter e) Filtrate collected in vial f) Transferred filtrate into GC vial g) Samples loaded on GC plate h) Column with samples i) Shimadzu TQ 8040 system and equipped with a capillary column (30 × 0.25 × 0.25m)

5.4 Stereo-Microscopy

Twenty male adult *G. mellonella* and *A. grisella* moths were collected for the stereomicroscopy experiment. The insect's wings were separated from the insect's body

using forceps and scissors. The wings were soaked in a 10 per cent potassium hydroxide solution for approximately 12 hours at room temperature.

The specimens were removed from the KOH and immersed for 15 minutes in 10 percent acetic acid. KOH helps remove the wings' organic impurities, scales, and debris. Acetic acid aids in the relaxation of wings. The specimen was removed from the acetic acid after 15 minutes and placed in 70 per cent ethanol. The wings were fixed in 70 percent ethanol, and `then dehydrated in a series of 75, 80, 90, 96, and 100 percent ethanol and water solutions for 10 minutes (Zohry & El-Sayed, 2019).

5.5 Scanning Electron Microscopy (SEM)

The forewings of *G. mellonella* and *A. grisella* were analyzed using Scanning Electron Microscopy. The specimens were preserved and fixed in a 70 per cent ethanol solution. After fixing the material with 70 per cent ethanol, it was subjected to a 10 minute dehydration process in which the ethanol/water concentration was increased sequentially from 75 to 90 to 96 to 100 per cent (Zohry & El-Sayed, 2019). Due to the extreme sensitivity of their wings to cleaning agents, cleaning the wings prior to SEM analysis is unnecessary.

Any effort to clean the wings caused damage or curling. Wing damage was difficult to avoid, even with an alcohol series. At the Central Instrumental Facility, Lovely Professional University (CIF, LPU), samples were mounted on holders, sputter-coated with gold, and analyzed with FESEM coupled to an EDS detector, Au Sputter Coater (FE-SEM: JEOL EDS, Oxford EDS, LNS free).

5.6 Protocol for Behavioral Bioassay in Female, *G. mellonella* and *A. grisella*

The relative efficacy of the synthetic blend and extracted blend of male *G. mellonella* and *A. grisella* with different concentration were studied. The response of female *G. mellonella* and *A. grisella* towards different concentrations of pheromone blend was observed in an enclosed acrylic chamber under controlled conditions. These bioassays can provide insight into the response of wax moths towards the stimuli provided, can help in understanding the ecology, physiology and behaviour.

5.6.1 Chemical Solutions

All compounds utilized as authentic standards in the laboratory bioassay experiments or the chromatographic analysis were > 97% pure by GC analysis and were stored at -10°C until they were required. Nonanal, 1- nonanol, heptadecane, undecanal, 1-Undecanol, Cis-9- hexadecenal and heneiocosane was purchased from Sigma-Aldrich (Germany).

5.6.2 Lure Preparation

The compounds were combined in various proportions, as outlined in Table 5.2 and 5.3, and diluted in hexane. The resulting solution was then carefully transferred onto filter paper using a pipette. Prior to utilization, all solutions were heated to ambient temperature and stirred to dissolve any crystals.

5.6.3 Insect Source

The population was reared and maintained under controlled laboratory conditions at 28–31°C temperature and 60 percent humidity in a BOD incubator. Male and female pupae were segregated from the stock and kept separated till the emergence of adults. Female pupa represents the cloven sterna forming copulatrix aperture while the male pupa represent the round knobs called Phallomeres in male pupa (Smith 1965) (Figure 5.7). The virgin female moths from this stock were selected for conducting laboratory bioassay experiments.

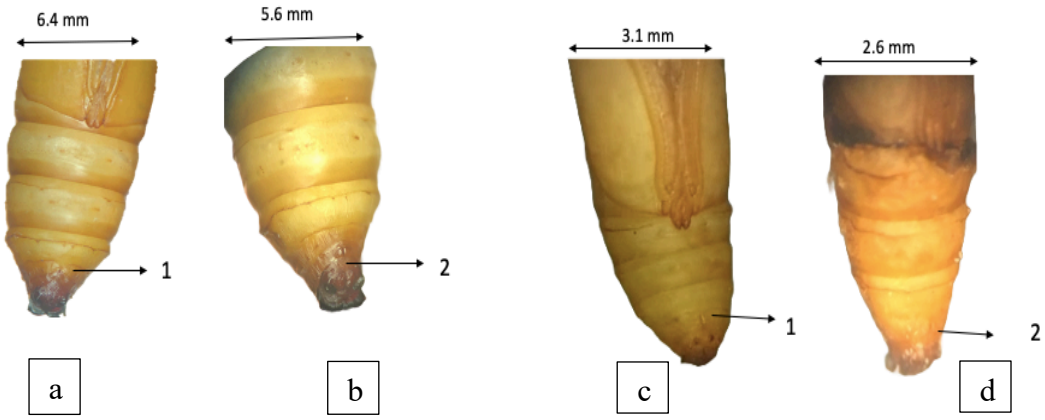


Figure 5.7: Stereomicroscopic images of Pupa at 18X magnification (Stereo Zoom) a) Female pupa, *G. mellonella* b) Male pupa, *G. mellonella* c) Female pupa, *A. grisella* d) Male pupa, *A. grisella* 1) Represents the cloven sterna forming copulatrix aperture in female pupa 2) Represent the round knobs called Phallomeres in male pupa

5.6.4 Behavioral Bioassay Setup

Female *G. mellonella* and *A. grisella* responses to component mixtures were observed in a laboratory wind tunnel. The behavioural observations were made in a 916.9 x 22.86 x 19.05 cm laboratory wind tunnel (Figure 5.8). An enclosed acrylic chamber with an inlet for the placement of the extract and release of the test insects. The exhaust tube on one end facilitates flushing out of air from the glass chamber. Further, Pheromone contamination between successive treatments was avoided by washing and rinsing the chamber surfaces with ethanol and the exhaust system removed the pheromone from the tunnel. Females were maintained in laboratory condition at 30°C on a 16:8, light: dark, photoperiod regime on wax collected from the bee apiaries.

A treatment-impregnate Whatman No. 1 filter paper (5x5 cm) was placed in the centre of the acrylic platform situated on the wind tunnel floor from the upwind end (Figure 5.9). It was allowed to dry for 2 minutes. Pupae separated by sexes emerged into virgin females (n=5) were taken in the wind tunnel room through the inlet chamber. Ten seconds were allowed for the females to acclimatize. Moths once used were not used again. An observation of 30 minutes was taken. All the experiment was done similar conditions.

Each female was scored for exhibiting the behaviours, usually occurring in this order taking upward flight, flight to 10 cm arena ovipositor display, and timings of orientation to the given concentrations described in Table 5.2 and 5.3. For the extracted blend the behavioural observations were done on 15 ppm concentration in *G. mellonella* and 10.7 ppm concentration in *A. grisella* only with females (n=5) was executed. This specific concentration was selected as a base as per the prior experiment done in order to identify and isolate the pheromones where effluvium of 10 males was collected for analysed quantitatively by GC MS.

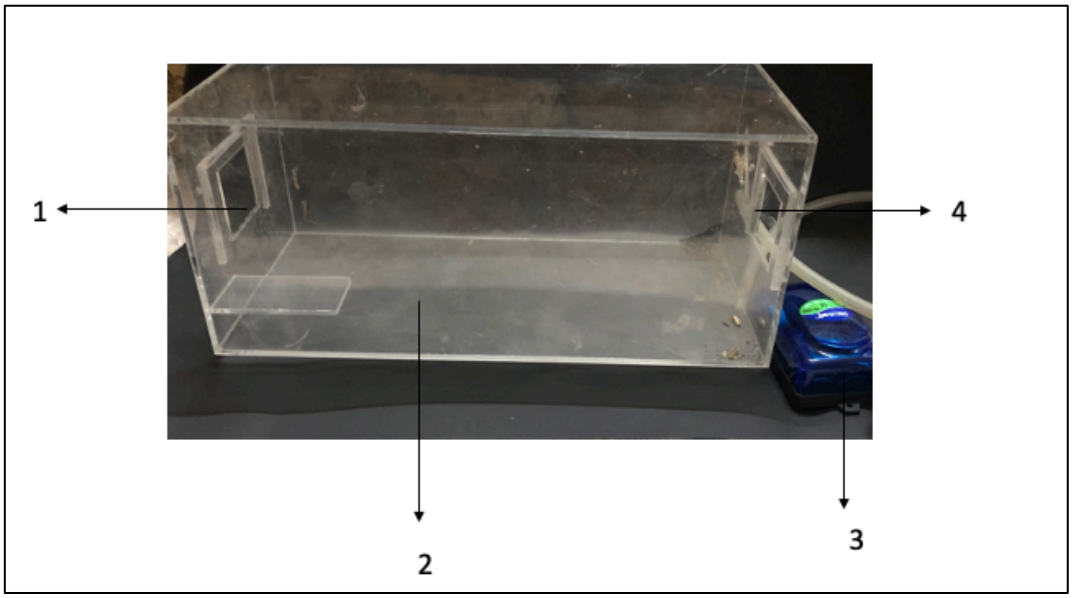


Figure 5.8: Bioassay chamber for assessing the behaviour of *G. mellonella* and *A. grisella* 1) Inlet chamber 2) Observational area 3) Vacuum pump 4) Access window

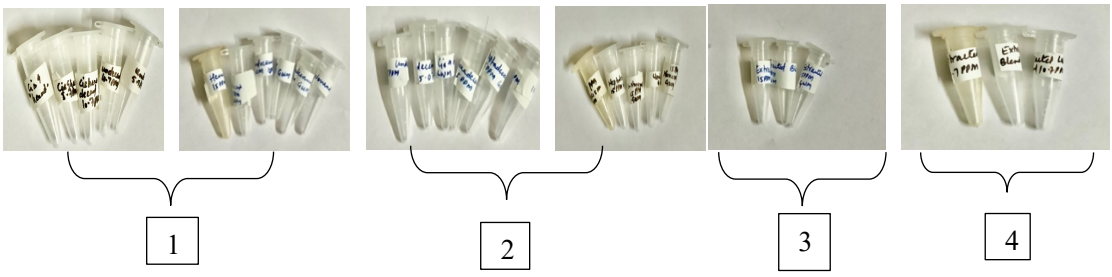


Figure 5.9: Blends for assessing the response of female *G. mellonella* and *A. grisella* 1) *A. grisella* (Synthetic blend) 2) *G. mellonella* (Synthetic blend) 3) *G. mellonella* (Extracted blend) 4) *A. grisella* (Extracted blend)

Table 5.2: Odour Stimuli with synthetic compound blends used in a behavioural bioassay with female *Galleria mellonella* (n=5)

Sr. No.	Compound	Concentrations of different compounds in preparation of Synthetic Blend				
		15 ppm	10 ppm	5 ppm	1 ppm	0.5 ppm
1	Undecanal ($C_{11}H_{22}O$)	7.6	5.1	2.5	0.5	0.25
2	Nonanal ($C_9H_{18}O$)	5.2	3.5	1.7	0.3	0.17
3	1-Nonanol ($C_9H_{20}O$)	1.2	0.8	0.4	0.08	0.04
4	1-Undecanol ($C_{11}H_{24}O$)	0.5	0.3	0.16	0.03	0.017
5	Heptadecane ($C_{17}H_{36}$)	0.2	0.1	0.06	0.01	0.007
6	Heneiocosane ($C_{21}H_{44}$)	0.3	0.2	0.1	0.02	0.01

Table 5.3: Odour Stimuli with synthetic compound blends used in a behavioural bioassay with female *Achroia grisella* (n=5)

Sr. No.	Compound	Concentrations of different compounds in preparation of Synthetic Blend				
		10.7 ppm	5.7 ppm	0.7 ppm	0.2 ppm	0.1ppm
1	Undecanal ($C_{11}H_{22}O$)	8.8	4.7	0.6	0.2	0.08
2	Cis-9-hexadecenal ($C_{16}H_{30}O$)	1.9	1.0	0.1	0.03	0.017

5.7 Protocol for Field Evaluation of Pheromonal Blends

5.7.1 Study Area and Site Selection

Apiaries with *Apis mellifera* were selected based on their proximity to non-managed conditions and the presence of wax moth populations. Three distinct bee farms were chosen for the experiment namely Alwaz Honey Bee Farm, Krishna Bee Farm, and Vicky Bee Farm, all situated in Phillour, Jalandhar. The first installation site was Alwaz Honey Bee Farm, situated at the coordinates 31°17'39.588" N and 75°32'34.98" E. The second site, Krishna Bee Farm located at 3°47'53.52" N and 76°1'51.6" E, and the third site, Vicky Bee Farm was located at 30°16'56.928" N and 76°37'33.78" E (Figure 5.10).

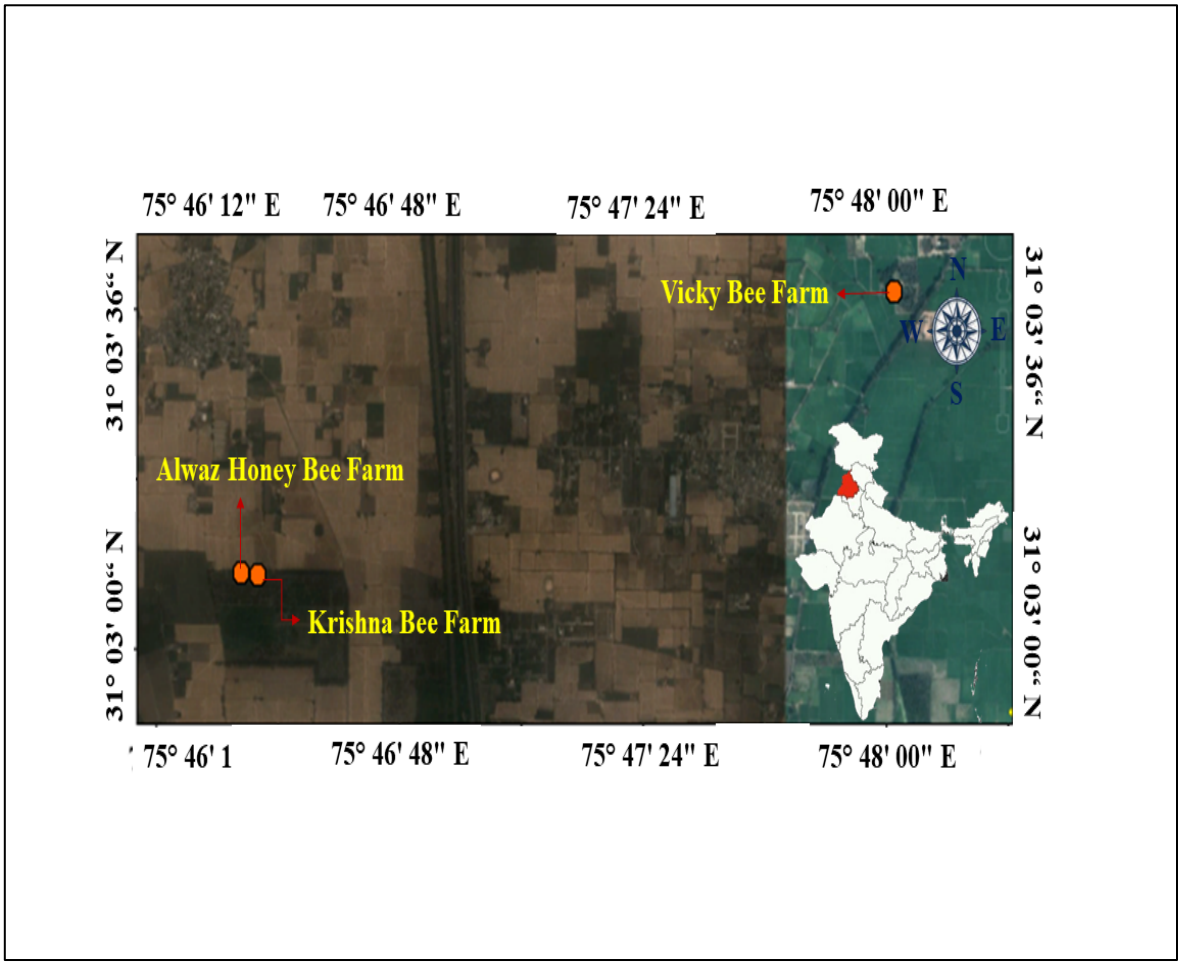


Figure 6.12: Geographical representation of the different bee farms i.e. Alwaz Honey Bee Farm, Krishna Bee Farm and Vicky Bee Farm, Phillour, Jalandhar

5.7.2 Duration of the Experiment

The trapping experiment spanned from July to November, covering late summer to late autumn. Commercially available insect traps and rubber septum were employed in the field conditions in all *Apis mellifera* apiaries, as depicted in Figure 5.11. The experiment was employed in triplicates.

5.8 Trap Design and Installation

The traps comprised a plastic top and with yellow bottom and are commercially available in the market. The upper lid from the bottom to top of the trap has a height of 15 cm and diameter 16 cm. The lower lid of the trap has a diameter of larger circular base of 16 cm. The height is 8 cm with the central opening has a diameter of 5 cm. There is a central point at the top with 1 cm height for hanging. The rubber septum has height of 2 cm, base diameter 0.7 cm and top diameter 0.2 cm respectively (Figure 5.11). Pheromone concentrate sources synthetic as well as extracted were impregnated at different concentrations (15 ppm to 100 ppm) diluted in hexane, loaded per rubber septa among commercially available rubber septa dispensers from Sigma-Aldrich. They were suspended by strings. Pheromone lures were hung inside the top plastic lid, positioned 5 cm from the lid. Traps were baited with *G. mellonella* and *A. grisella* male sex pheromone lures that attract only females. The lures were held in the centre of the traps by pheromone holders and hung inside the top plastic lid.

Thirteen traps for *G. mellonella* and thirteen traps for *A. grisella* were strategically installed in each of the three selected bee farms. Pheromone traps were installed 50 cm above the ground near the bee hives with the distance of 2-3 m apart from each other, and all traps were re-randomized. Treatments were triplicated. Extracted and Synthetic blend of pheromones were employed containing the sex pheromones of male, *G. mellonella* and sex pheromones of male *A. grisella* adult moth.

5.9 Pheromone Doses and Experimental Replication

The dosages of both extracted and synthetic pheromones were applied to assess their impact on trapping effectiveness. The dose ranges from 15 ppm to 100 ppm (extracted

and synthetic). Pheromone blend was used in the field along with the control hexane (15 ppm) for *G. mellonella* and *A. grisella* species. The hexane was used per ppm of the blend concentration in the experiment (Fu et al., 2022). The experiment included three replications in each of the three bee farms *i.e.* Alwaz Honey Bee Farm, Krishna Bee Farm, and Vicky Bee Farm.

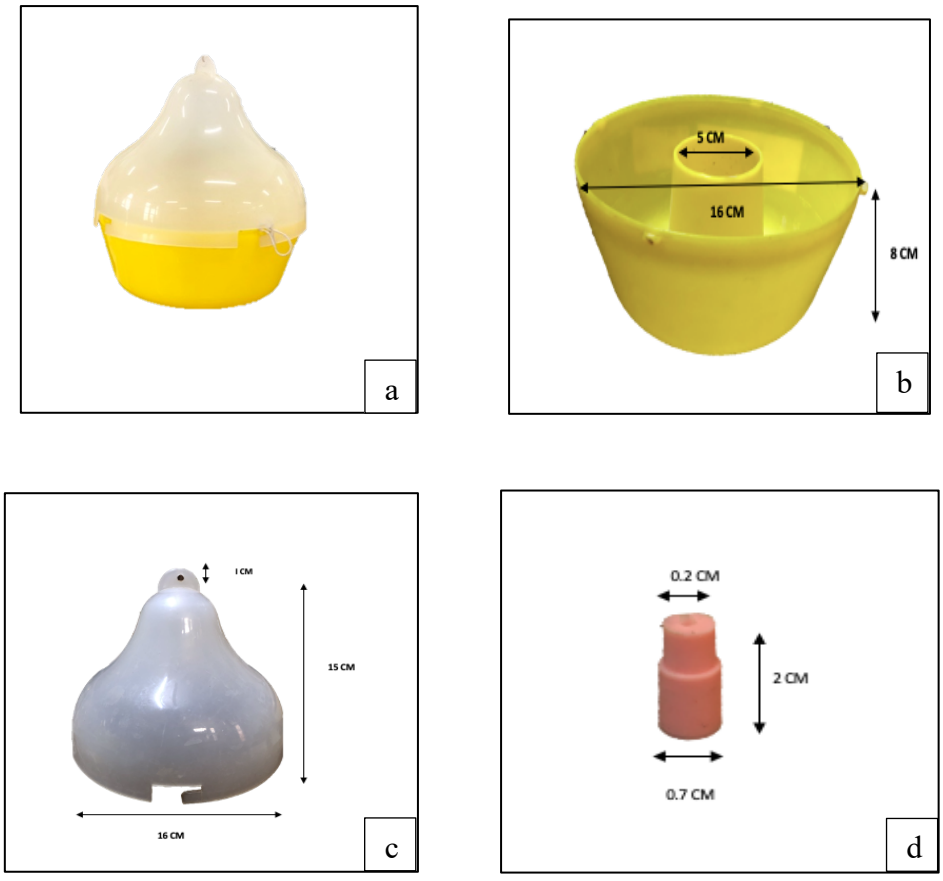


Figure 5.11: Image depicting pheromonal trap and rubber septa a) Pheromonal trap intact b) Lower lid of the trap c) Upper lid of the trap d) Rubber septa

5.10 Trap Monitoring and Data Collection

Data on wax moth population levels and the effectiveness of different pheromones blends were collected through regular monitoring of the installed traps. Adults in traps were counted every two days, after which water in the bottoms was replaced, and the reservoirs in the traps were refilled (Figure 5.12).

The pheromone lures in all the traps were replaced every 2 days in the case of synthetic and extracted blends to minimize possible temporal variance in the concentration of the pheromone plume. Traps were baited with blends of synthetic and extracted pheromones on rubber septum 0.2 x 2 x 0.7 cm before 0800 hours. After 2 days, female moths trapped in the traps were counted, and discarded. Pheromone traps were installed 50 cm above the ground near the hives. All traps were rerandomized. Treatments were triplicated.

Figure 5.12: Installed pheromone traps in different bee farms a) Vicky Bee Farm b) Krishna Bee Farm c) Alwaz Honey Bee Farm d) Vicky Bee Farm

Data Analysis

The statistical program Statistix 10 was utilized to perform a factorial analysis of variance (ANOVA) on the data. Tukey's adjustment for multiple comparisons was used to compare the means. A significance level of $P<0.05$ was deemed statistically significant. The error term was determined using the type III sum of squares. The trials were repeated three times, and the results were presented as the average value plus or minus the standard error. The results were considered statistically significant if the p-values were 0.05 or lower.

CHAPTER 6

RESULTS AND DISCUSSION

The pheromones of *Galleria mellonella* (*G. mellonella*) and *Achroia grisella* (*A. grisella*) were examined and identified qualitatively and quantitatively by Gas Chromatography-Mass Spectrometry (GC-MS) to establish the chemical constituents of the pheromones produced by males. To determine the behavioural bioassays, experiments were carried out in a controlled laboratory condition to measure the response of female moths to the detected pheromones. Moreover, field experiments were also conducted to determine the effectiveness of these pheromones in the natural environment.

Prior to the research study, a comprehensive knowledge of the androconial glands of male *G. mellonella* and *A. grisella* was acquired through the stereomicroscope and Scanning Electron Microscopy (SEM). Androconial glands are studied because of a few significant reasons. These are specialized structures found in male insects and produce and secrete pheromones which are important in mating behaviour as they aid attraction and courtship. Androconial glands produce pheromones that can be species-specific, allowing precise species-specific interactions and behaviours to be identified. In pest management, the study of these glands may yield pheromones that can be used in traps or integrated pest management (IPM) plans, attracting the pests, decreasing their numbers and the damage they cause to the economy.

6.1 Stereomicroscopic Wing Morphometry

The androconial gland (Figure 6.1) on the underside of the meso-wing is bulb-shaped and is present in both the greater wax moth (*G. mellonella*) and the lesser wax moth (*A. grisella*) (Smith, 1965). The body of the male *G. mellonella* measures 10 mm in length, with forewings measuring 18 mm in length, 5 mm in width and 0.07 mm in thickness whereas the body of the male *A. grisella* measures 8 mm in length, with forewings measuring 15 mm in length, 3 mm in width and 0.03 mm in thickness. The hind wings are smaller than the forewings in both *G. mellonella* and *A. grisella* (Figure 6.2).

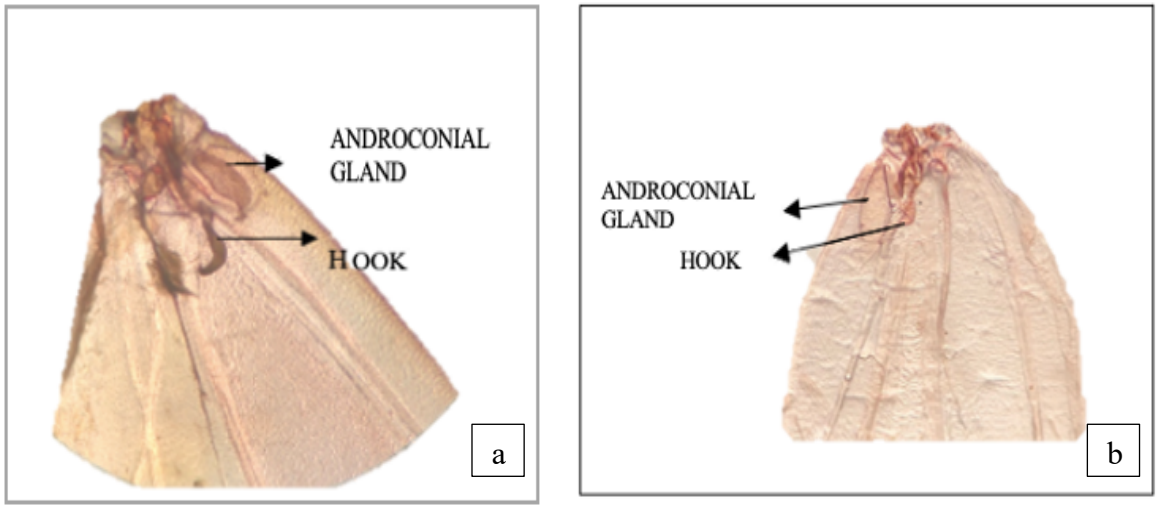


Figure 6.1: Androconial gland on the lower proximal side of forewing with the hook in male under stereomicroscope (Stereo-Zoom) at 18X magnification a) *G. mellonella* b) *A. grisella*

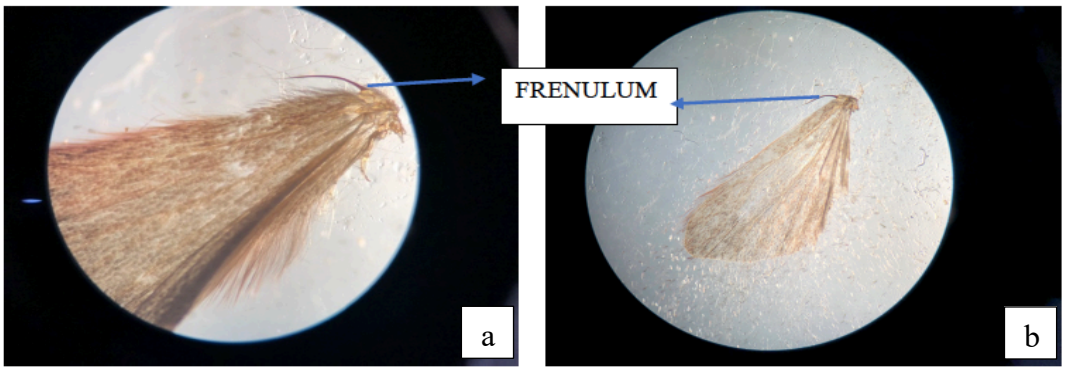


Figure 6.2: Metawing of male under stereomicroscope (Stereo-Zoom) at 18X magnification a) *G. mellonella* showing Frenulum b) *A. grisella* showing Frenulum

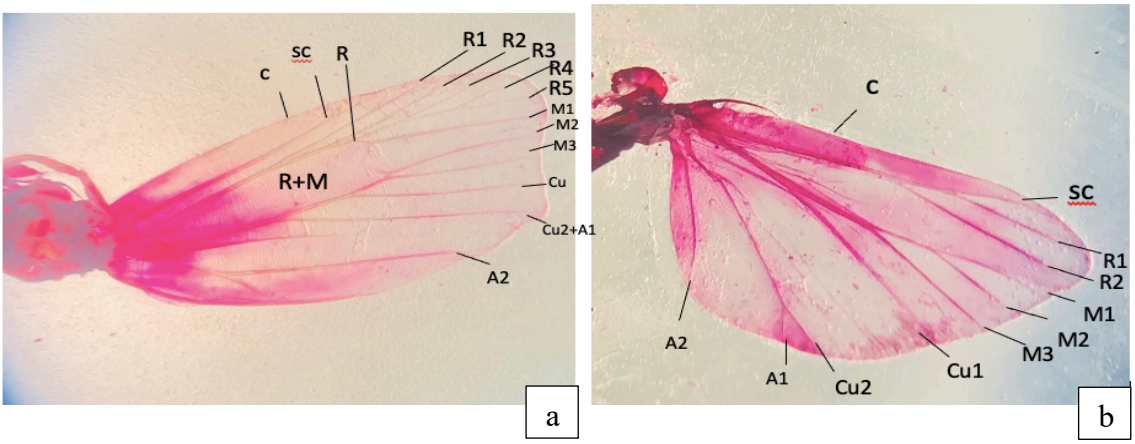


Figure 6.3 Metawing venation of *G. mellonella* under stereomicroscope (Stereo-Zoom) at 18X magnification a) Mesowing venation of male *G. mellonella* b) Metawing venation of male *G. mellonella*

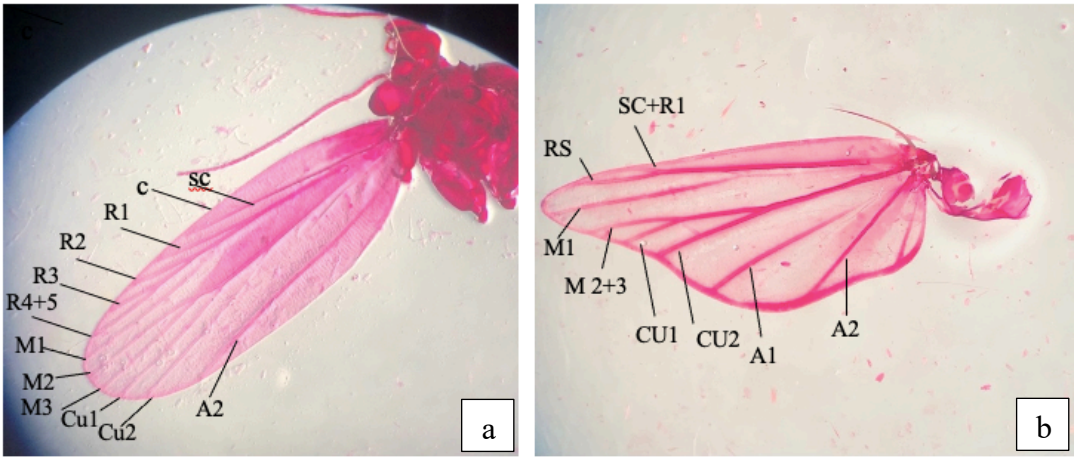


Figure 6.4 a) Mesowing venation of *G. mellonella* under stereomicroscope (Stereo-Zoom) at 18X magnification Mesowing venation of male *A. grisella* b) Metawing venation of male *A. grisella*

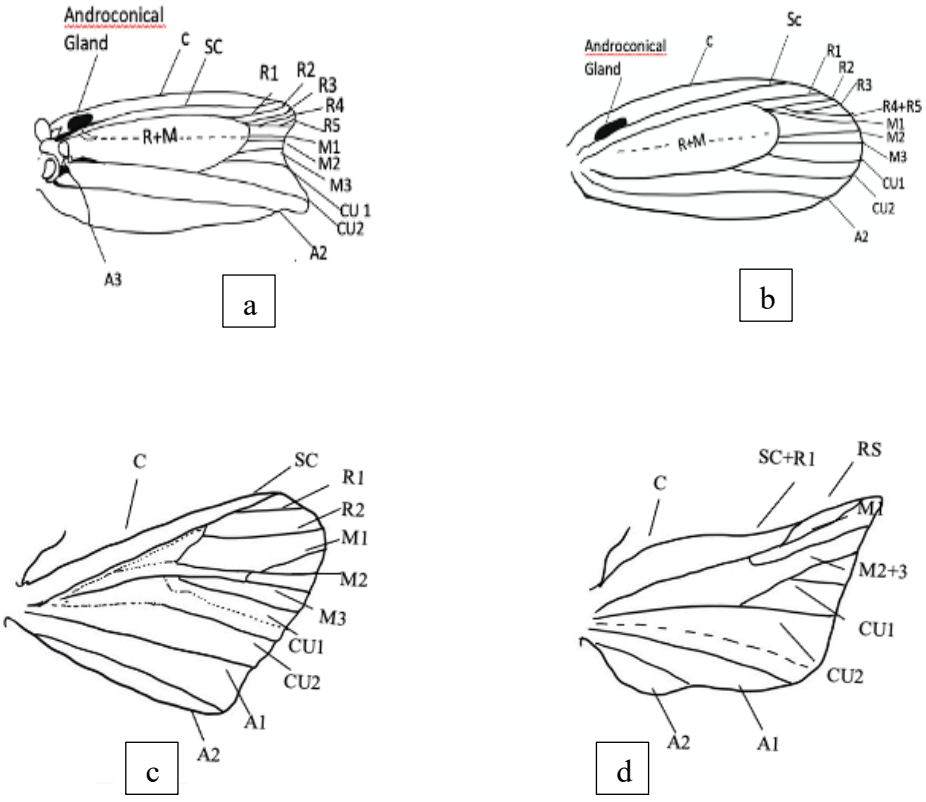


Figure 6.5: a) Mesowing venation and androconial gland of male, *G. mellonella* b) Mesowing venation of male *A. grisella* c) Metawing venation of male *G. mellonella* d) Metawing venation of male *A. grisella*

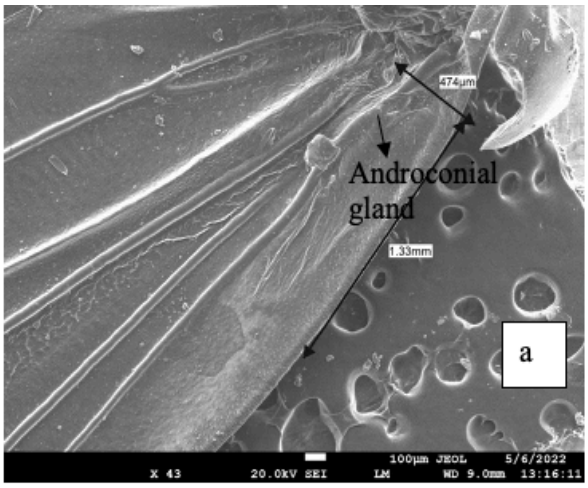
The hindwing of *G. mellonella* measures 9 mm in length, 8 mm in width and 0.02 mm in thickness whereas the hindwing of *A. grisella* measures 5 mm in length, 4 mm in width and 0.01 mm in thickness. Furthermore, the basal portion of the hind wings is folded and much hairier than the forewing and has a long, hair-like structure known as the frenulum. There are two marginal veins, which are referred to as the costal vein and the anal vein. The sub-coastal region is immediately adjacent to the coastal region, the leading marginal vein (Figure 6.5). Costal and Sub costal do not branch off into any other veins. The central stem R+M gradually moves into the middle position and splits off into the branches R and M. All the radius branches are subdivided into separate ones, namely R1, R2, R3, R4, and R5. On the other hand, M is composed of subunits designated as M1, M2, and M3. (It has been determined that any other structures on the wing membrane resembling veins are indentations). The cubitus is a longitudinal vein near the base of the wing that forms two principal branches, Cu1 and Cu2. In addition, an anal vein is a phenomenon that has never been seen before. It is an unbranched vein behind the cubitus. There is only a relatively short first branch of the anal vein (A3) and a relatively long A2 (Smith, 1965).

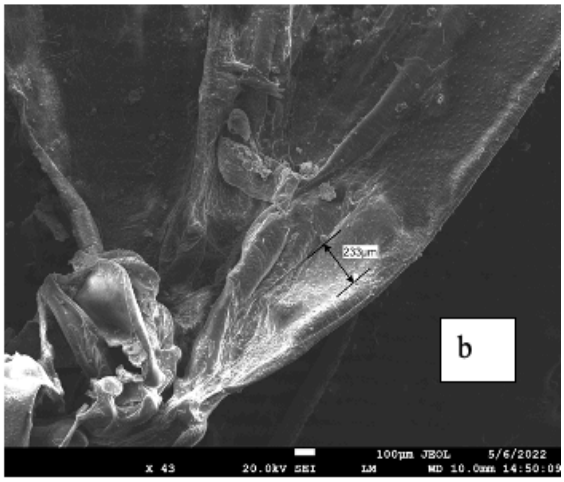
6.2 SEM Analysis of Mesowings

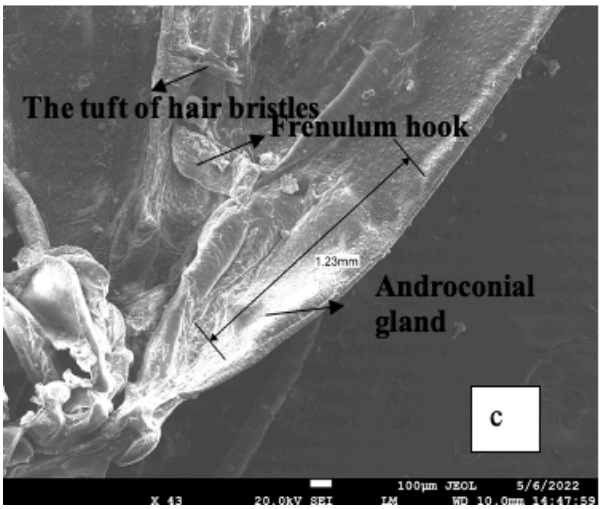
On the notch costal margin of the forewing, a row of hairs is present (Figure 6.6). The pheromone gland in both the Greater Wax Moth and Lesser Wax Moth is bulb-shaped and present on the underside of the meso-wing of the male in the proximal area of the costal cell. The length of the gland of the Greater Wax Moth is 1.33 mm, and the breadth is 4.74 mm. The length of the Lesser Wax Moth is 1.23 mm, and its breadth is 2.33 mm.

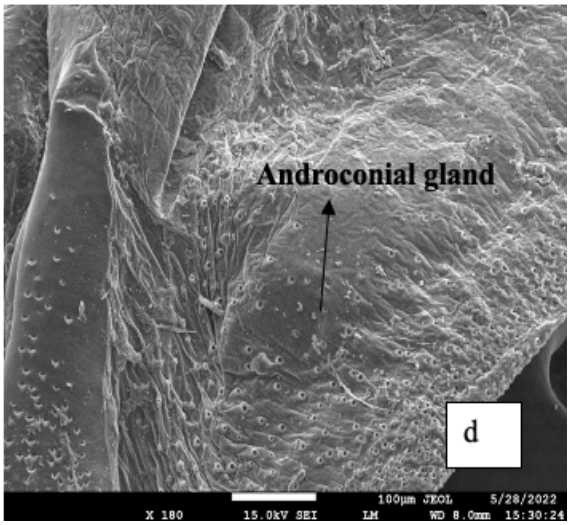
There are a large number of minute pores outside the margin of the gland as compared to those on the gland's surface in both species. The male's hind wing has a frenulum made up of a single prominent spine that develops from the humeral lobe at the humeral angle of the wing. This frenulum is only present on the male's hind wing. It takes up approximately one-third of the total length of the wing's costal surface length. The coupling device known as the frenulum hook or the retinaculum is attached to the forewing and helps to maintain the wings together when the insect is in flight.

They are kept in place by the jugal bristles, hair-like projections that grow from the jugal lobe of the forewing. It can be found closer to the body, towards the end of the cubital vein. The male also has a tuft of bristles on the underside of the mesowing. There are numerous hairy structures on the wings, just like everywhere else on the insect's body. Some are sensory hairs, but most are spines or microtrichia that do not contain nerve endings. Most touch-sensitive sensory hairs are found at the edges of the wings (Vogel, 1911; Yoshida & Emoto, 2010). Other hair sensilla are specialized to encode airborne vibrations and are likely involved in the frequency stability of wing beats.









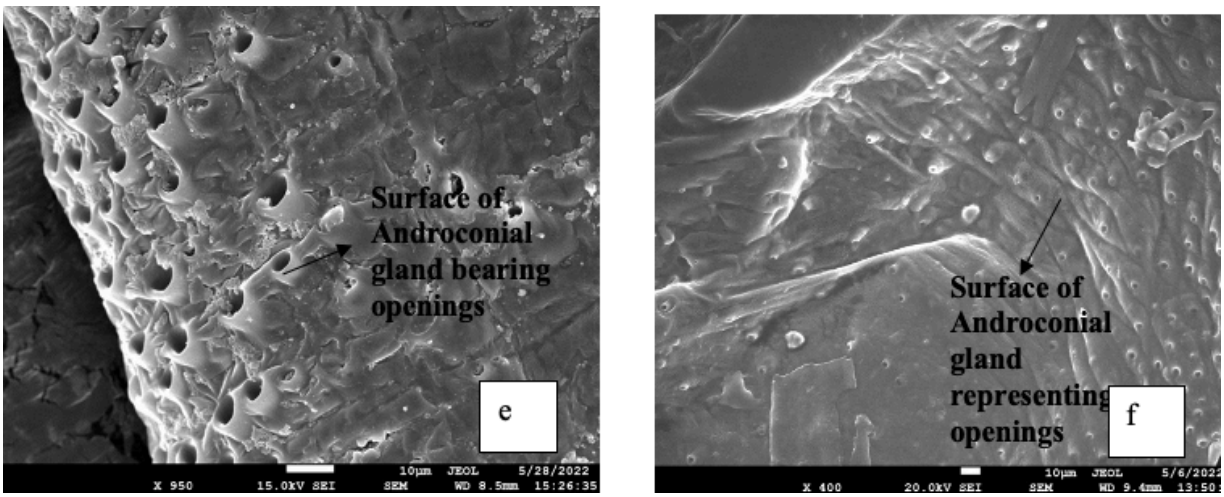
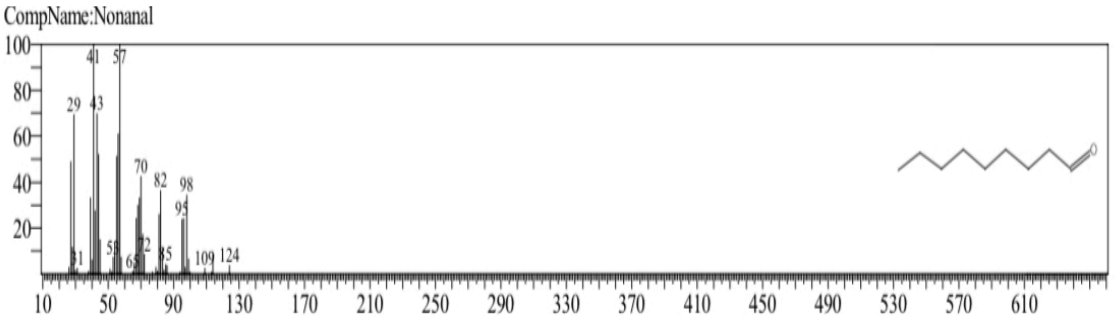


Figure 6.6: Scanning Electron Microscope (SEM) images of the mesowing of *G. mellonella* and *A. grisella* a) Gland representation in *G. mellonella* b-c) Gland in *A. grisella* d-e) Androconial gland surface of *G. mellonella* f) Androconial gland surface of *A. grisella*

6.3 Qualitative Analysis

Male *G. mellonella* and *A. grisella* samples were analyzed using a Shimadzu-TQ8040 gas chromatograph with a 30 x 0.25 x 0.25 m capillary column. The carrier gas was helium, and the temperature of the injector was 240°C. The oven temperature was programmed to increase from 35°C (held for 1 minute) to 230°C at a rate of 10°C per minute. Using mass spectral library data, the compounds were identified as two aldehydes: [nonanal (9.729 min RT), undecanal (12.299 min RT)], alkane: [heptadecane (13.537min RT), heneiocosane (16.053 min (21.785 min RT)], two alcohols,[1- undecanol (13.208 min RT) and 1-nonanol (18.768 min RT)]. Heptadecane, and heneiocosane, are the compounds reported for the first time from *G. mellonella* (Figures 6.7 and 6.8). Aldehydes: undecanal (12.302 min RT) and Cis- 9-Hexadecenal (20.393 min RT) have been identified as volatile compounds of *A. grisella* (Table 6.1 and Table 6.2). Cis-9-hexadecenal has not previously been reported from *A. grisella* in scientific literature.


CompName:Nonanal
100
80
60
40
20
41
57
29
43
70
82
98
95
109
124
10
50
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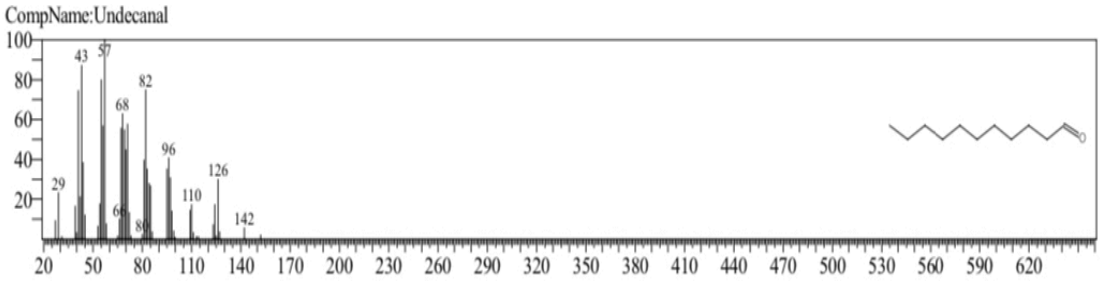
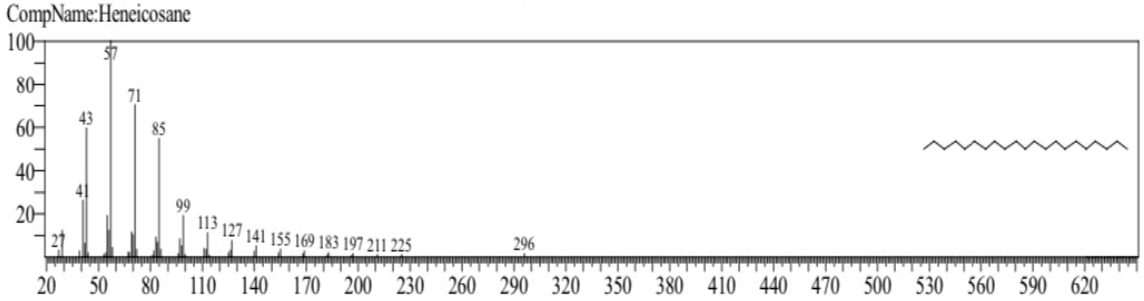
210
250
290
330
370
410
450
490
530
570
610

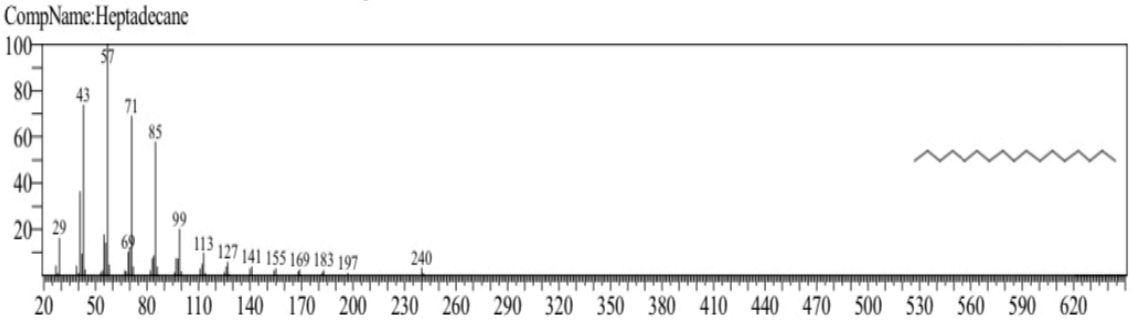
a


CompName:Undecanal
100
80
60
40
20
43
57
82
68
96
126
29
110
142
20
50
80
110
140
170
200
230
260
290
320
350
380
410
440
470
500
530
560
590
620

b


CompName:Heneicosane
100
80
60
40
20
57
71
43
85
41
99
113
127
141
155
169
183
197
211
225
296
27
20
50
80
110
140
170
200
230
260
290
320
350
380
410
440
470
500
530
560
590
620

c


CompName:Heptadecane
100
80
60
40
20
57
43
71
85
29
99
69
113
127
141
155
169
183
197
240
20
50
80
110
140
170
200
230
260
290
320
350
380
410
440
470
500
530
560
590
620

d

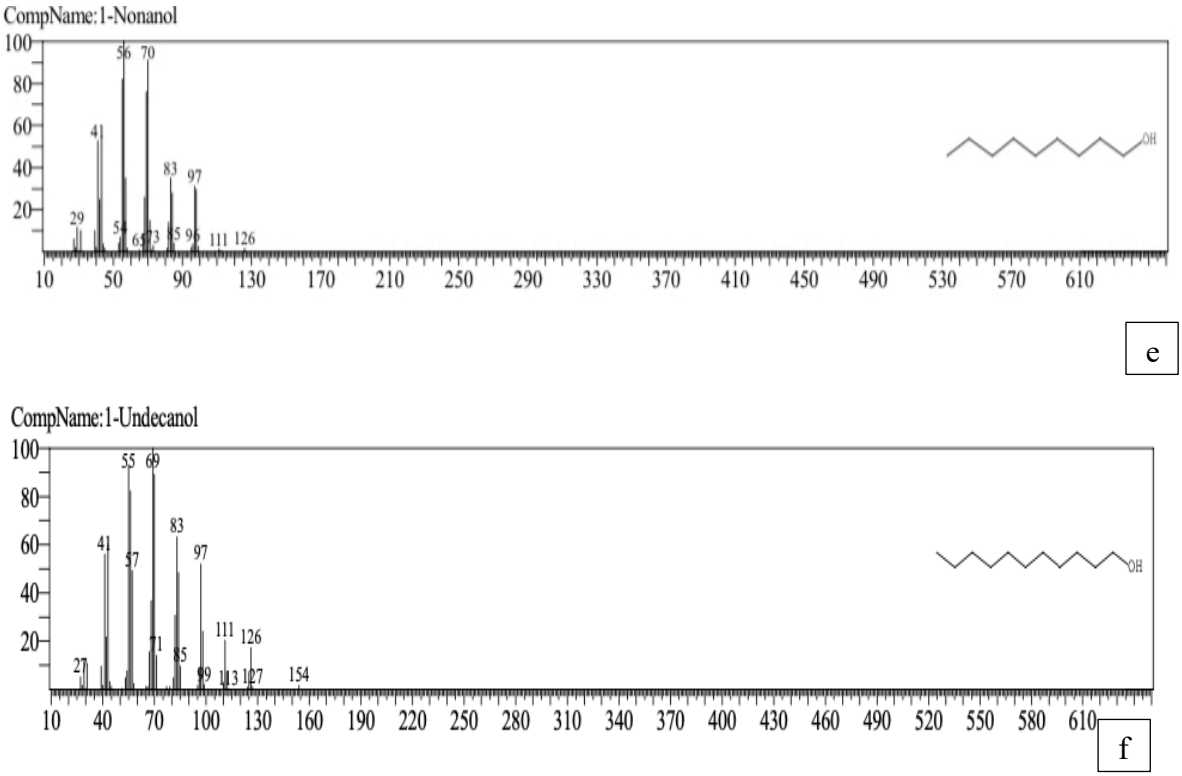


Figure 6.7: Mass spectra of the volatile compounds in male *G. mellonella* a) Nonanal b) Undecanal c) Heneiocosane d) Heptadecane e) 1- Nonanol f) 1- Undecanol

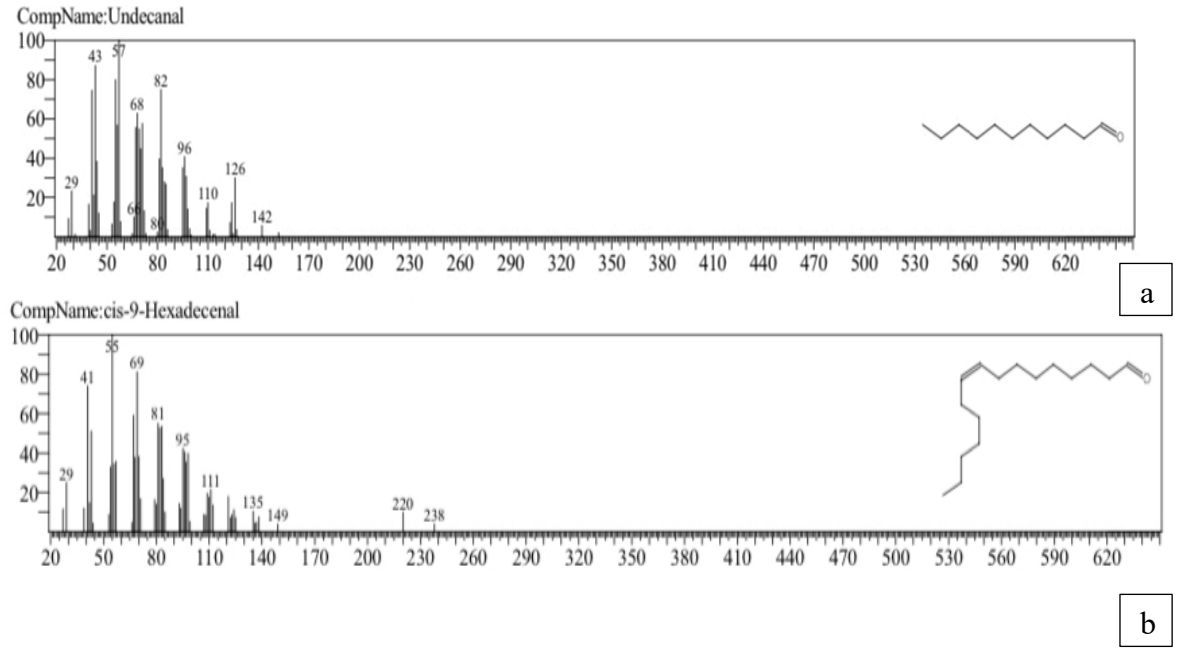


Figure 6.8: Mass spectra of the volatile compounds in male *A. grisella* a) Undecanal b) Cis 9 hexadecenal

Table 6.1:Qualitative analysis of the volatile compounds released by male *G. mellonella*

Sr.No.	Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Retention Time (min)	Nature of the Compound
1	Nonanal	($C_9H_{18}O$)	142.24	0.68	9.279	Fatty Aldehyde
2	1-Nonanol	($C_9H_{20}O$)	144.26	0.28	10.326	Fatty Alcohol
4	Undecanal	($C_{11}H_{22}O$)	170.29	0.67	12.299	Aldehyde
5	1-Undecanol	($C_{11}H_{24}O$)	172.31	0.47	13.208	Fatty Alcohol
6	Heptadecane	($C_{17}H_{36}$)	240.471	0.20	13.537	Hydrocarbon
7	Heneiocosane	($C_{21}H_{44}$)	296.583	0.19	16.053	Hydrocarbon

Table 6.2: Qualitative analysis of the compounds released by male, *A. Grisella*

Sr.No.	Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Retention Time (min)	Nature of the Compound
1	Undecanal	($C_{11}H_{22}O$)	170.29	0.17	12.302	Aldehyde
2	Cis -9 - hexadecenal	($C_{16}H_{30}O$)	238.41	14.52	20.393	Aldehyde

Table 6.3: Quantitative analysis of the volatile compounds released by male *G. mellonella* gland

Sr. No.	Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Retention Time (min)	ppm	Nature of the Compound
1	Undecanal	$C_{11}H_{22}O$	170.29	0.67	12.299	7.162	Aldehyde
2	Nonanal	$C_9H_{18}O$	142.24	0.68	9.279	5.218	Fatty Aldehyde
3	1-Nonanol	$C_9H_{20}O$	144.26	0.28	10.326	1.181	Fatty Alcohol
4	1-Undecanol	$C_{11}H_{24}O$	172.31	0.47	13.208	0.486	Fatty Alcohol
5	Heptadecane	$C_{17}H_{36}$	240.471	0.20	13.537	0.203	Hydrocarbon
6	Heneiocosane	$C_{21}H_{44}$	296.583	0.19	16.053	0.267	Hydrocarbon

Table 6.4: Quantitative analysis of the volatile compounds released by male *A. grisella* gland

Sr. No.	Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Retention Time (min)	ppm	Nature of the Compound
1	Undecanal	$C_{11}H_{22}O$	170.29	0.17	12.302	8.745	Aldehyde
2	Cis-9-Hexadecenal	$C_{16}H_{30}O$	238.41	14.52	20.393	1.819	Aldehyde

Table 6.5: Quantitative analysis result table of standard compounds

Sr.No.	Name	Conc	R. Time	m/z	Area
1.	Nonanal	1 ppm	6.177	57.00	229568
		3 ppm	6.180	57.00	745213
		5 ppm	6.179	57.00	1308573
2.	1-Nonanol	1 ppm	6.914	56.00	29003
		3 ppm	6.907	56.00	427100
		5 ppm	6.905	56.00	914587
		1 ppm	6.937	56.00	954113
3.	Heneiocosane	1 ppm	22.115	57.00	4272089
		3 ppm	22.116	57.00	11369537
		5 ppm	22.118	57.00	1690143
4.	Heptadecane	1 ppm	18.001	57.00	5893316
		3 ppm	18.003	57.00	12721988
		5 ppm	18.003	57.00	17836192
5.	Undecanal	1 ppm	12.252	43.00	1342125
		3 ppm	12.250	43.00	3994000
		5 ppm	12.251	43.00	7636529
6.	Cis- 9 - hexadecenal	1 ppm	19.190	55.00	310471
		2 ppm	19.189	55.00	1019441
		3 ppm	19.189	55.00	1777392

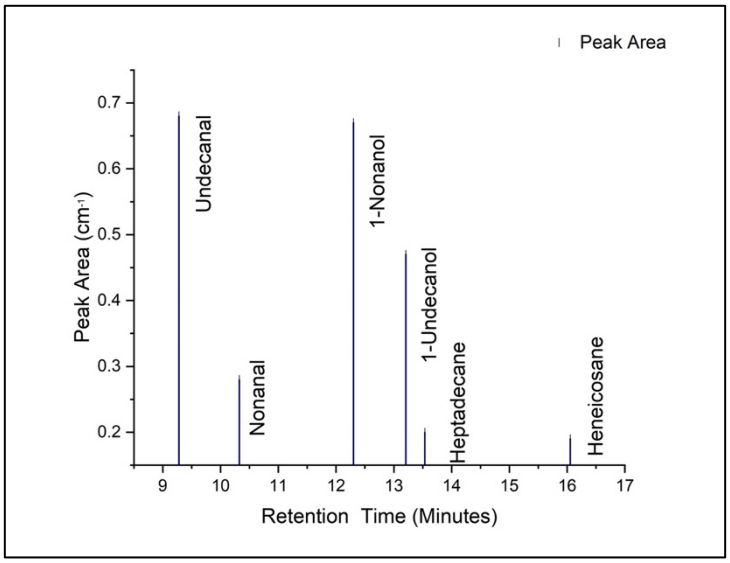


Figure 6.9: Gas chromatography (GC) chromatogram showing the retention times and peak areas of various compounds, including undecanal, nonanal, 1-nonanol, 1-undecanol, heptadecane, and heneicosane. The retention times are represented on the x-axis in minutes, while the corresponding peak areas are plotted on the y-axis in cm^2 in male, *G. mellonella*

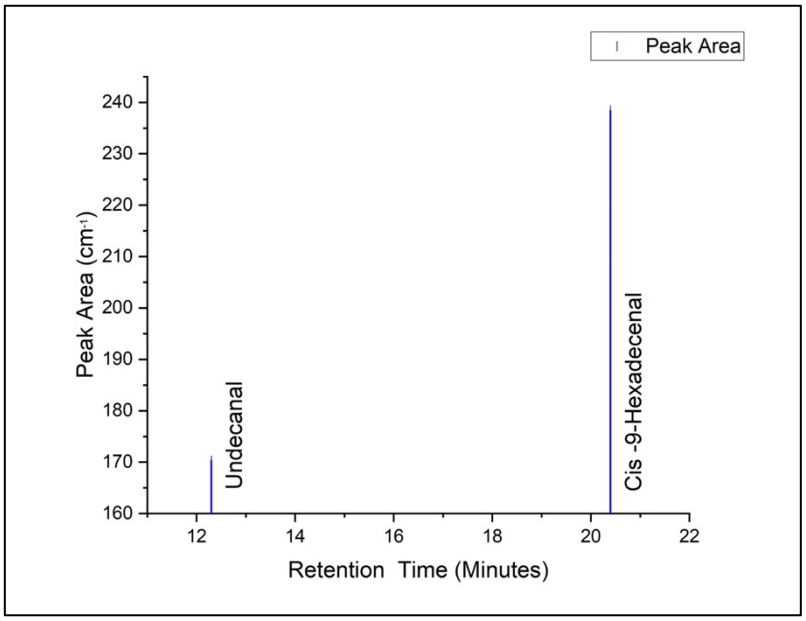
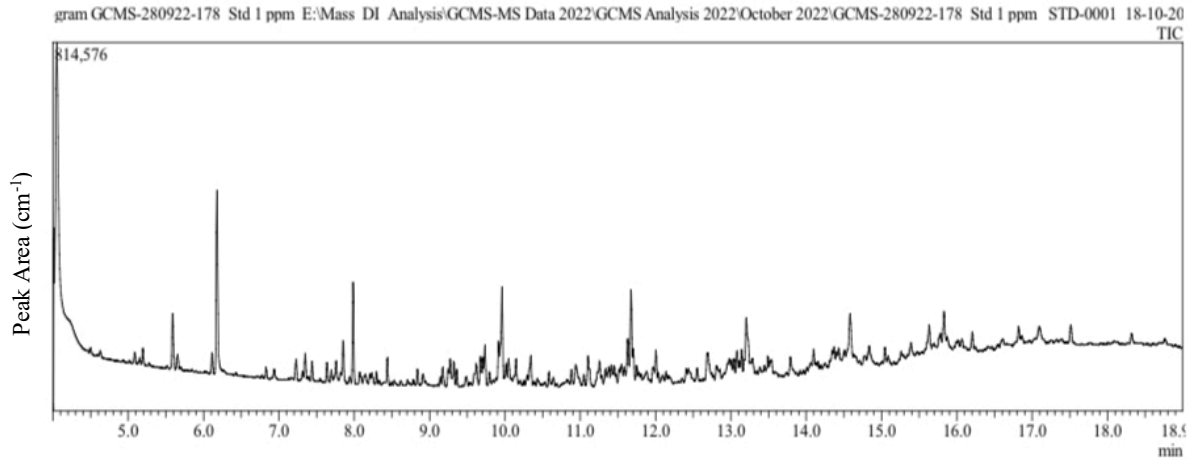
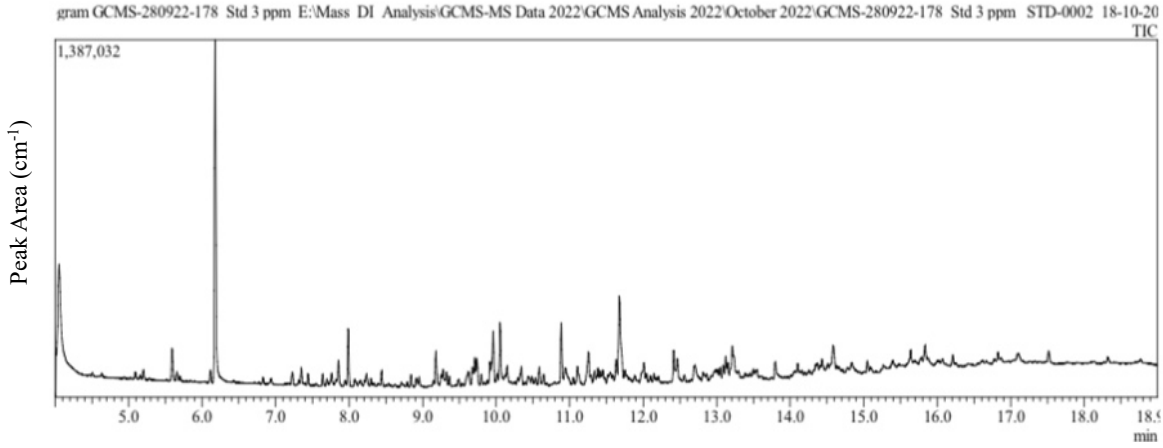


Figure 6.10: The gas chromatography (GC) chromatogram illustrates the retention times and peak sizes of undecanal and cis-9-hexadecenal. The retention times are represented on the x-axis in minutes, while the matching peak areas are displayed on the y-axis in square centimeters in male *Achroia grisella*



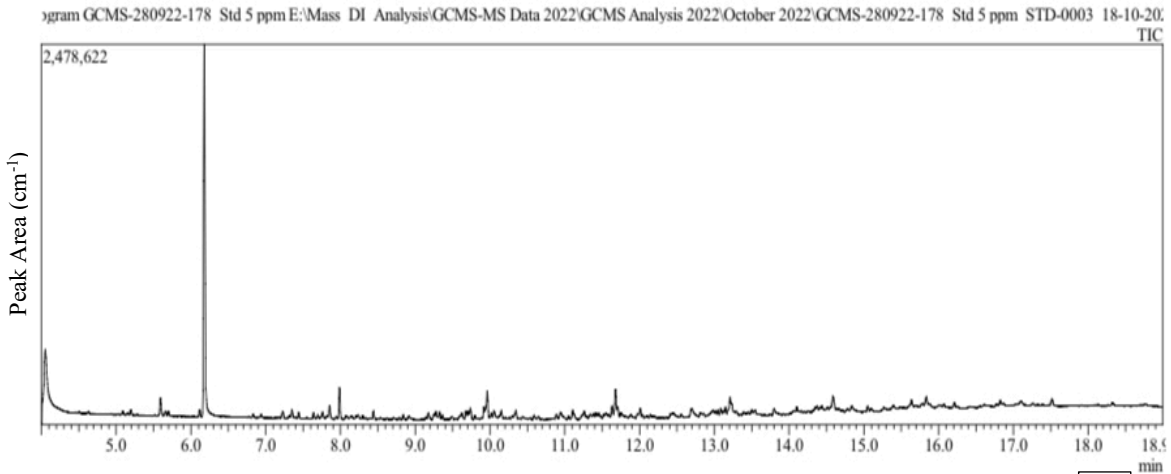
Retention Time (Minutes)

a



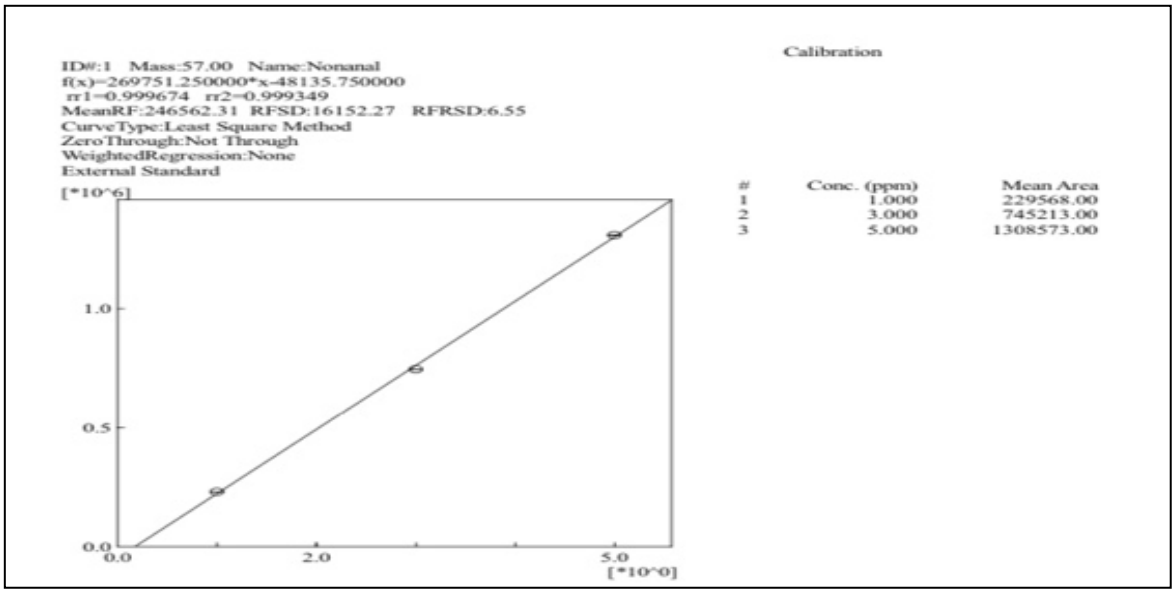
Retention Time (Minutes)

b



Retention Time (Minutes)

c



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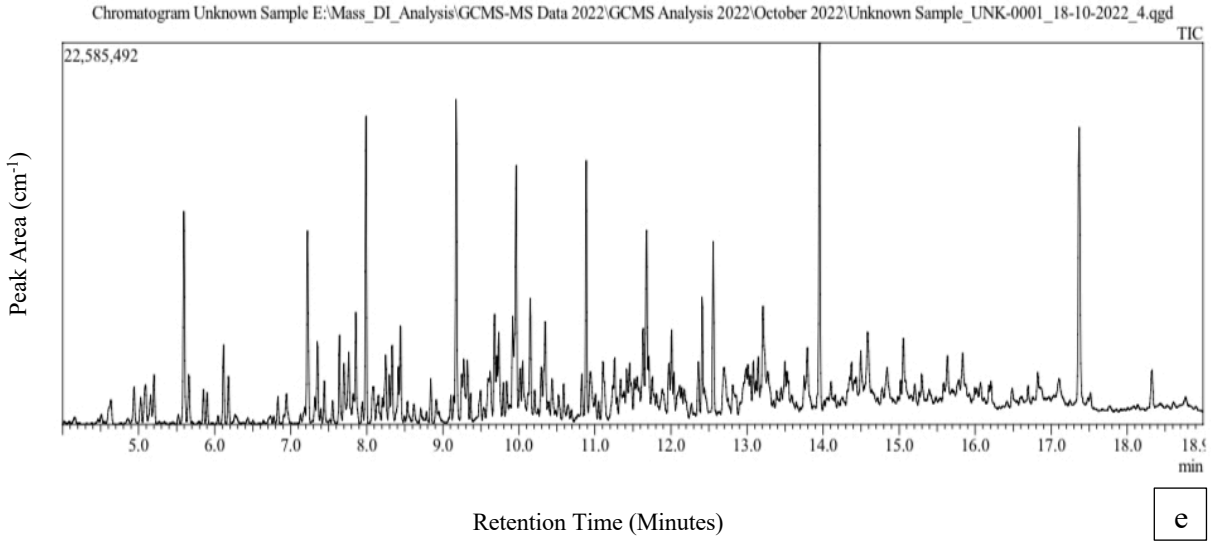
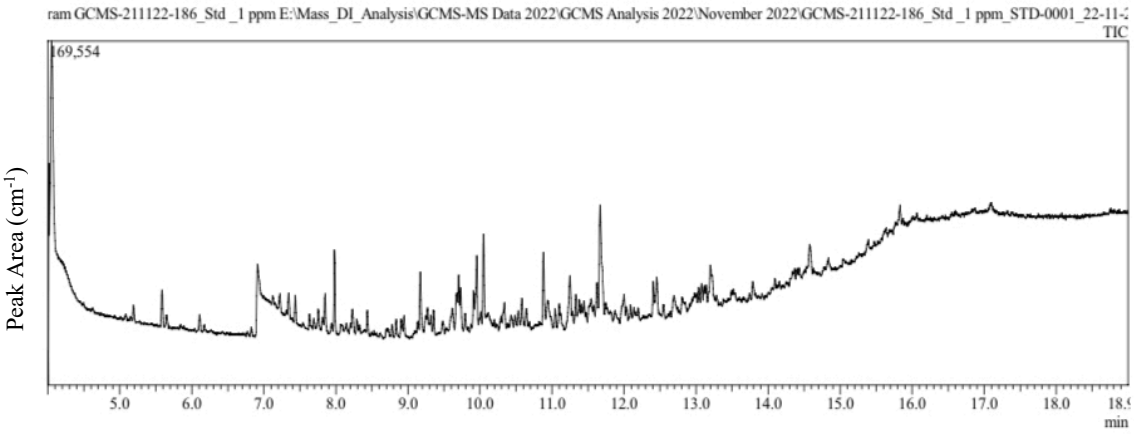


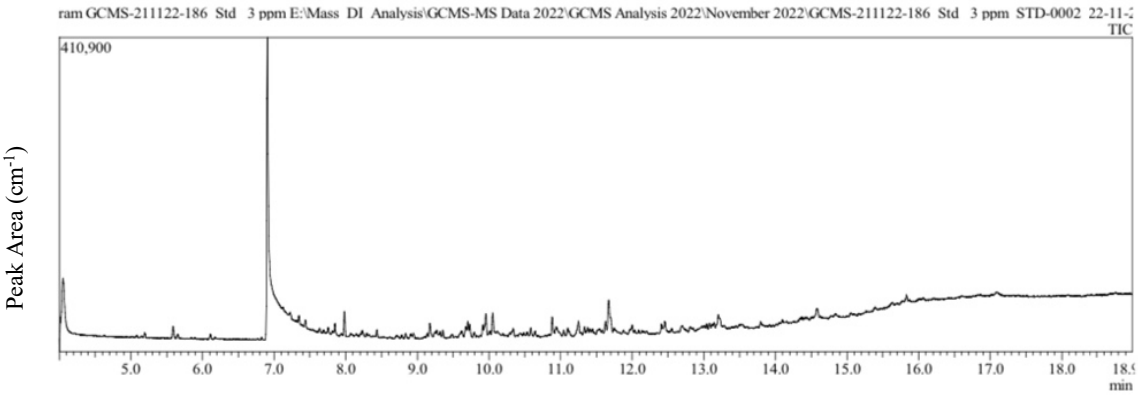
e

Figure 6.11: Quantitative analysis of the compound nonanal (a) TIC Chromatogram of nonanal standard (1ppm) (b) TIC Chromatogram of nonanal standard (3 ppm) (c) TIC Chromatogram of nonanal standard (5 ppm) (d) Calibration curve of nonanal standard (e) TIC Chromatogram of nonanal in male *G. mellonella*



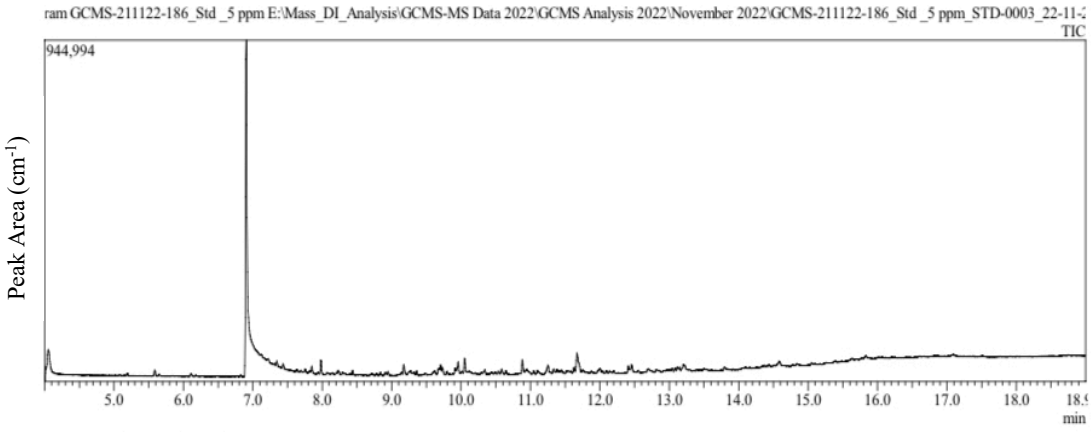
Retention Time (Minutes)

a



Retention Time (Minutes)

b



Retention Time (Minutes)

c

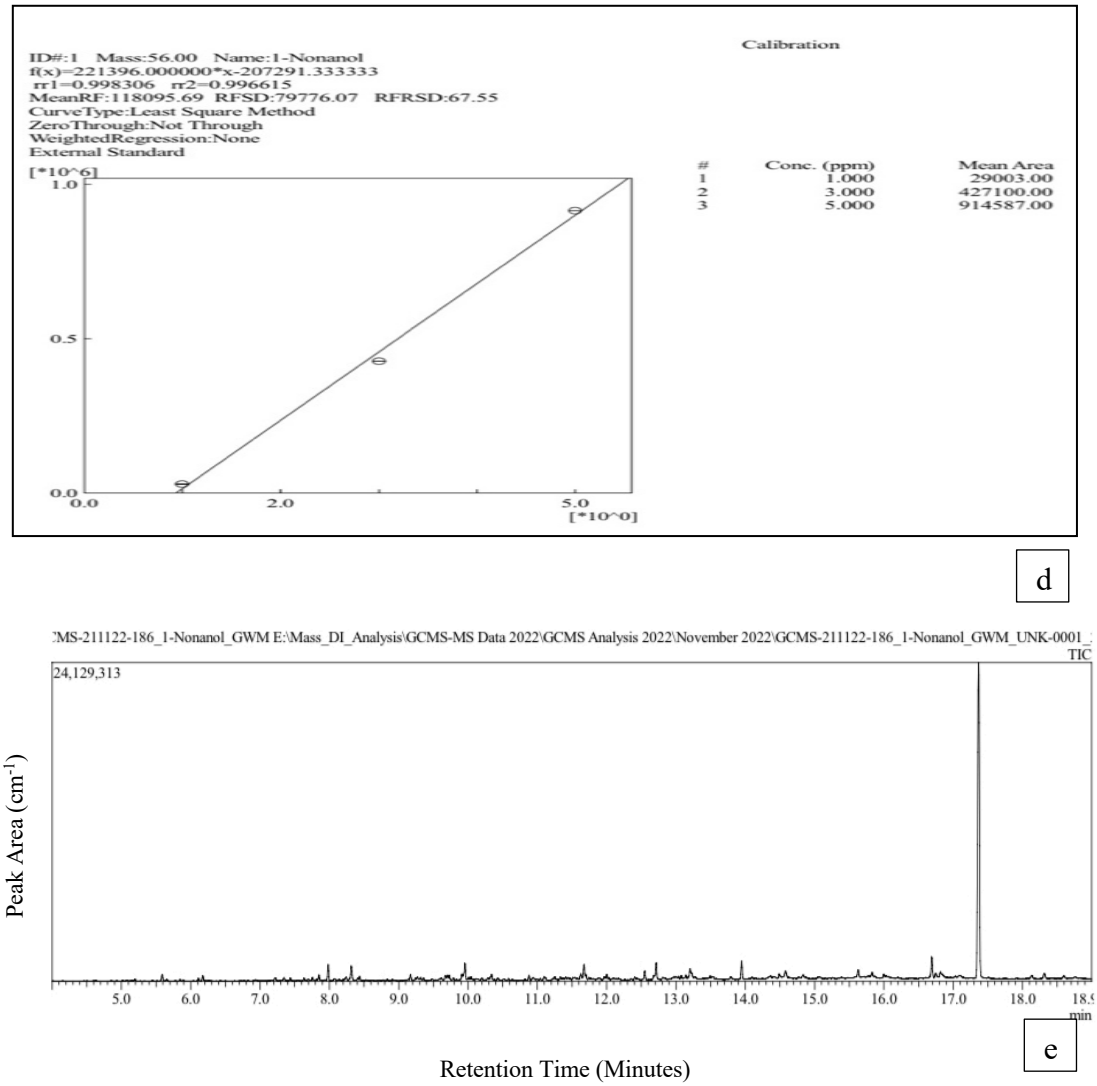
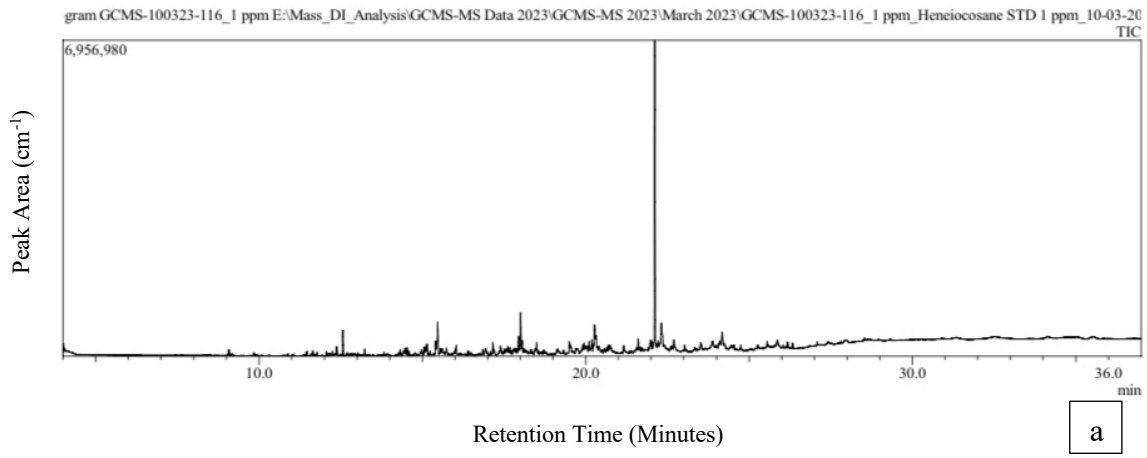


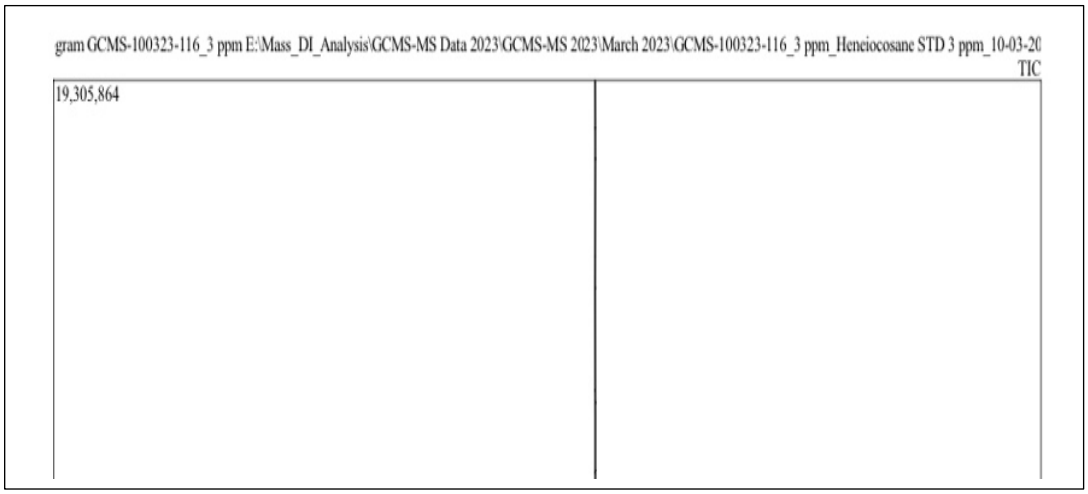
Figure 6.12: Quantitative analysis of the compound 1-nonanol (a) TIC Chromatogram of 1-nonanol standard (1ppm) (b) TIC Chromatogram of 1-nonanol standard (3 ppm) (c) TIC Chromatogram of 1-nonanol standard (5 ppm) (d) Calibration curve of 1- nonanol standard (e) TIC Chromatogram of 1-nonanol in male *G. mellonella*



Peak Area (cm^{-1})

Retention Time (Minutes)

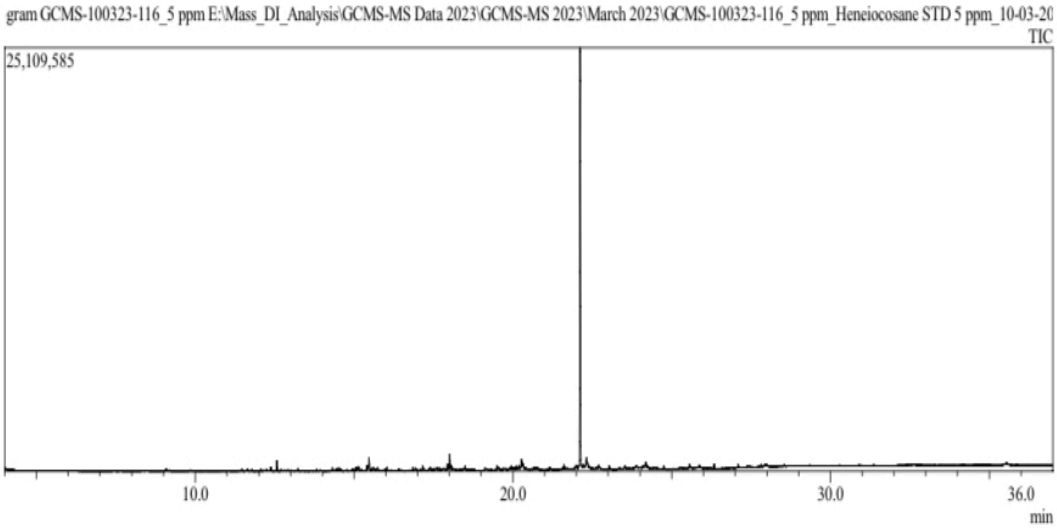
a



Peak Area (cm^{-1})

Retention Time (Minutes)

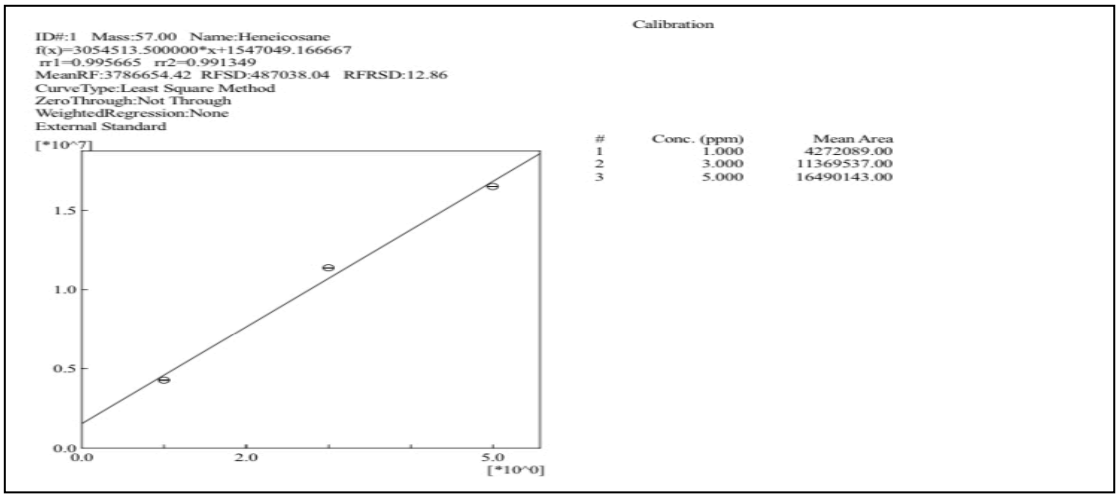
b



Peak Area (cm^{-1})

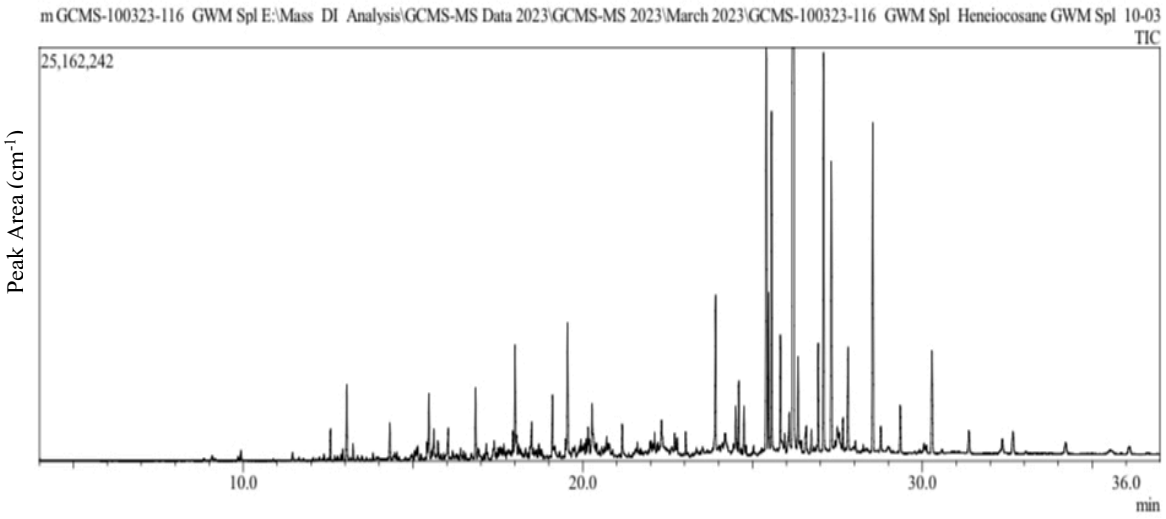
Retention Time (Minutes)

c



Retention Time (Minutes)

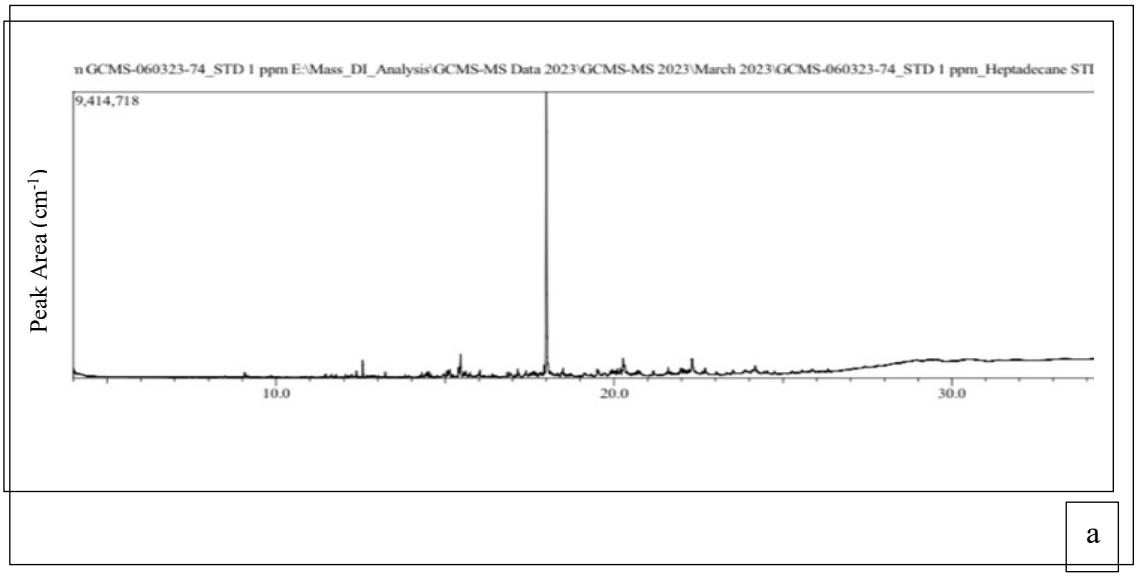
d



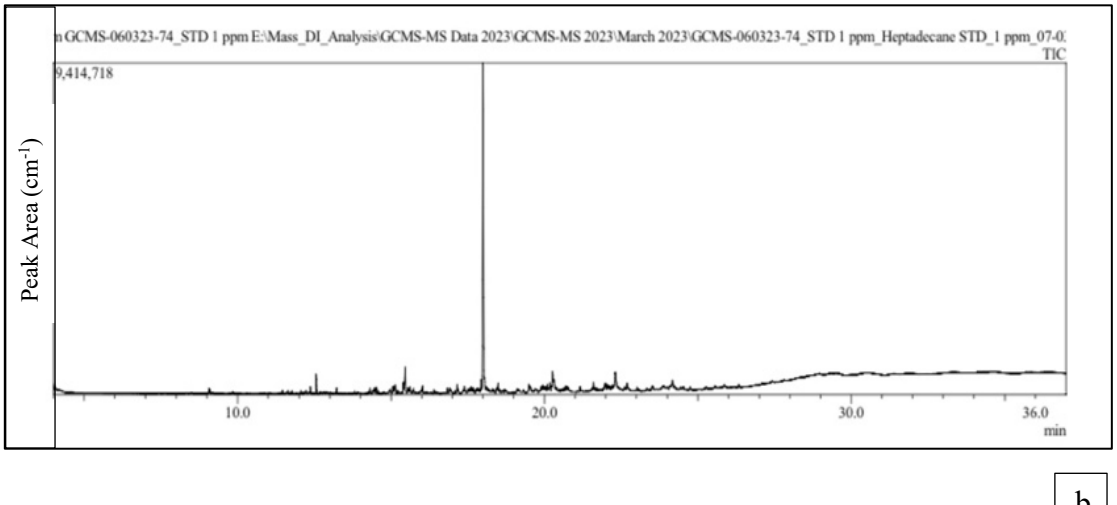
Retention Time (Minutes)

e

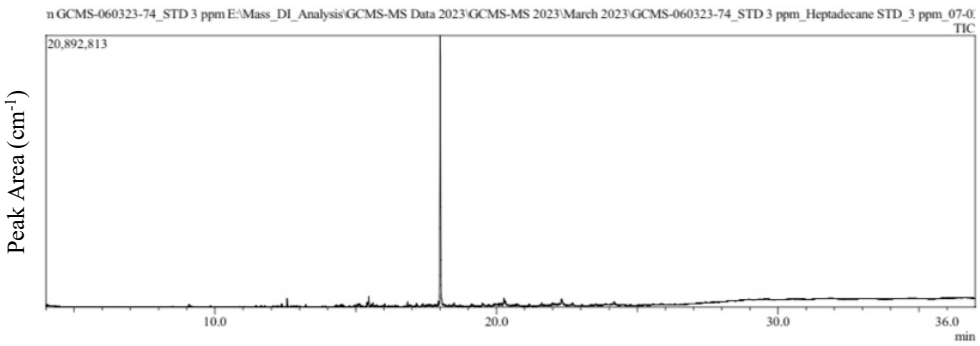
Figure 6.13: Quantitative analysis of the compound heneiocosane (a) TIC Chromatogram of heneiocosane standard (1ppm) (b) TIC Chromatogram of heneiocosane standard (3 ppm) (c) TIC Chromatogram of heneiocosane standard (5 ppm) (d) Calibration curve of heneiocosane standard (e) TIC Chromatogram of heneiocosane in male *G. mellonella*



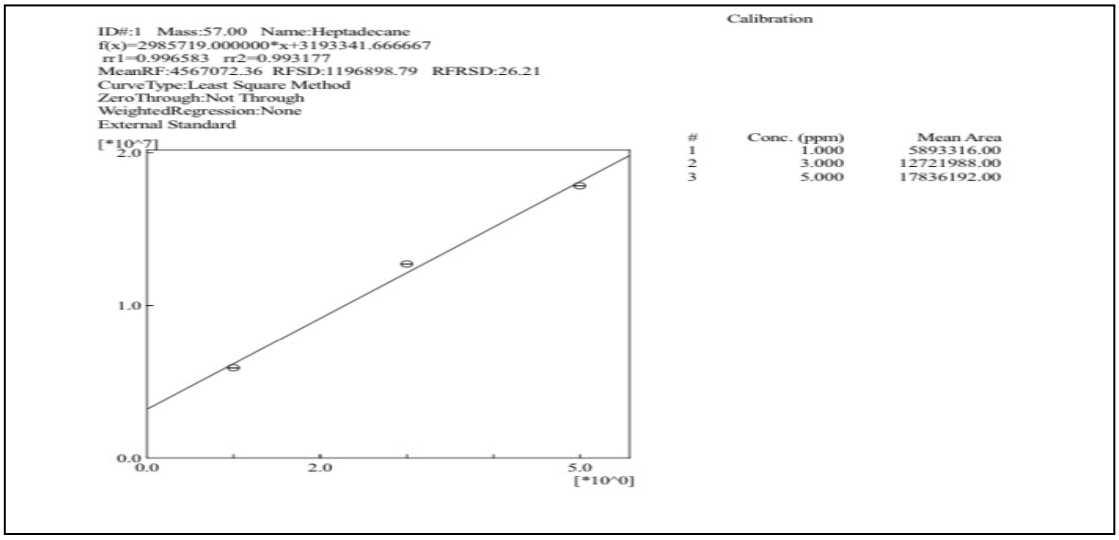
Retention Time (Minutes)



Retention Time (Minutes)



c



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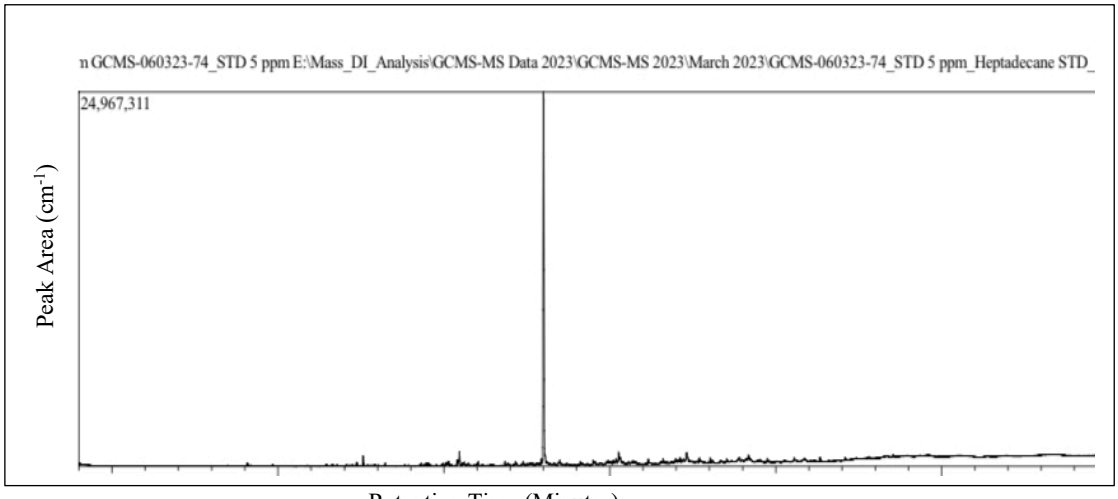
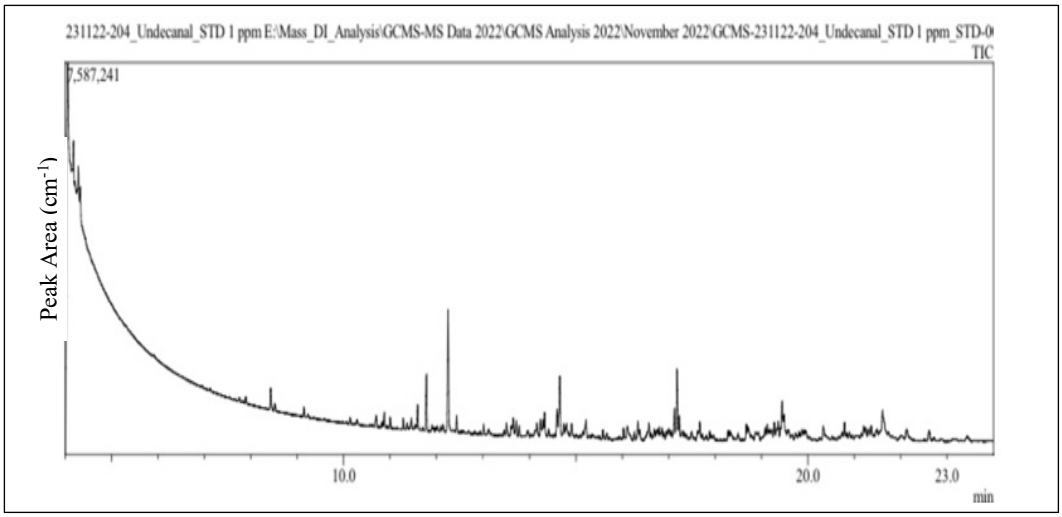


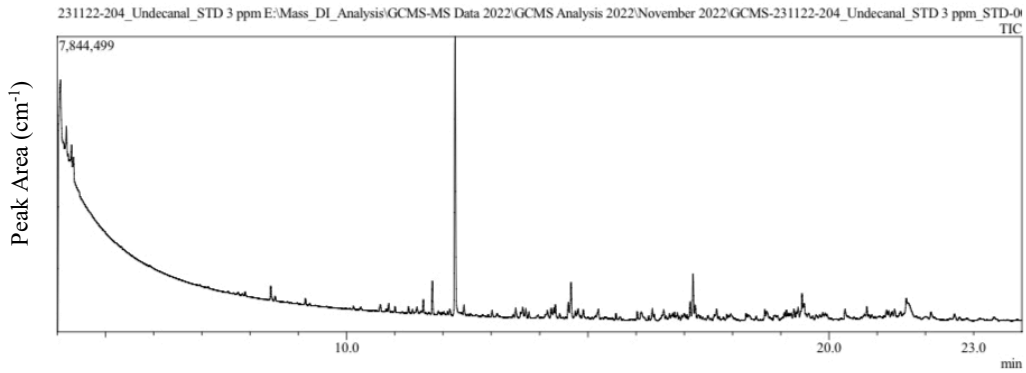
e

Figure 6.14: Quantitative analysis of the compound heptadecane (a): TIC Chromatogram of heptadecane standard (1ppm) (b) TIC Chromatogram of heptadecane standard (3 ppm) (c) TIC Chromatogram of heptadecane standard (5 ppm) (d) Calibration curve of heptadecane standard (e) TIC Chromatogram of heptadecane in male *G. mellonella*



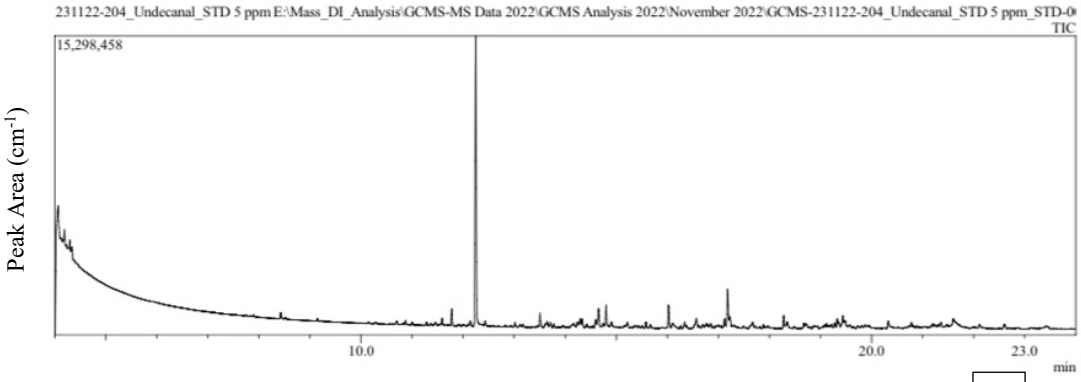
Retention Time (Minutes)

a



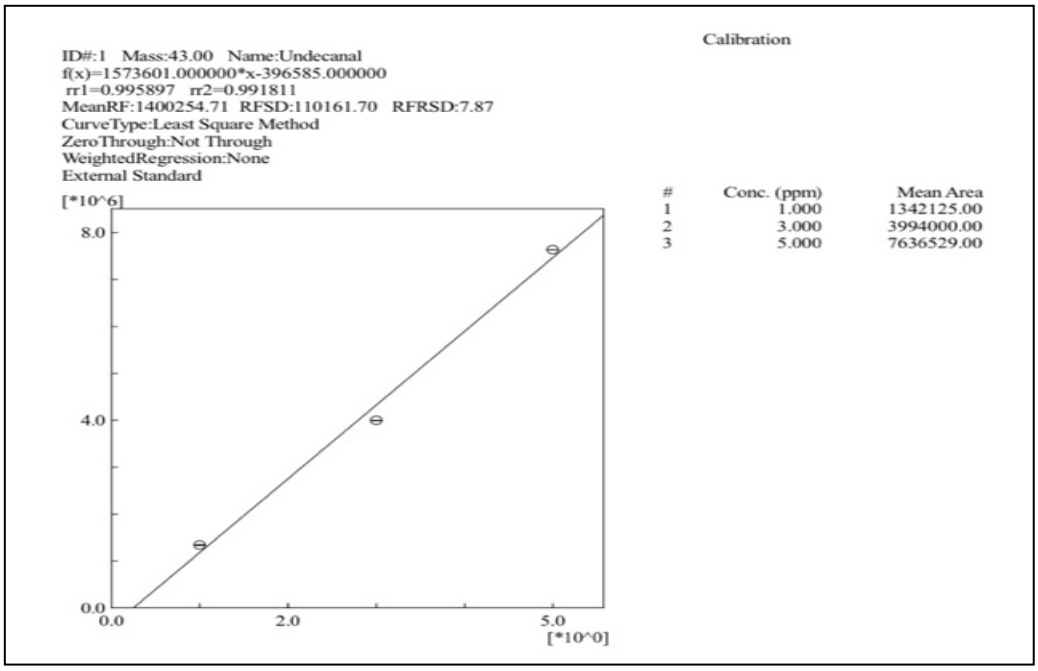
Retention Time (Minutes)

b

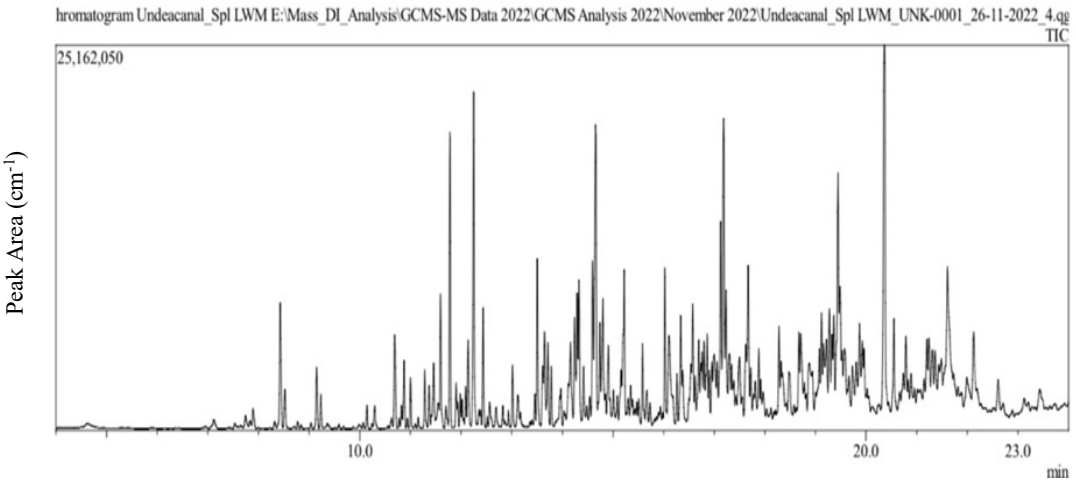


Retention Time (Minutes)

c



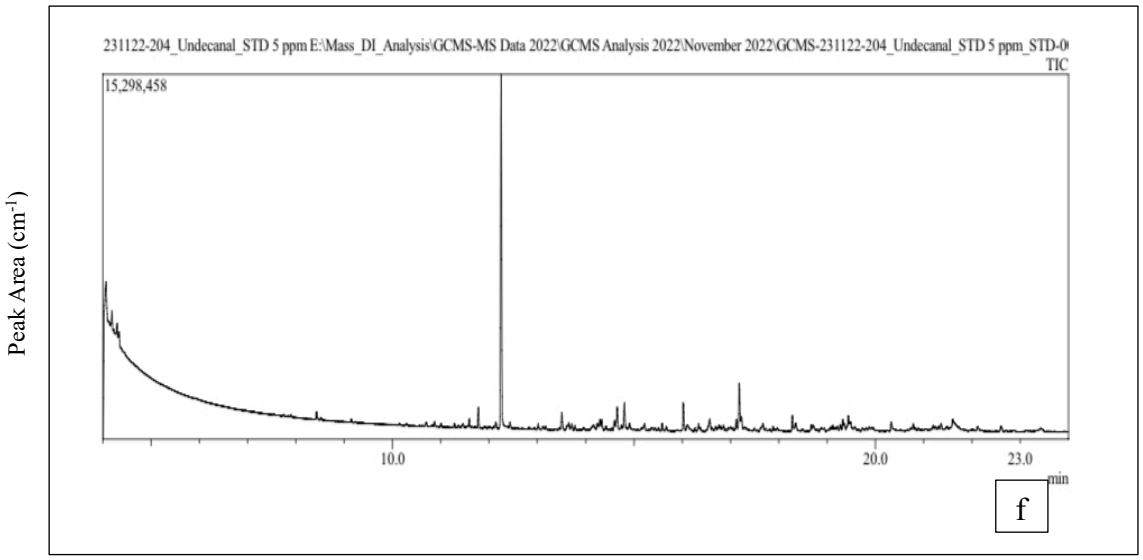
d



Peak Area (cm^{-1})

Retention Time (Minutes)

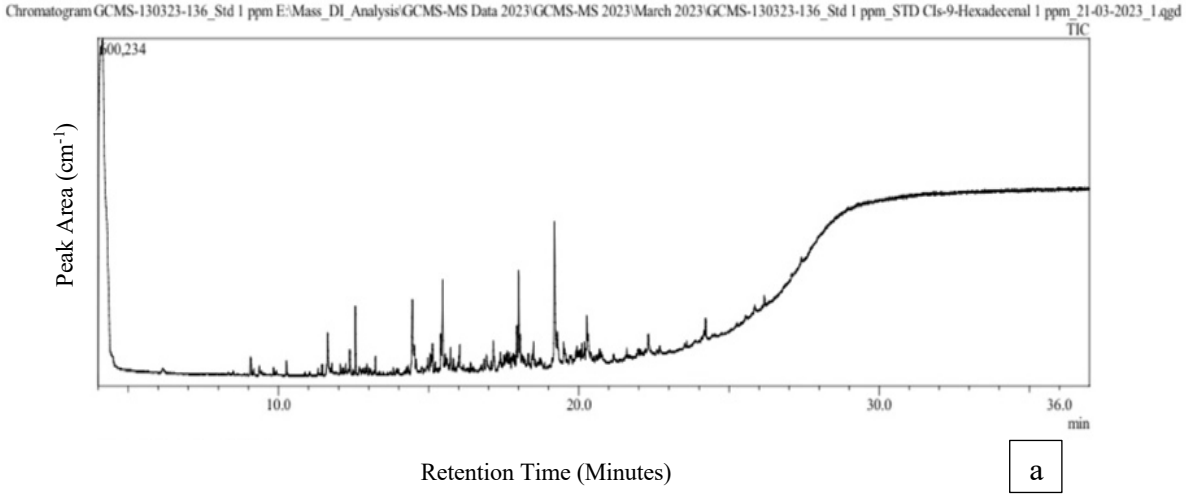
e



Peak Area (cm^{-1})

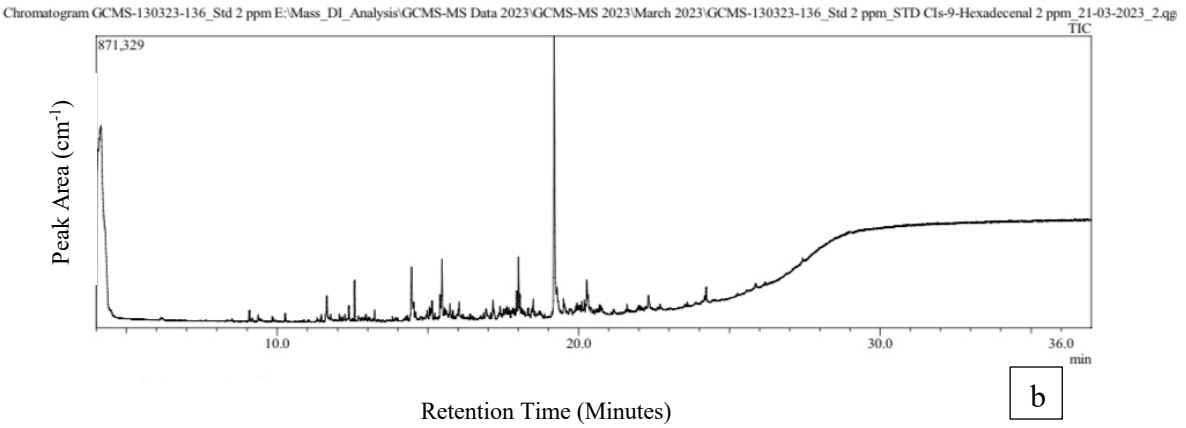
Retention Time (Minutes)

Figure 6.15: Quantitative analysis of the compound undecanal (a) TIC Chromatogram of undecanal standard (1 ppm) (b) TIC Chromatogram of undecanal standard (3 ppm) (c) TIC Chromatogram of Undecanal standard (5 ppm) (d) Calibration curve of Undecanal Standard (e) TIC Chromatogram of undecanal in male *A. grisella* (f) TIC Chromatogram of undecanal in male *G. mellonella*



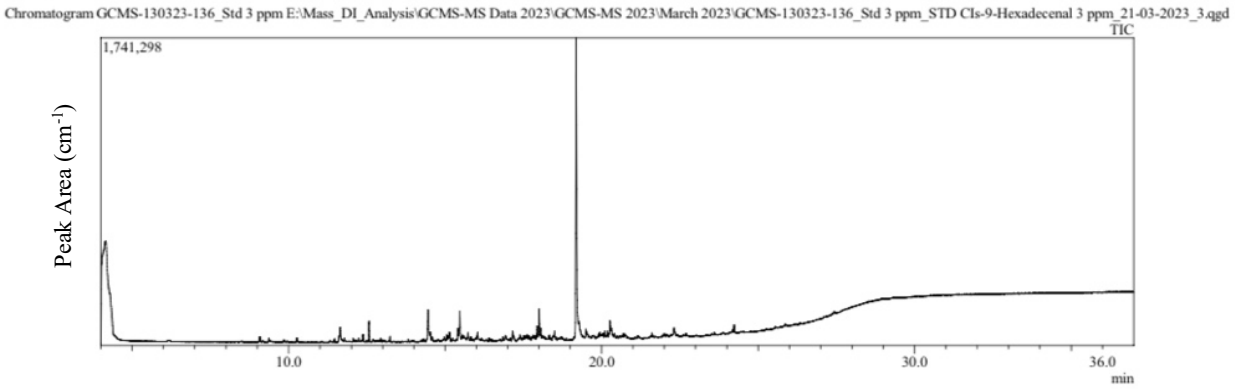
Peak Area (cm^{-1})

Retention Time (Minutes)



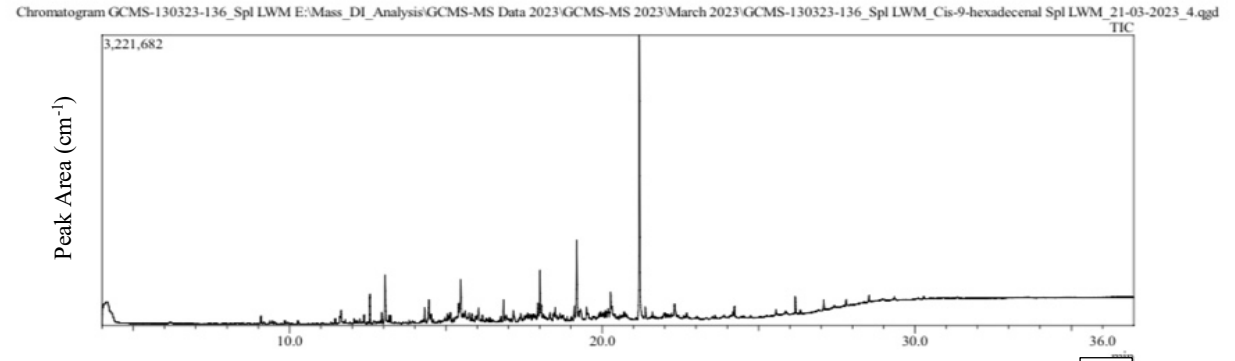
Peak Area (cm^{-1})

Retention Time (Minutes)



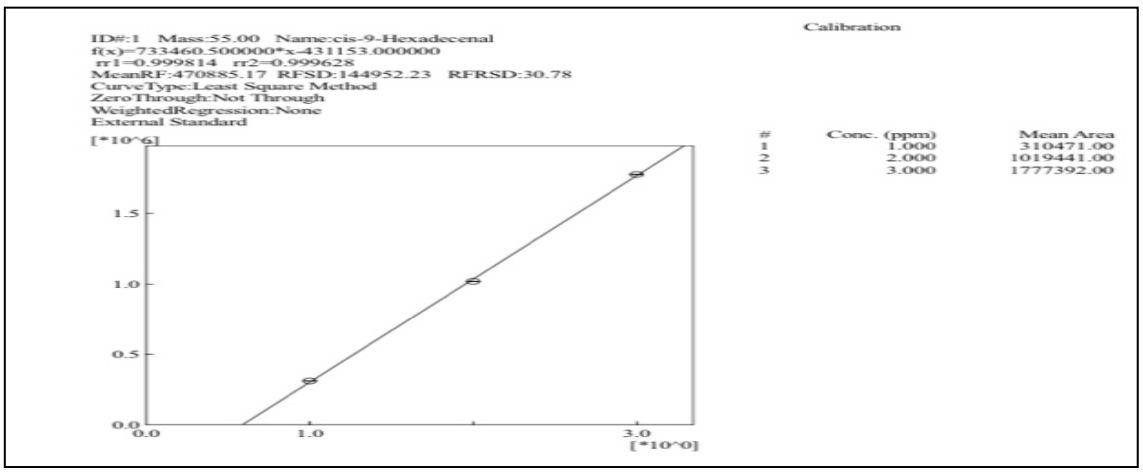
Retention Time (Minutes)

c



Retention Time (Minutes)

d



e

Figure 6.16: Quantitative analysis of the compound cis 9 hexadecenal (a) TIC Chromatogram of cis 9 hexadecenal standard (1 ppm) (b): TIC Chromatogram of cis 9 hexadecenal standard (2 ppm) (c) TIC Chromatogram of cis 9 hexadecenal standard (3 ppm) (d) Calibration curve of cis 9 hexadecenal Standard (e) TIC Chromatogram of cis 9 hexadecenal in male *A. grisella*

Male *G. mellonella* and *A. grisella* samples and standards were analyzed with a Shimandzu -TQ8040 gas chromatograph. Compounds were quantitatively analysed using a calibration curve and mass spectral libraries.

The data provided in the Table 6.1, 6.2, 6.3, 6.4 and 6.5 contains information about various compounds found in wax moths, particularly the greater wax moth (*Galleria mellonella*). The table lists the name, concentration, retention time (R. Time), mass-to-charge ratio (m/z), and peak area for each compound. The compounds analysed were Nonanal, 1-Nonanol, Heneiocosane, Heptadecane, Undecanal and Cis 9 Hexadecenal (Figure 6.9 and 6.10).

Nonanal has consistent retention times of around 6.177 to 6.180 minutes (min) and an m/z value of 57.00. The peak area increases with the concentration, showing a direct relationship as the area was at 1, 3 and 5 ppm concentration, respectively. At 1 ppm the Area = 229568, At 3 ppm: Area = 745213, At 5 ppm: Area = 13085731(Figure 6.11). 1-Nonanol has retention times ranging from 6.905 to 6.914 min with an m/z of 56.00. At 1 ppm: Area = 29003, At 3 ppm: Area = 427100, At 5 ppm: Area = 914587 (Figure 6.12). Heneiocosane shows a consistent retention time of around 22.115 to 22.118 min with an m/z of 57.00. Peak areas again increase with concentration. At 1 ppm: Area = 4272089, At 3 ppm: Area = 11369537, At 5 ppm: Area = 1690143 (Figure 6.13). Heptadecane has retention times are around 18.001 to 18.003 min with an m/z of 57.00. The peak area shows a clear increase with concentration. At 1 ppm: Area = 5893316, At 3 ppm: Area = 12721988, At 5 ppm: Area = 17836192 (Figure 6.14). Undecanal has consistent retention times around 12.250 to 12.252 min with an m/z of 43. The peak area increases with concentration. At 1 ppm: Area = 1342125, At 3 ppm: Area = 3994000, At 5 ppm: Area = 7636529 (Figure 6.15). Cis 9 hexadecenal has retention times are around 19.189 to 19.190 min with an m/z of 55.00. The peak area increases with concentration. At 1 ppm: Area = 31047, At 2 ppm: Area = 1019441, At 3 ppm: Area = 1777392 (Figure 6.16). For each compound, the retention time remains relatively consistent across different concentrations, indicating stable chromatographic conditions. The peak area generally increases with concentration, suggesting a linear response of the detector to the concentration of compounds within the measured range. This is crucial for quantitative analysis. Mass Spectral Data (m/z): The m/z values are

specific to each compound, assisting in their identification. These values should match known standards for accurate identification.

The compounds quantified in male wax moth *Galleria mellonella* were Aldehydes: nonanal (5.218 ppm, 6.182 min RT, 1359472 area), undecanal (7.162 ppm, 12.251 min RT, 10873560 area), heptadecane (0.203 ppm,18.005 min RT, area 3799665), heneiocosane (0.267 ppm, 22.118 min RT, area 749917) and alcohols: 1-nonanol (1.181ppm, 6.937 min RT, area 954113). **Heptadecane and heneiocosane, are the newly reported compounds in male, *G. mellonella.* The compound cis- 9-hexadecenal has not been previously reported in the scientific literature in male, *A. grisella***.

The chemical analysis of volatiles of male *A. grisella* resulted in the isolation and identification of 2 components, out of which one has not been identified in previous literature. The major compounds quantified in *G. mellonella* are nonanal (5.218 ppm) and undecanal (7.162 ppm) while other minor compounds are 1-nonanol (1.181ppm), heneiocosane (0.267 ppm) and heptadecane (0.203 ppm). In *A. grisella* two compounds are reported undecanal (8.745 ppm) and cis 9 hexadecenal (1.819 ppm). Aldehydes: undecanal (12.302 min RT) and cis 9 hexadecenal (20.393 min RT) have been identified as volatile compounds of *A. grisella* (Table 6.1 and Table 6.2).

6.4 Behavioural Bioassay in female *Galleria mellonella* (Greater wax moth)

The mean behavioural responses of female, *G. mellonella* moths under different treatments in a bioassay chamber (Figure 6.18), were associated with specific pheromone doses or control conditions with Whatman No. 1 filter paper (5x5 cm). In the control treatment, with no pheromone stimulus, the moths exhibited no response across all measured parameters whereas in the extracted blend treatment 15 ppm dose was used as a stimulus and the synthetic blend included 15, 10, 5, 1.0 and 0.5 ppm doses respectively (Figure 6.17).

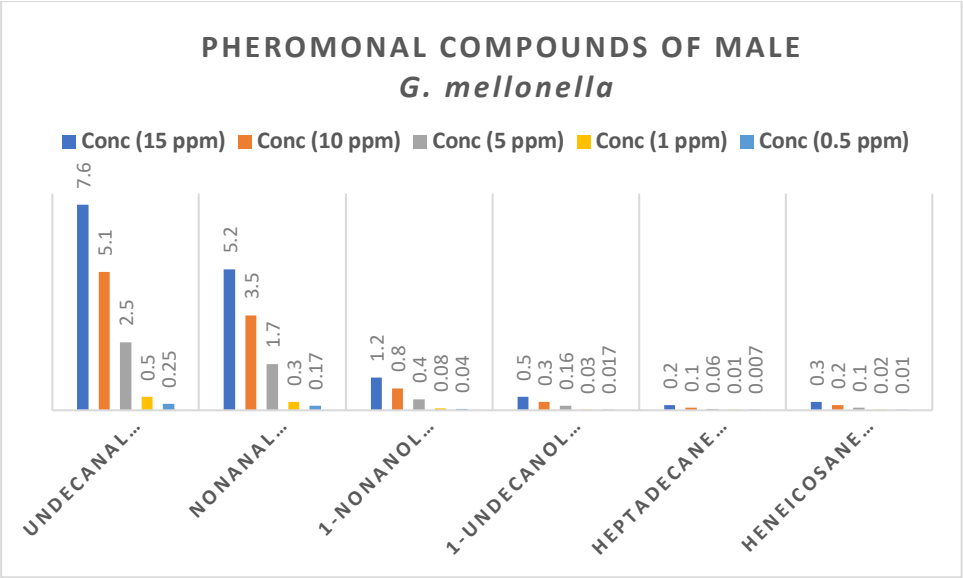


Figure 6.17: Pheromonal compound concentrations in male *Galleria mellonella* at different exposure levels (15 ppm, 10 ppm, 5 ppm, 1 ppm, and 0.5 ppm). The graph shows the relative abundance of specific compounds, including undecanal, nonanal, 1-nonanol, 1-undecanol, heptadecane, and heneicosane, indicating variations in response to increasing concentrations

6.4.1 Threshold to incite response in female *G. mellonella*

6.4.1.1 Untreated Control (without any treatment) and Hexane Treatment

No females exhibited the observed behaviours in response to the untreated control or hexane treatments.

6.4.1.2 Extracted Blend

The number of females observed beginning upward flight was 5.00 ± 0. All females flew to within 10 cm of the arena, with 5.00 ± 0 displaying this behaviour. 5.00 ± 0 of the females displayed their ovipositor. Those making the closest approach to the filter paper of 5.00 ± 0 cm. On average, all females oriented themselves for 28.33 ± 1.53 min. The extracted blend elicited a consistent response from the female *G. mellonella*. All five females observed began upward flight, flew to within 10 cm of the arena, and displayed their ovipositor, and made the closest approach to filter paper.

6.4.1.3 Synthetic Blend (15 ppm)

The number of females observed beginning upward flight was 5.00 ± 0. The percentage of females flying to within 10 cm of the arena was 5.00 ± 0. 5.00±0 of the females displayed their ovipositor. Females made the closest approach to the filter paper of 5.00 ± 0. On average, all females oriented themselves for 28.33 ± 2.08 min. The 15 ppm synthetic blend elicited the same consistent response as the extracted blend. All five females observed exhibited the same behaviours, approaching the filter paper to a 5.00 ± 0 and orienting themselves for an average of 28.33 ± 2.08 min.

6.4.1.4 Synthetic Blend (10 ppm)

The number of females observed beginning upward flight was 4.67 ± 0.58. The number of females flying to within 10 cm of the arena was also 4.67 ± 0.58. 4.00 ± 1 displayed their ovipositor. Females have the closest approach to the filter paper 4.00 ± 1. On average, all females oriented themselves for 26.67 ± 1.53 min. At a concentration of 10 ppm, the synthetic blend elicited a slightly reduced response compared to the higher concentration.

6.4.1.5 Synthetic Blend (5 ppm)

The number of females observed beginning upward flight was 4.00 ± 0. The number of females flying within 10 cm of the arena was also 4.00 ± 0. Females displayed their ovipositor were 3.00 ± 1. The females showing the closest approach to the filter paper was 3.67 ± 0.58. On average, the orientation time for all females was 23.33 ± 2.08 min. At a concentration of 5 ppm, the synthetic blend elicited a further reduced response. On average, 4.00 ± 0 females began upward flight, with 4.00 ± 0 flying to within 10 cm. 3.00 ± 1 displaying their ovipositor.

6.4.1.6 Synthetic Blend (1 ppm)

The number of females observed beginning upward flight was 3.00 ± 1. The number of females flying to within 10 cm of the arena was 3.67 ± 0.58. The females displayed their ovipositor were 3.00 ± 1. The closest approach to the filter paper was 2.67 ± 0.58. On average, the orientation time for all females was 22.00 ± 1.73 min. At the lowest concentration of 1 ppm, the synthetic blend elicited the weakest response.

6.4.1.7 Synthetic Blend (0.5 ppm)

None of the released females *G. mellonella* moths exhibited the observed behaviours in response to the 0.5 ppm synthetic blend.

Overall, the extracted blend and the highest concentration of the synthetic blend (15 ppm) elicited the most consistent and intense responses from the female *G. mellonella*. As the concentration of the synthetic blend decreased, the intensity of the observed behaviours also decreased, with fewer females exhibiting the behaviours and approaching the filter paper at a greater distance. The orientation time also decreased with lower concentrations.

The provided analysis of variance (ANOVA) results was based on a completely randomized design (CRD) where the experimental units are randomly assigned to the treatments. The ANOVA table is used to partition the total variation into the variation explained by the treatments and the residual variation. The results are presented for each factor separately, with the main focus on the effects of the treatments on the response variable. The results of the completely randomized ANOVA indicate that the different doses of the synthetic and extracted pheromone blends had a highly significant impact on various behavioural responses of female moths, including upward flight, flight to a 10 cm arena, displaying ovipositor, closest approach to filter paper, and total orientation time.

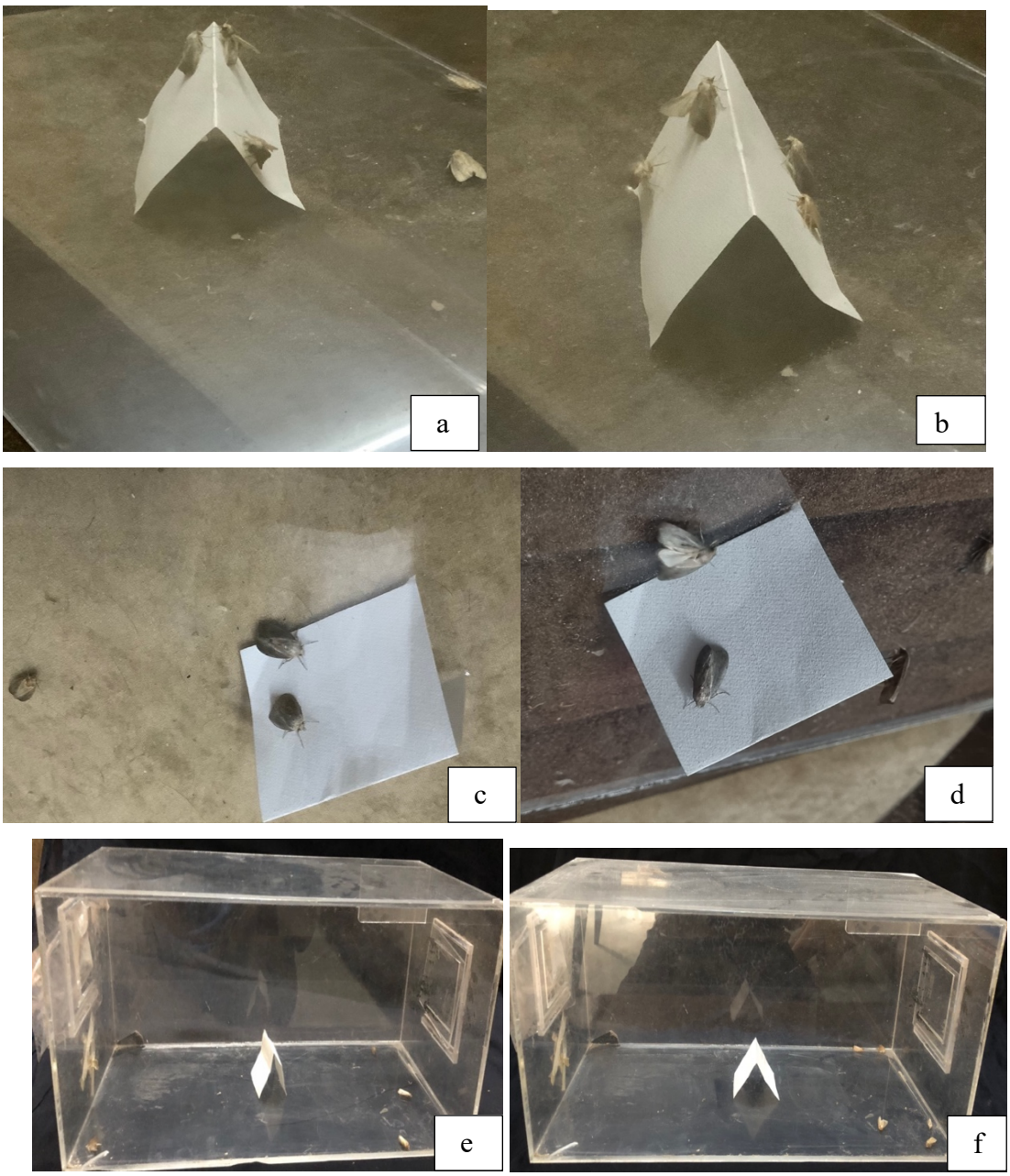


Figure 6.18: Behavioural response of Female *Galleria mellonella* a) represents the initiation of response with three females' approach on filter paper with synthetic pheromone b) Maximum females approach towards synthetic pheromonal blend c) initiation of response with two females approach on filter paper for extracted blend d) Females approaching towards extracted pheromonal blend e) untreated control f) hexane as control

6.4.2 Observed Behavioural Responses towards the Different Treatments

6.4.2.1 Upward Flight of Females

For upward flight behaviour, the ANOVA revealed a highly significant effect of pheromone dose ($F=112.94$, $p<0.0001$). The large F-value and the mean square for dose (16.73) being much larger than the error mean square (0.148) suggest a strong effect size and that the model explains a large portion of the variability in this behaviour. The grand mean was 2.41 with a CV of 15.99%, indicating moderate variability in the data.

The Tukey HSD test for upward flight identified three homogeneous groups. The extracted blend, synthetic blend 15 ppm, and synthetic blend 10 ppm treatments had the highest mean upward flight and were not significantly different from each other. Synthetic blend 5 ppm formed a separate group, while synthetic blend 1 ppm was in a lower group. The hexane 0.5 ppm, hexane 15 ppm, synthetic blend 0.5 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.19).

6.4.2.2 Flight to 10 cm Arena

For flight to the 10 cm arena, the ANOVA also showed a highly significant effect of pheromone dose ($F=231.87$, $p<0.0001$). The very large F-value and the dose mean square (17.18) being much larger than the error mean square (0.074) indicate a strong effect size and that the model explains a large portion of the variability in this behaviour. The grand mean was 2.48 with a CV of 10.97%, suggesting lower variability compared to upward flight.

The Tukey HSD test for flight to the 10 cm arena identified four homogeneous groups. The extracted blend and synthetic blend 15 ppm treatments had the highest means and were not significantly different. synthetic blend 10 ppm formed a separate group, followed by synthetic blend 5 ppm and synthetic blend 1ppm in lower groups. The 0.5 ppm hexane, 15 ppm hexane, synthetic blend 0.5 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.20).

6.4.2.3 Displaying Ovipositor

The results for displaying ovipositor while walking also showed a highly significant effect of pheromone dose ($F=44.50$, $p<0.0001$). The large F-value and the dose mean square (14.83) being much larger than the error mean square (0.33) indicate a strong effect size and good model fit. The grand mean was 2.22 with a CV of 25.98%, suggesting higher variability compared to the previous behaviours.

The Tukey HSD test for ovipositor display revealed three homogeneous groups. The extracted blend and synthetic blend 15 ppm treatments had the highest means and were not significantly different. A synthetic blend 10 ppm formed a separate group, followed by a synthetic blend of 1 ppm and a synthetic blend of 5 ppm in a lower group. The 0.5 ppm hexane, 15 ppm hexane, synthetic blend 0.5 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.21).

6.4.2.4 Closest Approach to Whatman Paper

For the closest approach to filter paper, the ANOVA indicated an extremely significant effect of pheromone dose ($F=141.33$, $p<0.0001$). The very large F-value and the dose mean square (15.70) being much larger than the error mean square (0.11) suggest a very strong effect size and excellent model fit. The grand mean was 2.30 with a CV of 14.52%, indicating moderate variability.

The Tukey HSD test for edge behaviour identified four homogeneous groups. The extracted blend and synthetic blend 15 ppm treatments had the highest means and were not significantly different. A synthetic blend of 10 ppm formed a separate group, followed by a synthetic blend of 5 ppm in a lower group. Synthetic blend 1 ppm was in an even lower group, while the 0.5 ppm hexane, 15 ppm hexane, synthetic blend 0.5 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.22).

6.4.2.5 Orientation Time

The results for total orientation time revealed a massive significant effect of pheromone dose ($F=311.12$, $p<0.0001$). The extremely large F-value and the dose mean square (564.62) being much larger than the error mean square (1.82) indicate a very strong

effect size and that the model explains a very large portion of the variability in this behaviour. The grand mean was 14.30 with a CV of 9.42%, suggesting low variability.

The Tukey HSD test for orientation time identified four homogeneous groups. The extracted blend and synthetic blend 15 ppm treatments had the highest means and were not significantly different. The synthetic blend of 10 ppm formed a separate group, followed by a synthetic blend of 5 ppm in a lower group. synthetic blend 1 ppm was in an even lower group, while the 0.5 ppm hexane, 15 ppm hexane, synthetic blend 0.5 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.23).

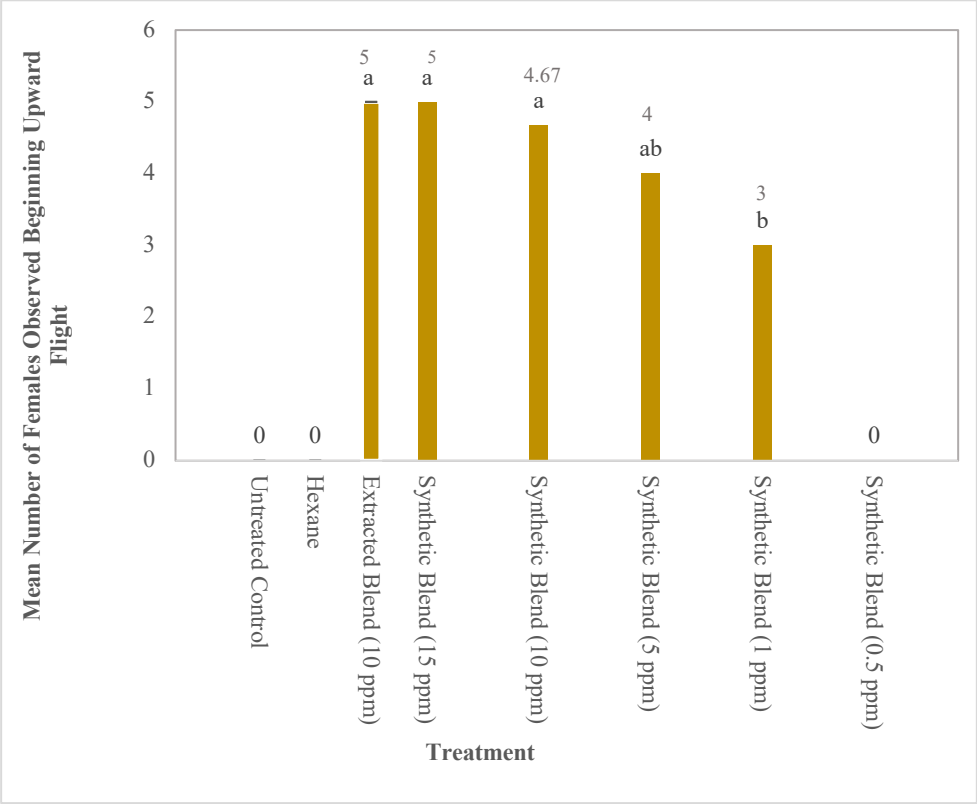


Figure 6.19: This graph shows the number of female *G. mellonella* observed beginning an upward flight in response to different treatments. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The y-axis represents the number of females observed, and the x-axis lists the various treatments, including Untreated Control, Hexane, Extracted Blend, and Synthetic Blend at different concentrations (15 ppm, 10 ppm, 5 ppm, 1 ppm, 0.5 ppm). Untreated Control and Hexane attracts 0 females: Both the untreated control and hexane treatments resulted in 0 females initiating upward flight, indicating no response from these treatments. Extracted Blend (15 ppm) and Synthetic Blend (15 ppm, 10 ppm): These treatments showed the highest number of females beginning upward flight, marked with 'a'. There is no significant difference between these treatments, indicating they are equally effective in eliciting upward flight. Synthetic Blend (5 ppm): This treatment showed a

slightly lower response compared to the highest responses, marked with 'ab'. This indicates it is statistically different from the untreated control and hexane but not significantly different from the higher responses marked 'a'. Synthetic Blend (1 ppm): This dose showed a lower response, marked with 'b', indicating a statistically significant lower response compared to the treatments marked with 'a'. Synthetic Blend (0.5 ppm): This treatment also resulted in 0 females initiating upward flight, indicating no response similar to the untreated control and hexane.

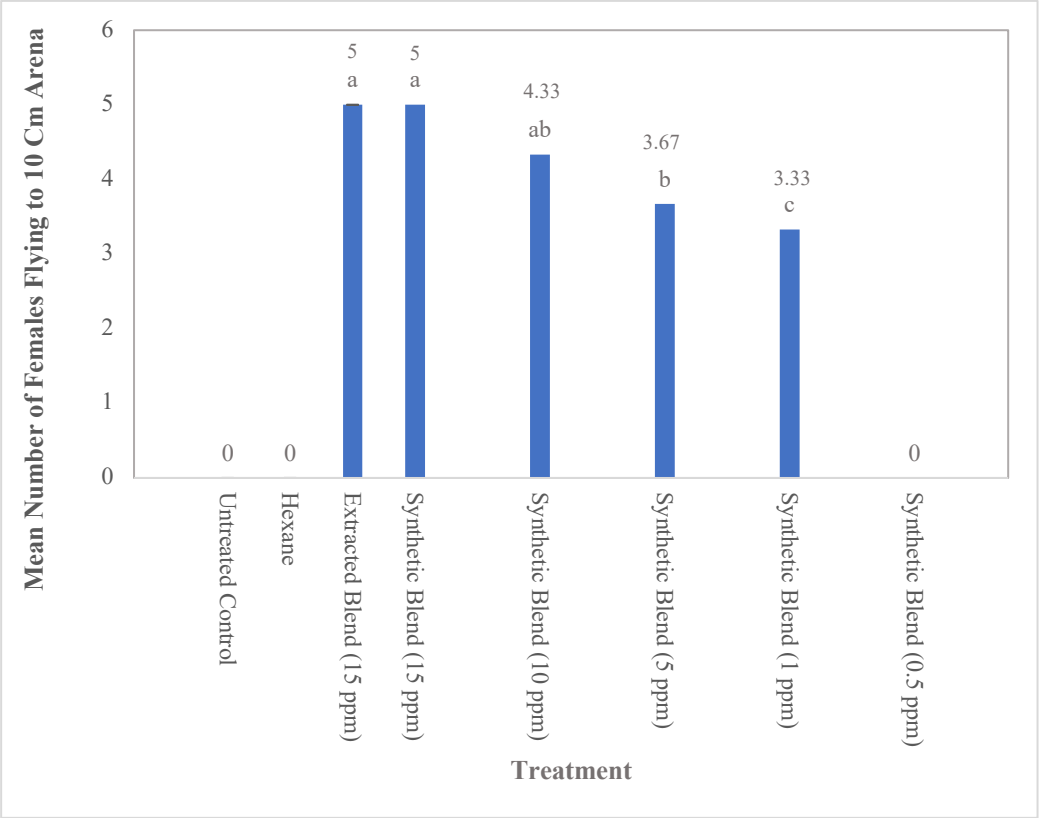


Figure 6.20: The graph represents the average number of female *G. mellonella* moths that flew to within 10 centimetres of the arena under various treatment conditions. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The Vertical Axis (Y-Axis): The number of females flying within 10 centimetres of the arena. The Horizontal Axis (X-Axis) includes the list of different treatments applied like hexane as a Solvent used as a control, extracted Blend and synthetic blend tested at various concentrations:15 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm. Extracted Blend and Synthetic Blend (15 ppm): These treatments had the highest number of females flying to within 10 cm of the arena, marked with the letter "a", indicating no significant difference between them. Synthetic Blend (10 ppm): This treatment showed a moderate number of females flying to within 10 cm, marked with "ab", indicating it is not significantly different from both "a" and "b" groups. Synthetic Blend (5 ppm): This treatment had fewer females flying to within 10 cm compared to the 10-ppm synthetic blend, marked with "bc", indicating it is not significantly different from "b" and "c". Synthetic Blend (1 ppm): This treatment had the least number of females flying to within 10 cm among the treatments that had any flight activity, marked with "c", indicating a significant difference from "a" treatment. Synthetic blends at 15 ppm were most effective in prompting female *G. mellonella* moths to fly within 10 cm of the arena. Statistical significance is indicated by the letters, where treatments sharing the same letter are not significantly different, while those with different letters are significantly different.

The extracted blend 15 ppm and 15 ppm synthetic blend treatments resulted in the highest activity, whereas lower concentrations of synthetic blend (1 ppm and 0.5 ppm) were less effective

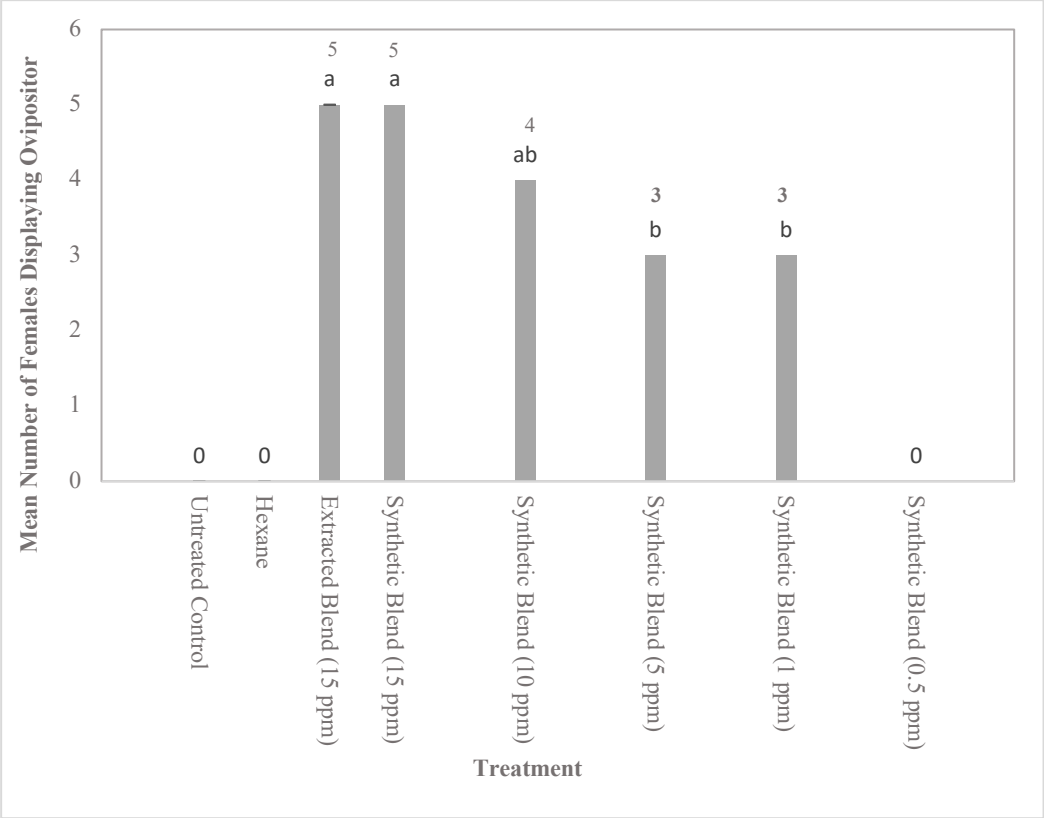


Figure 6.21: The graph shows the number of female *G. mellonella* moths displaying their ovipositor while under various treatment conditions. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The number of displays is measured and the data are presented with standard error (± SE) to indicate variability. The Vertical Axis (Y-axis) represents females displaying ovipositor and the horizontal Axis (X-axis) represents lists of different treatments applied like hexane as Solvent used as a control, and extracted Blend. Synthetic blend tested at various concentrations:15 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm. In the untreated control, hexane, and synthetic blend at 0.5 ppm treatments, no females were observed displaying ovipositor. Extracted Blend and Synthetic Blend (15 ppm): These treatments had the highest number of females displaying ovipositor, marked with the letter "a", indicating no significant difference between them. Synthetic Blend (10 ppm): This treatment showed a moderate number of females displaying ovipositor, marked with "ab", indicating it is not significantly different from both "a" and "b" groups. Synthetic Blend (5 ppm and 1 ppm): These treatments had fewer females displaying ovipositor compared to the higher concentrations, marked with "b", indicating they are significantly different from those marked "a"

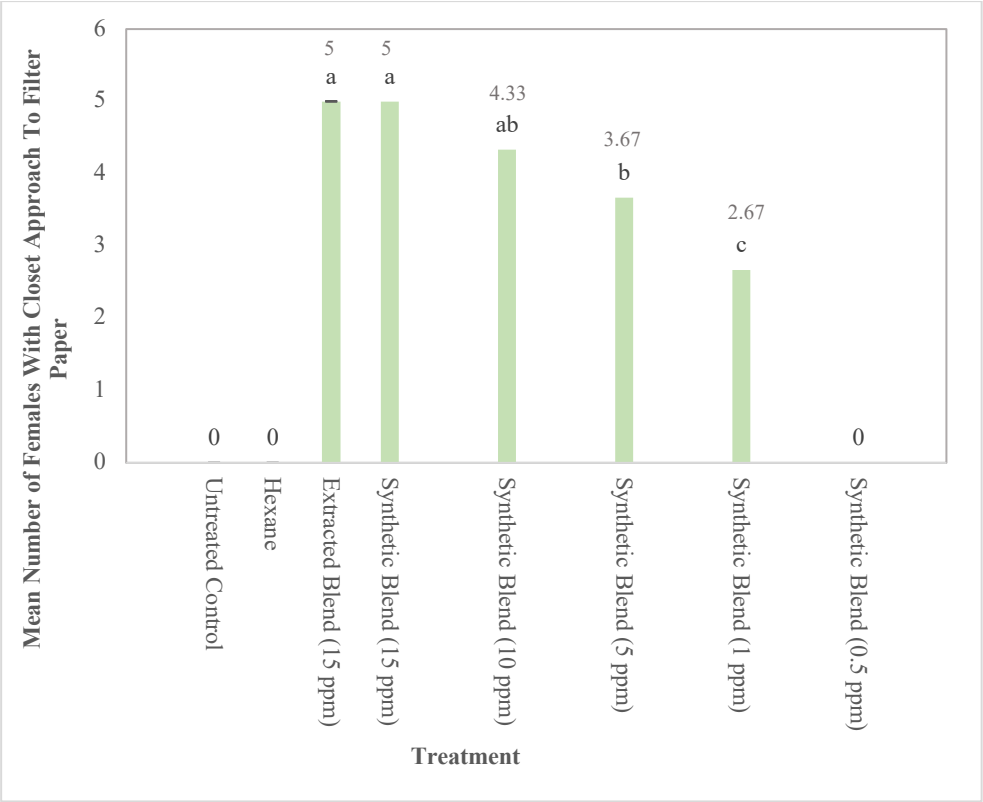


Figure 6.22: This graph illustrates the closest approach to filter paper by female *G. mellonella* moths that flew to the edge of their environment under various treatments. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The Vertical Axis (Y-axis) represents the number of females with the closest approach to filter paper and the horizontal Axis (X-axis) represents lists of different treatments applied like hexane as the solvent used as a control and extracted Blend. Synthetic blend tested at various concentrations:15 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm. No Approach to Filter Paper: In the untreated control, hexane, and synthetic blend at 0.5 ppm treatments, no females were observed approaching the filter paper (indicated by 0 cm). Extracted Blend (15 ppm) and Synthetic Blend (15 ppm): These treatments had the closest approach distances, marked with the letter "a", indicating no significant difference between them. Synthetic Blend (10 ppm): This treatment showed a moderate approach distance, marked with "ab", indicating it is not significantly different from "a" or "b" treatments. Synthetic Blend (5 ppm): This treatment had a greater approach distance than the 10 ppm synthetic blend, marked with "b", indicating a significant difference from "a" but not from "ab". Synthetic Blend (1 ppm): This treatment had the farthest approach distance among those that had any activity, marked with "c", indicating a significant difference from both "a" and "b" treatments

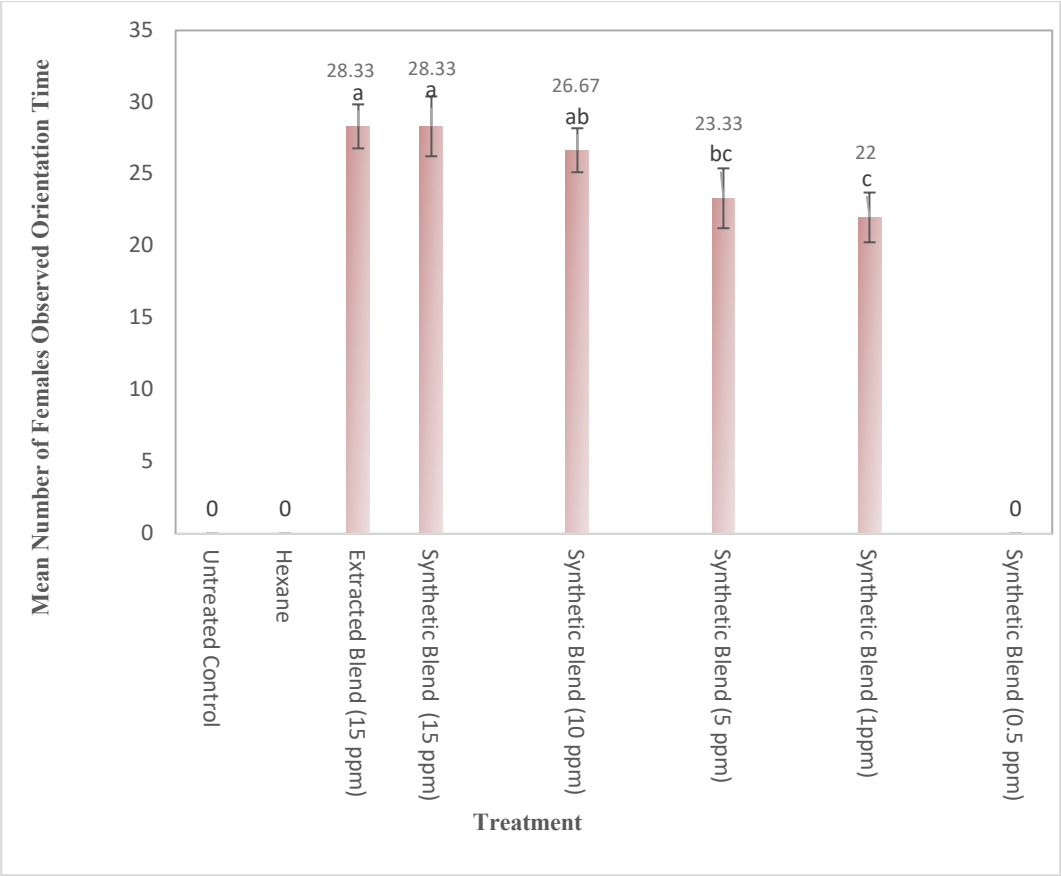


Figure 6.23: This graph shows the average orientation time in minutes for female *G. mellonella* moths under various treatment conditions. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The error bars represent the standard error (± SE) of these measurements, providing an indication of variability. The Vertical Axis (Y-axis) represents Orientation time in minutes and the horizontal Axis (X-Axis) represents lists of different treatments applied like hexane as Solvent used as a control, and extracted Blend. Synthetic blend tested at various concentrations:15 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm. Untreated Control and Hexane: Both treatments resulted in an orientation time of 0 minutes, indicating no response from the females. Extracted Blend: This treatment elicited the longest orientation time, similar to the highest concentration of the Synthetic Blend (15 ppm), both marked with 'a', suggesting a strong and statistically significant response compared to other treatments. Synthetic Blends: The orientation time varied with concentration:15 ppm: The highest concentration of the Synthetic Blend had an orientation time similar to the Extracted Blend 15 ppm, marked with 'a', indicating a strong response. Synthetic Blend 10 ppm: This treatment had an intermediate orientation time, marked with 'ab', indicating it is statistically different from the highest and lowest responses but somewhat similar to both. 5 ppm: Showed a further decrease in orientation time, marked with 'bc', indicating a statistically intermediate response, different from both higher and lower concentration responses.1 ppm: This concentration had a lower orientation time than 10 ppm and 5 ppm, marked with 'c', indicating a statistically significant lower response.0.5 ppm: The lowest concentration had the shortest orientation time among the responsive treatments, marked with 'c', indicating the weakest response among the treatments tested

Overall, the results suggest that the higher concentrations of the synthetic and extracted blends were more effective in eliciting various behavioural responses in the moths, including upward flight, flight to the 10 cm arena, ovipositor display, closest

approach to filter paper, and orientation time. The data presented in Table 6.6 demonstrates the behavioural responses of female *G. mellonella* under different treatments, each associated with specific pheromone doses or control conditions The ANOVA results indicate significant differences in behavioural responses among the various treatments for all five behavioural measures (upward flight, flying to 10 cm arena, displaying ovipositor, closest approach to Whatman paper, and orientation time). The extremely low p-values (P=0.0000) across all measures suggest strong evidence against the null hypothesis, indicating that treatments have a significant impact on these behaviours.

The Tukey HSD test further clarifies these differences by grouping the treatments into homogeneous subsets. For all behavioural measures, the untreated control group (untreated control) and the hexane groups (0.5 ppm hexane, 15 ppm hexane) consistently show the lowest mean values, indicating minimal behavioural response. On the other hand, treatments such as Extracted Blend and higher concentrations of Synthetic blend consistently show the highest mean values, indicating a strong behavioural response the synthetic blends and the extracted blend had a significant impact on various behavioural responses of female moths, with the extracted blend and higher concentrations of Synthetic Blend (synthetic blend 15 ppm and synthetic blend 10 ppm) generally showing the highest levels of upward flight, flight to the 10 cm arena, ovipositor display, closest approach to filter paper, and orientation time. The results revealed that 1 ppm synthetic blend is the threshold as the concentration below which failed to elicit any kind of response in female *G. mellonella* in laboratory conditions.

Table 6.6: Behaviour of female *G. mellonella* in observation arena in response to Synthetic blend and Extracted blend provided on Whatman No. 1 filter paper

Treatment on Whatman paper No. 1	No. of females observed beginning upward flight	No. of females flying to 10 cm of arena	No. of females displaying ovipositor	No. of females having closet approach to filter paper	Observed orientation time (min) (± SE)
Untreated Control	0	0	0	0	0
Hexane (15 ppm and 0.5 ppm)	0	0	0	0	0
Extracted Blend (15ppm)	$5.00^{a} \pm 0$	$5^{a} \pm 0$	$5^{a} \pm 0$	$5.00^{a} \pm 0$	$28.33^{a} \pm 0.88$
Synthetic Blend (15 ppm)	$5.00^{a} \pm 0$	$5^{a} \pm 0$	$5^{a} \pm 0$	$5.00^{a} \pm 0$	$28.33^{a} \pm 1.20$
Synthetic Blend (10 ppm)	$4.67^{a} \pm 0.33$	$4.67^{ab} \pm 0.33$	$4.00^{ab} \pm 0.58$	$4.33^{ab} \pm 0.58$	$26.67^{ab} \pm 0.88$
Synthetic Blend (5 ppm)	$4.00^{ab} \pm 0$	$4.00^{bc} \pm 0$	$3.00^{b} \pm 0.58$	$3.67^{b} \pm 0.33$	$23.33^{bc} \pm 1.20$
Synthetic Blend (1 ppm)	$3.00^{b} \pm 0.58$	$3.67^{c} \pm 0.33$	$3.00^{b} \pm 0.58$	$2.67^{c} \pm 0.33$	$22.00^{c} \pm 0.99$
Synthetic Blend (0.5 ppm)	0	0	0	0	0

Mean values followed with different superscripts are significantly different ($p<0.05$) using Tukey's test. Statistical groupings (denoted by letters such as 'a', 'b', 'c', etc.) indicate significant differences among the mean values at various concentrations.

6.5 Behavioural Bioassay in Female *Achroia grisella* (Lesser wax moth)

The mean behavioural responses of female, *A. grisella* moths and under different treatments in a bioassay chamber (Figure 6.25), each stimulus was associated with specific pheromone doses or control conditions with Whatman No. 1 filter paper (5x5

cm). In the control treatment, with no pheromone stimulus given, the moths exhibited no response across all measured parameters whereas in the extracted blend treatment 10.7 ppm dose was used as a stimulus and the synthetic blend included 10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm and 0.1 ppm doses, respectively (Figure 6.24).

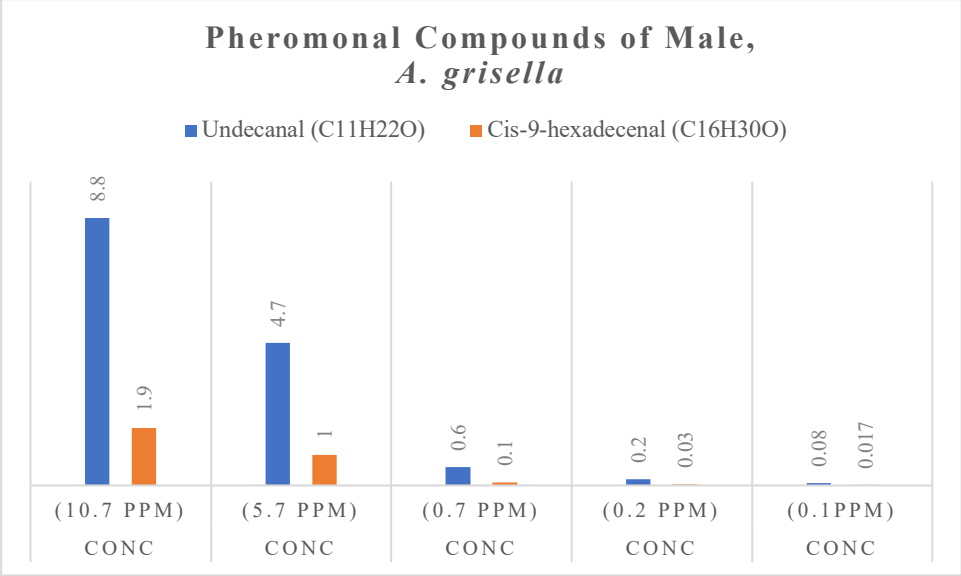


Figure 6.24: Relative abundance of Undecanal and Cis-9-hexadecenal pheromones in male *A. grisella* at varying concentrations (10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, and 0.1 ppm)

6.5.1 Threshold to incite response in female *A. grisella*

6.5.1.1 Untreated Control (without any treatment) and Hexane Treatment

No females exhibited the observed behaviours in response to the untreated control or hexane treatments.

6.5.1.2 Extracted Blend

The Number of females observed beginning upward flight was 5.00 ± 0. The number of females flying to 10 cm of the arena was 5 ± 0. The females displaying ovipositor were 5 ± 0. Females with the closet approach to filter paper 4.67 ± 1. The orientation time of females on filter paper on an average was 29.67 ± 0.58 min. The extracted blend elicited a consistent response from the female *A. grisella*. All 5 females observed began upward flight, flew to 10 cm within the arena, displayed the ovipositor.

6.5.1.3 Synthetic Blend (10.7 ppm)

The number of females observed beginning upward flight was 5.00 ± 0. Females flying to 10 cm of the arena were 5 ± 0. The number of females displaying ovipositor was 5 ± 0. Females with a closet approach to filter paper were 5 ± 0. The orientation time of filter paper on filter paper was 29.67 ± 0.58 min. The 10.7 ppm synthetic blend elicited the same consistent response as the extracted blend. All 5 females observed exhibited the same behaviours, approaching the filter paper to a distance of 5 ± 0 and orienting themselves for an average of 29.67 ± 0.58 min.

6.5.1.4 Synthetic Blend (5.7 ppm)

The number of females observed beginning upward flight was 4.00 ± 1 females. The number of females flying to 10 cm of arena was 4.33 ± 0.58. The number of females displaying ovipositor was 4.33 ± 1.15. The number of females with closet approach to filter paper was 4.00 ± 0.58. The orientation time of females on filter paper was 27.00 ± 1 min. At a concentration of 5.7 ppm, the synthetic blend elicited a slightly reduced response compared to the higher concentration. On average, 4.00 ± 1 females began upward flight, with 4.33 ± 0.58, flying to 10 cm. 4.33 ± 1.15 displayed the ovipositor. The females approached the filter paper 4.00 ± 0.58 and oriented themselves for an average of 27.00 ± 1 min.

6.5.1.5 Synthetic Blend (0.7 ppm)

The number of females observed beginning upward flight was 3.33 ± 0.58 females. The number of females flying to 10 cm of arena was 3.67 ± 0. The number of females displaying ovipositor was 2.67 ± 0.58. The females with a closet approach to filter paper were 3.67 ± 0.58. The orientation time of females on the filter paper was 24.67 ± 1.53 min. At a concentration of 0.7 ppm, the synthetic blend elicited a further reduced response. On average, 3.33 ± 0.58 females began upward flight, with 3.67 ± 0, flying to 10 cm. Of those, 2.67 ± 0.58 fanned while walking and displayed the ovipositor. The females approached the filter paper was 3.67 ± 0.58 and oriented themselves for an average of 24.67 ± 1.53 min.

6.5.1.6 Synthetic Blend (0.2 ppm)

The number of females observed beginning upward flight was 2.67 ± 0.58. The females flying to 10 cm of arena were 3.33 ± 0.58. Females displaying ovipositor were 2.67 ± 0.58. Females with closet approach to filter paper were 2.33 ± 0.58. The orientation time of females on filter paper was 22.33 ± 1.53 min. At the lowest concentration of 0.2 ppm, the synthetic blend elicited the weakest response. On average, 2.67 ± 0.58 females began upward flight, with 3.33 ± 0.58 flying to 10 cm. Of those, 2.67 ± 0.58 fanned while walking and displayed the ovipositor. The females approached the filter paper were 2.33 ± 0.58 and oriented themselves for an average of 22.33 ± 1.53 min.

6.5.1.7 Synthetic Blend (0.1 ppm)

None of the released *A. grisella* female moths exhibited the observed behaviours in response to the 0.1 ppm synthetic blend.

Overall the extracted blend and the highest concentration of the synthetic blend (10.7 ppm) elicited the most consistent and intense responses from the female *A. grisella.* As the concentration of the synthetic blend decreased, the intensity of the observed behaviours also decreased, with fewer females exhibiting the behaviours and approaching the filter paper at a greater distance. The orientation time also decreased with lower concentrations.

The analysis of variance (ANOVA) results presented here are derived from a completely randomized design (CRD), in which the experimental units are assigned to the treatments in a random manner. The ANOVA table is used to partition the total variation into the variation explained by the treatments and the residual variation. The results are presented for each factor separately, with the main focus on the effects of the treatments on the response variable.

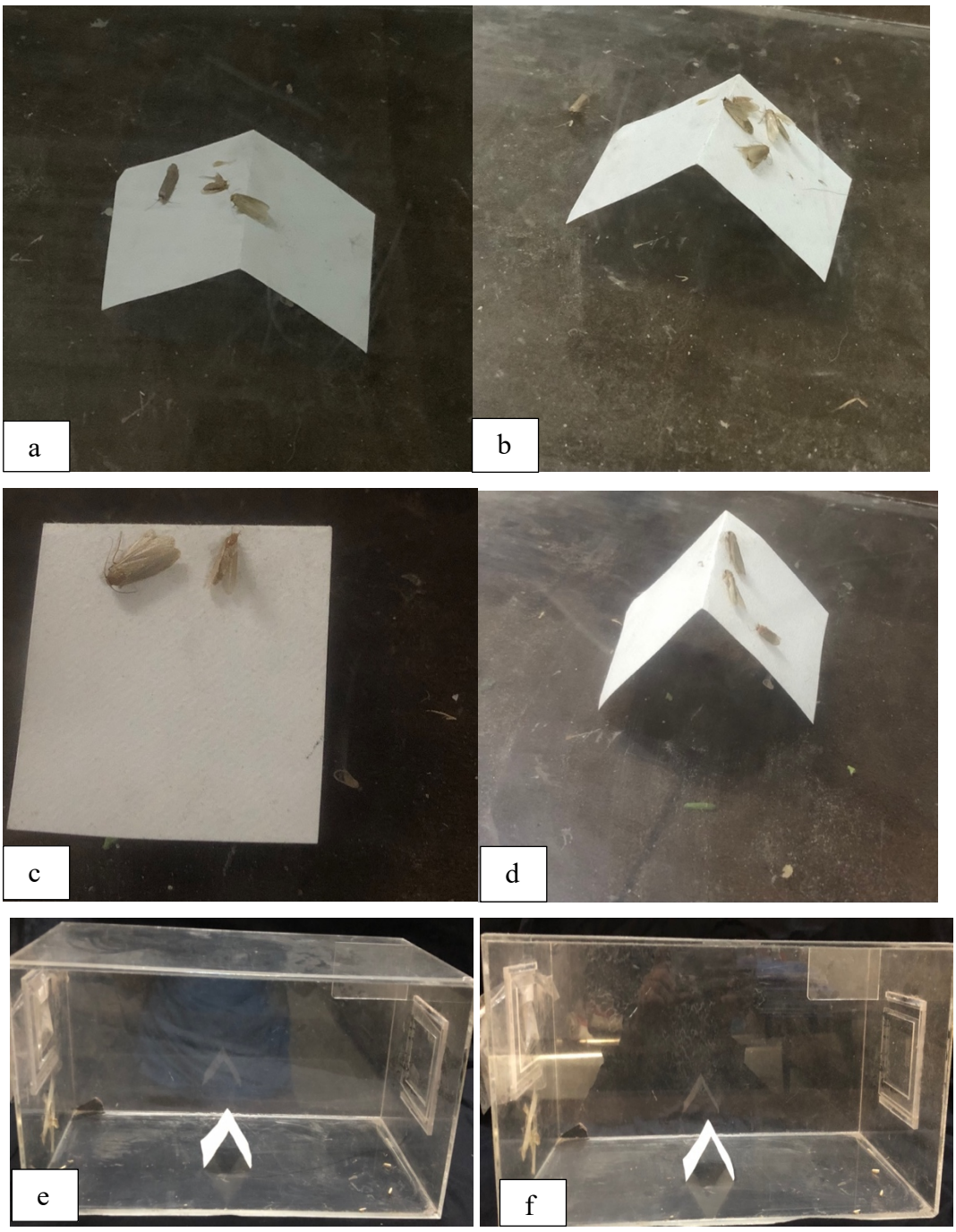


Figure 6.25: Behavioural response of Female *Achroia grisella* a) Onset of response towards synthetic pheromonal blend b) maximum females approaching towards filter paper with synthetic lure c) Females initiating the response in the beginning of placement of filter paper with extracted blend d) maximum females approaching towards filter paper with extracted pheromonal blend e) untreated control f) hexane as control

6.5.2 Observed Behavioural Responses towards the Different Treatments

6.5.2.1 Upward Flight of Females

The one-way ANOVA for the behaviour of females beginning upward flight revealed a highly significant effect of the pheromone treatment ($F=80.55$, $p<0.0001$). The large F-value and the treatment mean square (14.916) being much larger than the error mean square (0.19) suggest a strong effect size and that the model explains a large portion of the variability in this behaviour. The grand mean was 2.23 with a CV of 19.36%, indicating moderate variability.

The Tukey HSD test for upward flight identified four homogeneous groups. the extracted blend and synthetic blend 10.7 ppm treatments had the highest means and were not significantly different. synthetic blend 5.7 ppm formed a separate group, followed by a synthetic blend of 0.7 ppm in a lower group. synthetic blend 0.2 ppm was in an even lower group, while the 0.1 ppm hexane, 10.7 ppm hexane, synthetic blend 0.1 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.26).

6.5.2.2 Female Flight to 10 cm Arena

The one-way ANOVA for the behaviour of females flying to a 10 cm arena showed a highly significant effect of the pheromone treatment ($F=144.33$, $p<0.0001$). The very large F-value and the treatment mean square (16.037) being much larger than the error mean square (0.11) indicate a strong effect size and that the model explains a large portion of the variability in this behaviour. The grand mean was 2.37 with a CV of 14.06%, suggesting moderate variability.

The Tukey HSD test for flight to the 10 cm arena identified four homogeneous groups. The extracted blend and synthetic blend 10.7 ppm treatments had the highest means and were not significantly different. synthetic blend 5.7 ppm formed a separate group, followed by synthetic blend 0.7 ppm and synthetic blend 0.2 ppm in lower groups. the 0.1 ppm hexane, 10.7 ppm hexane, synthetic blend 0.1 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.27).

6.5.2.3 Displaying Ovipositor

The one-way ANOVA for the behaviour of displaying ovipositor also showed a highly significant effect of the pheromone treatment ($F=135.08$, $p<0.0001$). The large F-value and the treatment mean square (15.0093) being much larger than the error mean square (0.11) indicate a strong effect size and good model fit. The grand mean was 2.19 with a CV of 15.25%, suggesting moderate variability.

The Tukey HSD test for ovipositor display revealed three homogeneous groups. the extracted blend and synthetic blend 10.7 ppm treatments had the highest means and were not significantly different. synthetic blend 5.7 ppm formed a separate group, followed by synthetic blend 0.2 ppm and synthetic blend 0.7 ppm in a lower group. the 0.1 ppm hexane, 10.7 ppm hexane, synthetic blend 0.1 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.28).

6.5.2.4 Closest Approach to Whatman Paper

The one-way ANOVA results for the behaviour females with closest approach to Whatman paper revealed a highly significant effect of the pheromone treatment ($F=70.42$, $p<0.0001$). The large F-value and the mean square for treatment (15.64) being much larger than the error mean square (0.22) suggest a strong effect size and that the model explains a large portion of the variability in this behaviour. The grand mean was 2.26 with a coefficient of variation (CV) of 20.87%, indicating moderate variability in the data.

The Tukey HSD test for the edge and closest approach behaviour identified three homogeneous groups. the extracted blend, synthetic blend 10.7 ppm, and synthetic blend 5.7 ppm treatments had the highest mean values and were not significantly different from each other. The synthetic blend 0.7 ppm treatment formed a separate group, while the synthetic blend 0.2 ppm was in a lower group. the 0.1 ppm hexane, 10.7 ppm hexane, synthetic blend 0.1 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.29).

6.5.2.5 Orientation Time of Observed Females

The one-way ANOVA for the total orientation time of observed females showed an extremely significant effect of the pheromone treatment ($F=863.89$, $p<0.0001$). The remarkably large F-value and the treatment mean square (607.93) being much larger than the error mean square (0.704) indicate a very strong effect size and that the model explains a very large portion of the variability in this behaviour. The grand mean was 14.82 with a CV of 5.66%, suggesting low variability.

The Tukey HSD test for orientation time identified four homogeneous groups. the extracted blend and synthetic blend 10.7 ppm treatments had the highest means and were not significantly different. synthetic blend 5.7 ppm formed a separate group, followed by synthetic blend 0.7 ppm in a lower group. synthetic blend 0.2 ppm was in an even lower group, while the 0.1 ppm hexane, 10.7 ppm hexane, synthetic blend 0.1 ppm, and untreated control treatments had the lowest means and were not significantly different from each other (Figure 6.30).

In summary, the completely randomized ANOVA results demonstrate that the different pheromone treatments had a highly significant impact on various behavioural responses of female moths, including upward flight, flight to a 10 cm arena, displaying ovipositor flight to the edge and closest approach to Whatman paper, total orientation time. The large F-values, small p-values, and the Tukey HSD tests provide strong evidence for the effectiveness of the pheromone blends in eliciting specific behaviours. These findings have important implications for understanding moth mating behaviour and potential applications in pest management strategies.

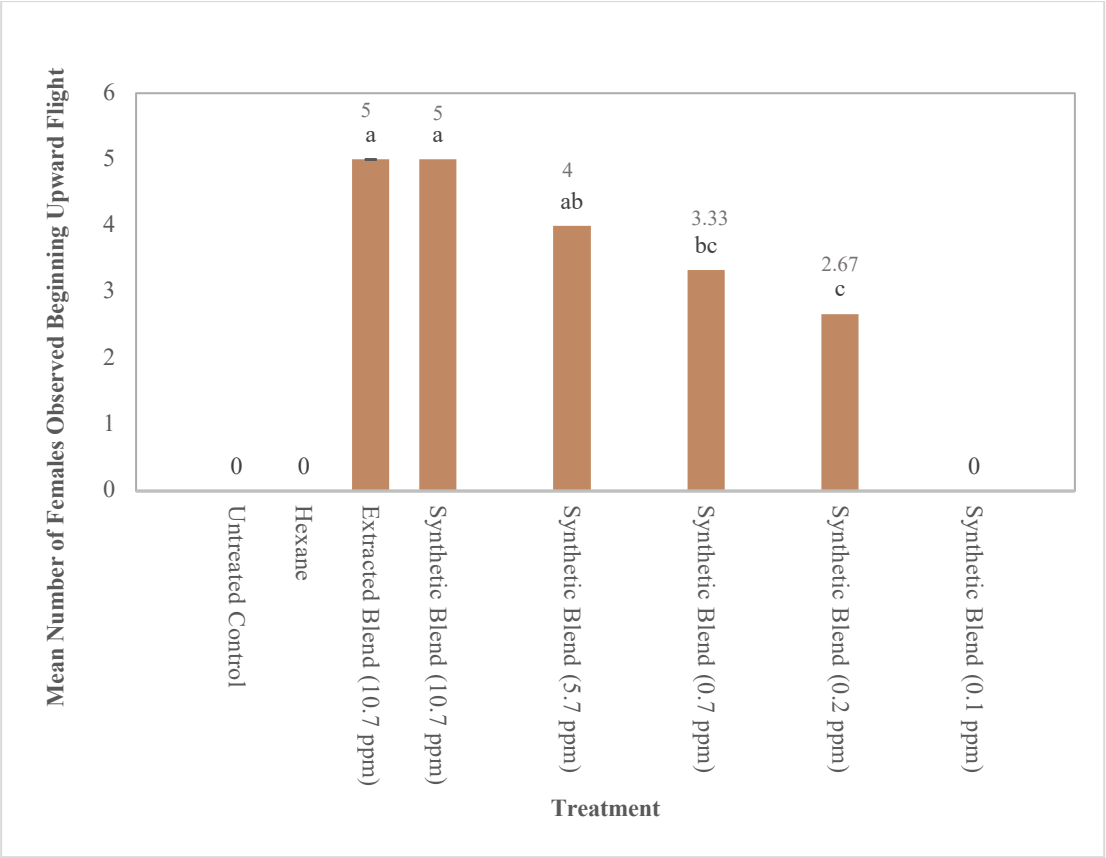


Figure 6.26: The graph displays the number of *A. grisella* females observed beginning upward flight in response to various treatments. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The treatments included an Untreated Control, Hexane, Extracted Blend, and Synthetic Blend at different concentrations (10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, and 0.1 ppm). The y-axis represents the number of females initiating upward flight, while the x-axis lists the different treatments. For both the Untreated Control and Hexane treatments, no females were observed beginning upward flight, indicating no response. The Extracted Blend (10.7 ppm) and the highest concentration of Synthetic Blend (10.7 ppm) had the highest number of females initiating upward flight, marked with 'a', showing no significant difference between these two treatments. The 5.7 ppm Synthetic Blend had an intermediate number of females beginning upward flight, marked with 'ab', while the 0.7 ppm concentration had fewer females, marked with 'bc'. The 0.2 ppm Synthetic Blend saw even fewer females initiating upward flight, marked with 'c'. Finally, the lowest concentration (0.1 ppm) of Synthetic Blend, like the control treatments, showed no upward flight activity. These results suggest that higher concentrations of synthetic blends and the extracted blend are more effective in inducing upward flight behaviour in female *A. grisella*

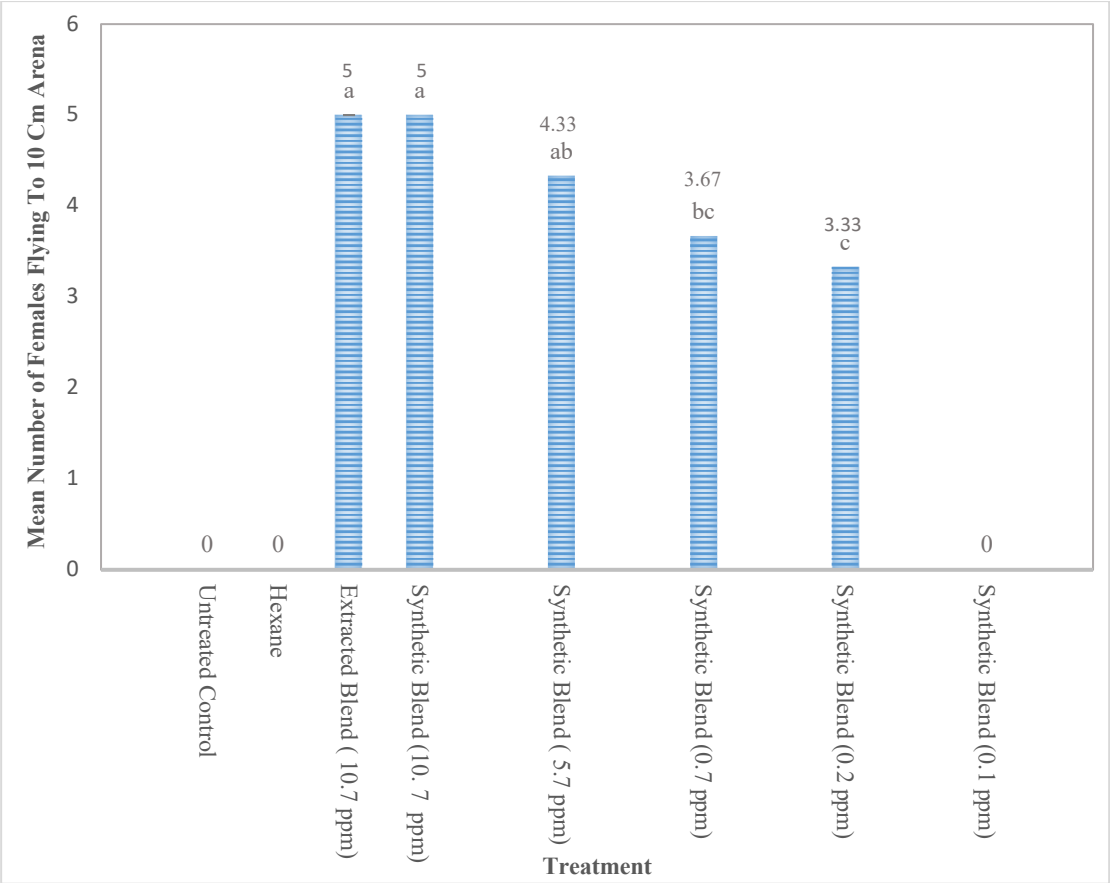


Figure 6.27: The graph highlights the effectiveness of different treatments in prompting female *A. grisella* moths to fly within 10 cm of the arena. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The treatments included an untreated control, hexane, extracted blend, and synthetic blend at different concentrations (10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, and 0.1 ppm). The axis indicates the number of females flying to within 10 centimetres of the arena. while the x-axis lists the different treatments. the treatments included an untreated control, hexane, extracted blend, and synthetic blend at different concentrations (10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, and 0.1 ppm). for both the untreated control and hexane treatments, no response was recorded. extracted blend (10.7 ppm) and synthetic blend (10.7 ppm): both treatments are labelled "a", indicating no significant difference between them. these treatments are significantly different from those labelled "b" or "c". Synthetic blend (5.7 ppm): this treatment is labelled "ab", indicating it is not significantly different from both "a" and "b" groups, but represents an intermediate significance level. Synthetic blend (0.7 ppm): this treatment is labelled "bc", indicating it is not significantly different from both "b" and "c" groups. synthetic blend (0.2 ppm): This treatment is Labelled "c", showing it is significantly different from "a" treatment but not from the "bc" group

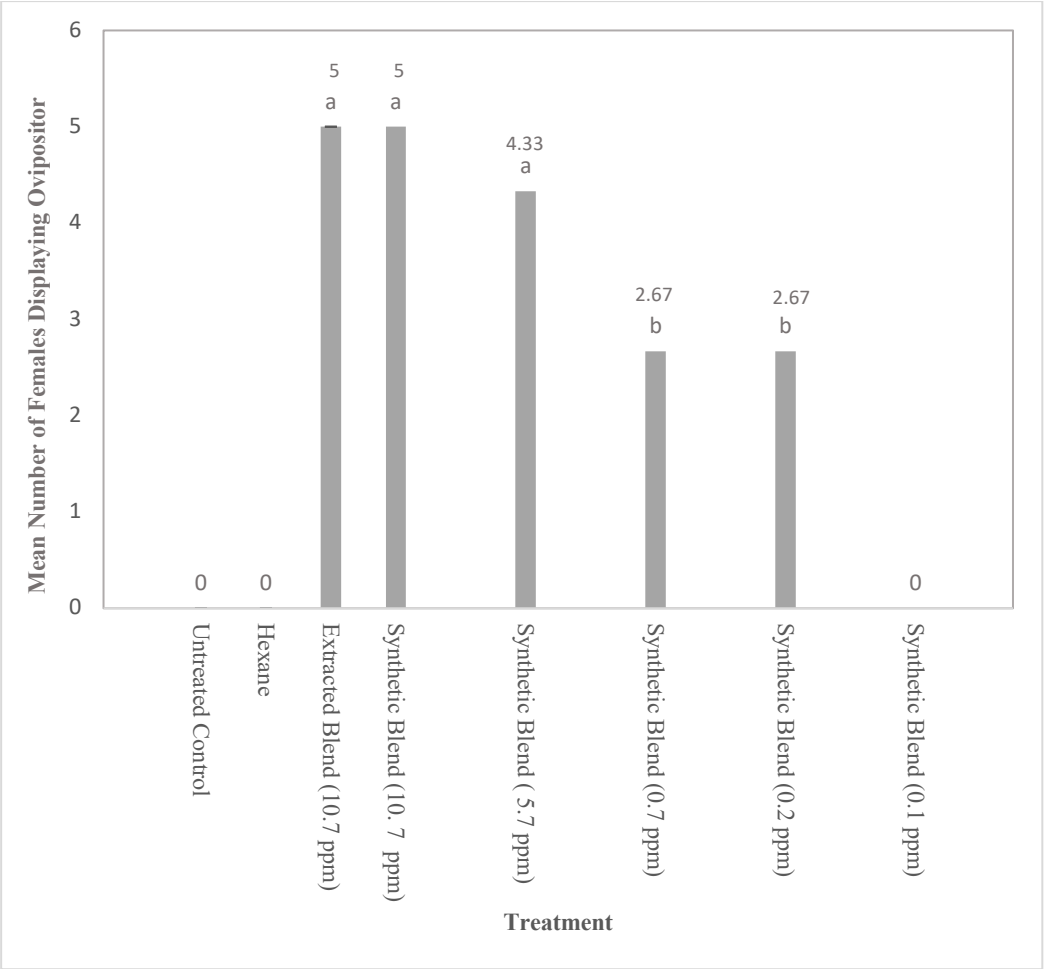


Figure 6.28: The graph shows that the extracted blend and synthetic blends at higher concentrations (10.7 ppm and 5.7 ppm) were more effective in inducing ovipositor display in female *A. grisella* compared to the control and lower concentration synthetic blends. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). Vertical Axis (Y-Axis): Number of females displaying ovipositor while walking and fanning. The Horizontal Axis (X-Axis) includes different treatments applied as follows Untreated Control, Hexane, Extracted Blend, Synthetic Blend at various concentrations:10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm and 0.1 ppm. In the untreated control, hexane, and synthetic blend at 0.1 ppm treatments, no females were observed displaying ovipositor (indicated by 0). Extracted Blend, Synthetic Blend (10.7 ppm, and 5.7 ppm): These treatments had the highest number of females displaying ovipositor, marked with the letter "a", indicating no significant difference among them. Synthetic Blend (0.7 ppm and 0.2 ppm): These treatments had a moderate number of females displaying ovipositor, marked with the letter "b", indicating they are significantly different from those marked "a".

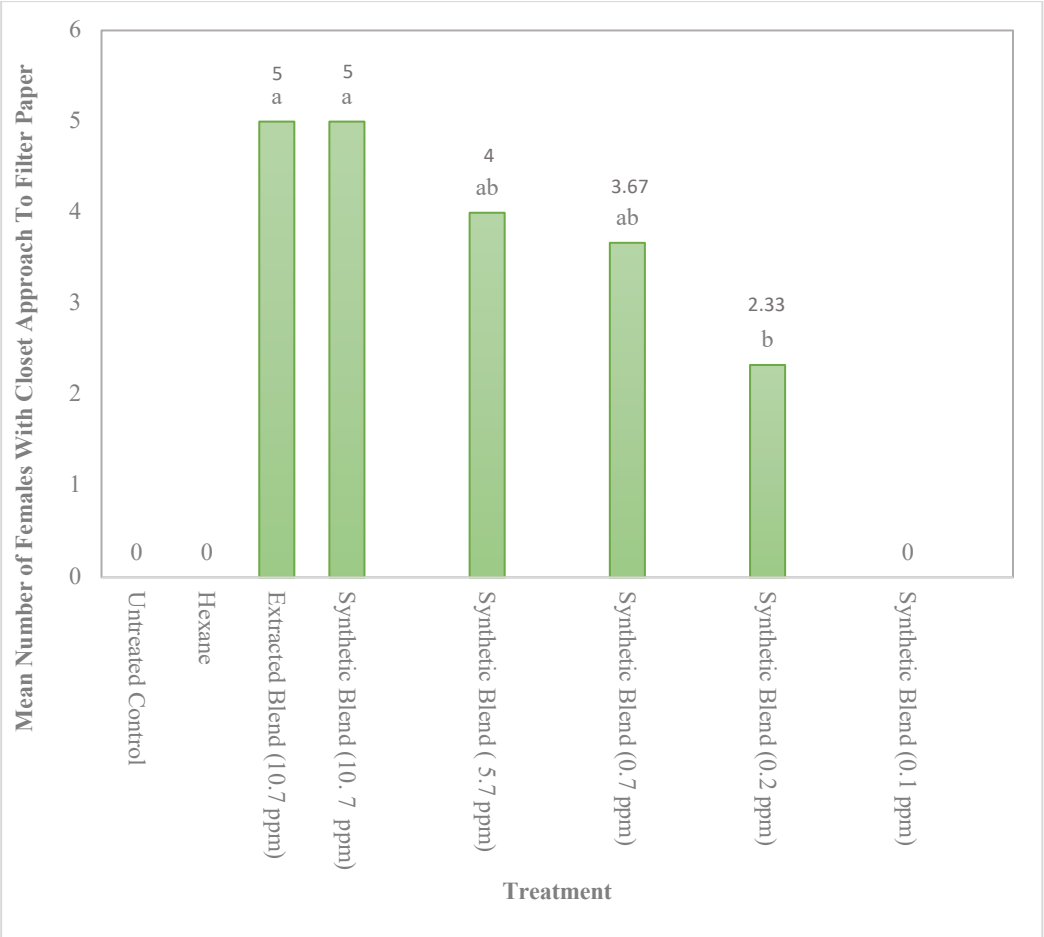


Figure 6.29: The graph illustrates *A. grisella* moths' closet approach to filter paper under various treatment conditions. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The approach distance is measured in centimetres, and the error bars represent the standard error (± SE) of these measurements. The vertical Axis (Y-Axis) indicates the distance in centimetres to the closest approach to the filter paper whereas the Horizontal Axis (X-Axis) includes lists the different treatments as Untreated Control, hexane, extracted blend, synthetic blend various concentrations 10.7 ppm, 5.7 ppm, 0.7 ppm, 0.2 ppm, 0.1 ppm. No Approach was observed in the untreated control, hexane, and synthetic blend at 0.1 ppm treatments, no females were observed approaching the filter paper (indicated by 0 cm). Approach to Filter Paper: Females showed varying degrees of approach in other treatments. Extracted Blend and Synthetic Blend (10.7 ppm) treatments tends the moth to approach the filter paper to the closest. Synthetic Blend (5.7 ppm and 0.7 ppm) treatments showed a moderate approach distance. Synthetic Blend (0.2 ppm): Moths showed a lesser approach compared to higher concentrations but still more than untreated control and hexane. Extracted Blend (10.7 ppm) and Synthetic Blend (10.7 ppm): Both treatments are labelled "a", indicating no significant difference between them. These treatments are significantly different from those labelled "b". Synthetic Blend (5.7 ppm and 0.7 ppm): These treatments are labelled "ab", indicating they are not significantly different from either "a" or "b". They represent an intermediate group. Synthetic Blend (0.2 ppm): This treatment is labelled "b", showing it is significantly different from "a" treatment but not from the "ab" group.

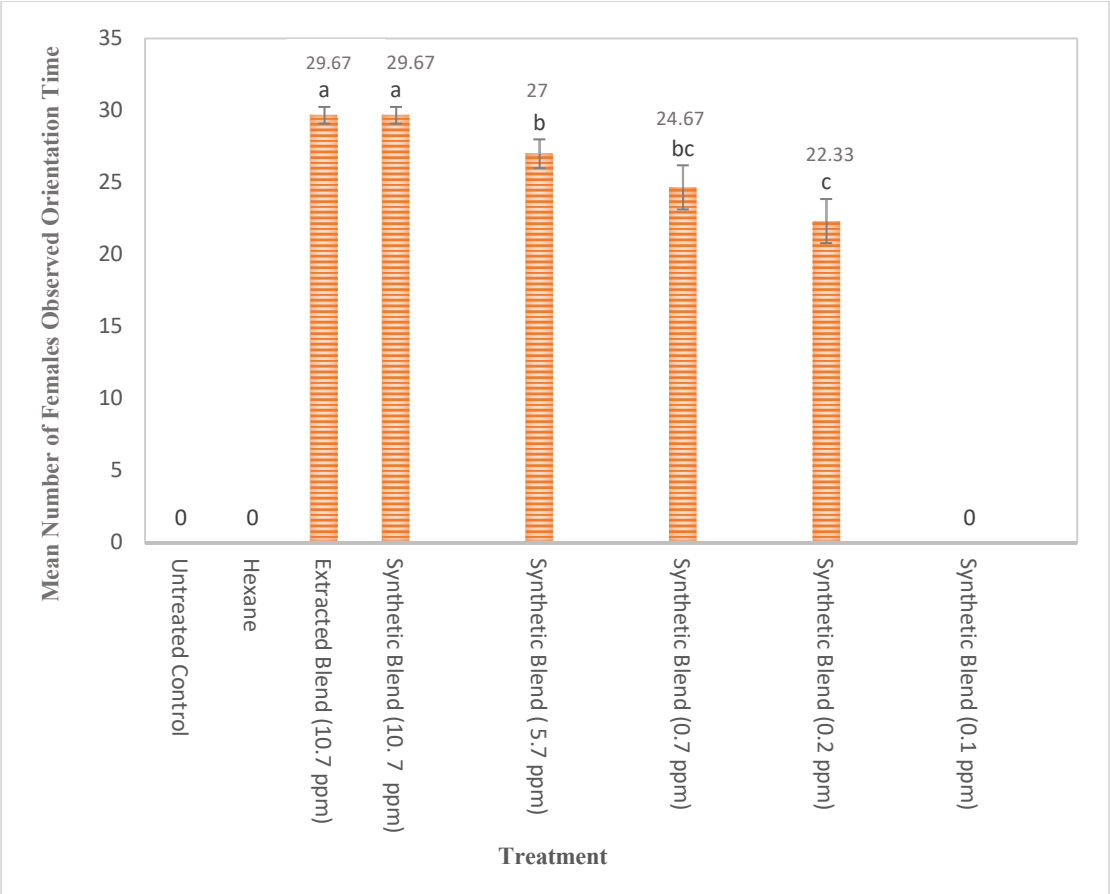


Figure 6.30: This graph illustrates how different treatments affect the orientation behaviour of female *A. grisella* showing that higher concentrations of synthetic blend and extracted blend elicit longer orientation times. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The Untreated Control and Hexane treatments both had an orientation time of 0 minutes, indicating no response. The Extracted Blend showed a high orientation time, similar to the highest concentration of Synthetic Blend at 10.7 ppm, and both were marked with 'a'. For the Synthetic Blends, the orientation time decreased with lower concentrations. The 5.7 ppm and 0.7 ppm treatments had intermediate times, with 5.7 ppm marked 'b' and 0.7 ppm marked 'bc'. The lowest concentration of Synthetic Blend at 0.1 ppm had the shortest orientation time among the responsive treatments, marked 'c'.

Table 6.7: Behaviour of *A. grisella* in observation arenas in response to mixtures of Synthetic blend and Extracted blend on Whatman No. 1 filter paper

Treatment on Whatman paper No.1	No. of females observed beginning upward flight	No. of females flying to 10 cm of arena	No. of females displaying ovipositor	No. of females having closet approach to filter paper	Observed orientation time (min) (± SE)
Untreated Control	0	0	0	0	0
Hexane (10.7 ppm and 0.1 ppm)	0	0	0	0	0
Extracted Blend (10.7 ppm)	$5.00^{a} \pm 0$	$5^{a} \pm 0$	$5^{a} \pm 0$	$4.67^{a} \pm 0.58$	$29.67^{a} \pm 0.33$
Synthetic Blend (10. 7 ppm)	$5.00^{a} \pm 0$	$5^{a} \pm 0$	$5^{a} \pm 0$	$5^{a} \pm 0$	$29.67^{a} \pm 0.33$
Synthetic Blend (5.7 ppm)	$4.00^{ab} \pm 0.58$	$4.33^{ab} \pm 0.33$	$4.33^{a} \pm 0.66$	$4.00^{ab} \pm 0.33$	$27.00^{b} \pm 0.58$
Synthetic Blend (0.7 ppm)	$3.33^{bc} \pm 0.33$	$3.67^{bc} \pm 0$	$2.67^{b} \pm 0.33$	$3.67^{ab} \pm 0.33$	$24.67^{bc} \pm 0.88$
Synthetic Blend (0.2 ppm)	$2.67^{c} \pm 0.33$	$3.33^{c} \pm 0.33$	$2.67^{b} \pm 0.33$	$2.33^{b} \pm 0.33$	$22.33^{c} \pm 0.88$
Synthetic Blend (0.1ppm)	0	0	0	0	0

Mean values followed with different superscripts are significantly different ($p<0.05$) using Tukey's test. Statistical groupings (denoted by letters such as 'a', 'b', 'c', etc.) indicate significant differences among the mean values at various concentrations.

The data presented in Table 6.7 demonstrates the behavioural responses of female *A. grisella* under different treatments, each associated with specific pheromone doses or control conditions. The results of the Factorial ANOVA consistently demonstrate the significant impact of the Dose factor on all the measured behaviours of *Achroia grisella*. The high F-values and low p-values indicate the strong and significant effects of the Dose factor across all behaviours. The relatively low Error MS values suggest

that the model explains a substantial portion of the variability in the behaviour, with the Dose factor accounting for most of the explained variance.

The grand means and CV values provide insights into the central tendency and variability of the behaviours across different doses. The CV values range from 5.87 to 27.79 per cent, with orient and arena having the lowest and highest variability, respectively. These findings contribute to a deeper understanding of how different doses influence the behaviours' of *Achroia* grisella, providing valuable insights for further research and practical applications.

This indicates that the nature of the pheromone and its specific effects on different aspects of moth behaviour are complex and context-dependent. The effect of volatile component quantity on the behaviour of the female *A. grisella* was assessed in windless arena bioassays. It highlights the significance of pheromone dosage in eliciting specific behavioural reactions. Understanding these dynamics is crucial for both ecological research and applications in pest management, as pheromones play a pivotal role in insect communication and mating behaviours. Further research may help unravel the intricate mechanisms behind pheromone signalling in moths and its broader ecological implications.

These results contribute to the broader understanding of insect-pheromone interactions and have practical implications for pest management and agricultural practices. Tailoring synthetic blends to mimic the attractive qualities of natural pheromones could offer effective tools for monitoring and controlling wax moth populations, reducing agricultural losses, and minimizing the need for conventional insecticides. The dose-dependent responses observed also pave the way for fine-tuning pheromone-based strategies for specific pest control applications.

6.6 Female *G. mellonella* and *A. grisella* Trapping Efficacy with Extracted and Synthetic Blends

The synthetic and extracted blends were tested for their ability to attract *G. mellonella* and *A. grisella* at varied concentrations at field level in beekeepers' apiaries with *Apis mellifera* species at Phillour, in three bee farms *i.e.* Alwaz Honey Bee Farm, Krishna Bee Farm, and Vicky Bee Farm. There were no geographical differences in trap catch

for both species. Monsoon to late autumn was covered by the trapping experiment from July to November. Commercial insect traps and rubber septum were used. To evaluate trapping efficiency, extracted and synthetic pheromone dosages were tested. Field trials employed extracted and synthetic pheromones at 15–100 ppm per µl hexane with each concentration and control hexane at 15 ppm for *G. mellonella* and control hexane 10.7 ppm for *A. grisella*. Regular trap monitoring provided wax moth population data and pheromone blend effectiveness. In synthetic and extracted mixes, all trap pheromone lures were replaced every 2 days to minimize temporal variation in plume concentration. Female moths in traps were counted and discarded after 2 days. Treatments were triplicated.

6.6.1 Trapping Efficiency of Female *G. mellonella* in Field Conditions

6.6.1.1 Control Groups and Baseline Observations

The control group, which included hexane and blend doses from 15 ppm to 35 ppm, showed no trapping of female *G. mellonella* for both the synthetic and extracted blends. This establishes a critical baseline, indicating that the solvent (hexane 15 ppm) and lower concentrations of the blends do not possess any significant attractive properties for the moths. This lack of response underscores the necessity of higher concentrations for eliciting an attraction response.

6.6.1.2 Effective Concentrations for Response Initiation

Starting from a dose of 40 ppm, a noticeable difference in the trapping efficacy of both synthetic and extracted blends is observed. At 40 ppm, the synthetic blend trapped a mean of (14.67±0.88) moths, while the extracted blend trapped a mean of (13.33±1.20) moths. This significant attraction at 40 ppm for the synthetic blend suggests a very strong initial response, whereas the extracted blend also starts to show noticeable activity.

6.6.1.3 Peak Trapping Efficiency and Optimal Dose Range

The most significant trapping efficiency was recorded within the dose range of 50 ppm to 70 ppm for both the synthetic and extracted blends. The synthetic blend at 50 ppm, exhibited a mean trapping of (23.00±1.53) moths, and the extracted blend showed a

mean of (22.67 ±0.88) moths. The synthetic blend at 60 ppm, achieved the highest mean trapping rate of (27.67 ±1.20) moths, while the extracted blend attracted (26.00±0.58) moths. The synthetic blend at 70 ppm trapped a mean of (21.33 ±1.20) moths and the extracted blend trapped (19.67±1.45) moths. These results suggest that doses within this range are optimal for attracting *G. mellonella*, demonstrating the highest levels of efficacy.

6.6.1.4 Decline in Attractiveness at Higher Doses

Beyond 70 ppm, there was a notable decline in the number of moths trapped, indicating a potential saturation point or deterrence at higher concentrations. The synthetic blend of 80 ppm trapped a mean of (22.00±0.58) moths, while the extracted blend trapped (20.33±1.20) moths. The synthetic blend at 90 ppm trapped (10.33±2.02) moths and the extracted blend trapped (9.33±1.20) moths. The synthetic blend at 100 ppm: trapped a mean of (10.00±1.15) moths, and the extracted blend trapped (9.00±1.15) moths. This trend suggests that extremely high doses may reduce the attractiveness of the blends, possibly due to oversaturation or repellent effects of the active compounds at high concentrations.

6.6.1.5 Comparative Efficacy and Analysis of Blends

Overall, the synthetic blend consistently showed higher trapping means compared to the extracted blend across most concentrations, particularly at the optimal range of 50 ppm to 70 ppm. This could be attributed to the purity and precise composition of synthetic blends, which might be more consistent in releasing the active attractant compounds as compared to the natural variability found in extracted blends. The highest trapping efficacy was observed at (27.67±1.20) with 60 ppm synthetic blend. At 50 ppm, Extracted Blend showed a high efficacy with (22.67±0.88) moths. The differences in their statistical groupings suggest variability in moth response to different concentrations, highlighting the importance of selecting the right dose to achieve the best trapping results.

6.6.1.6 Total Mean Comparison

The total mean trapping for the synthetic blend is 9.92, and for the extracted blend is 9.28, showing no significant overall difference between the two blends (Figure 6.33).

6.6.1.7 Analysis of Trap Catch in Relation to Blend, Dose, and Their Interaction in Female, *G. mellonella*

The data indicates significant differences in the mean number of moths trapped at different dose levels in apiaries with *Apis mellifera* species, providing insights into the attractiveness of both synthetic and extracted blends (Figure 6.31 and 6.32). The Table 6.8 shows the mean number of female *Galleria mellonella* moths trapped at different doses (ppm) of synthetic and extracted blends of pheromone components along with the standard error (SE) for each measurement. This study investigated the effect of synthetic and extracted blends at various concentrations on the trapping of *G. mellonella* (greater wax moth). The factorial analysis of variance (ANOVA) and subsequent Tukey HSD test results are presented to elucidate the effects of blend type, dose, and their interaction on the number of moths trapped.

The ANOVA analysis for the Dose factor demonstrated a highly significant effect on the trapping outcome ($F=271.97$ $p<0.0001$) indicating that the amount of pheromone used is crucial in determining the effectiveness of the trap. The blend factor showed no significant difference in the mean number of trapped moths between Synthetic Blend (9.92) and the Extracted Blend (9.28). The effect of blend type (synthetic vs. extracted) on the number of moths trapped was not statistically significant ($F=3.63$, $p=0.062$). Although the mean number of moths trapped was slightly higher for the synthetic blend (9.92) compared to the extracted blend (9.28), this difference did not reach statistical significance.

This suggests that, on average, the synthetic and extracted blends are similarly effective in attracting *G. mellonella* and there is no significant difference in attractiveness between the synthetic and extracted blends. Both blends performed similarly in attracting female *G. mellonella* moths. In the case of Blend*Dose Interaction, there is no significant interaction ($P = 0.98$) between blend type and dose, suggesting that the effect of dose on moth trapping is consistent across both synthetic and extracted blends. The interaction between Blend and Dose was not significant ($F=0.34$, $p = 0.9781$). This suggests that the effect of dose on trapping efficiency is consistent across both blend types, indicating no interaction effect. In other words, the

dose-response relationship for the synthetic and extracted blends does not differ significantly. The trapping efficacy was primarily influenced by the individual factors of Blend and Dose implying that the dose-response is consistent across both synthetic and extracted blends. Additionally, the grand mean of Trap catch was calculated to be 9.59, providing a central reference point for the data. The coefficient of variation was found to be 16.10 per cent, indicating a moderate level of variability in the Trap catch values.

The Tukey HSD pairwise comparisons grouped the doses based on their mean number of trapped moths. Dose 60 ppm had the highest mean (26.83), forming the top homogeneous group (Group A). This indicates that a dose of 60 ppm was the most effective in attracting female *G. mellonella* moths. Doses 50, 80, and 70 ppm formed the next group (Group B), with intermediate trapping rates significantly lower than the peak at Dose 60 ppm. Doses 40, 90, and 100 ppm formed Group C, with the lowest trapping rates, significantly lower than the optimal Dose 60 ppm. Doses 0, 15, 20, 25, 30, and 35 ppm did not result in any trapped moths, forming the lowest homogeneous group (Group E).

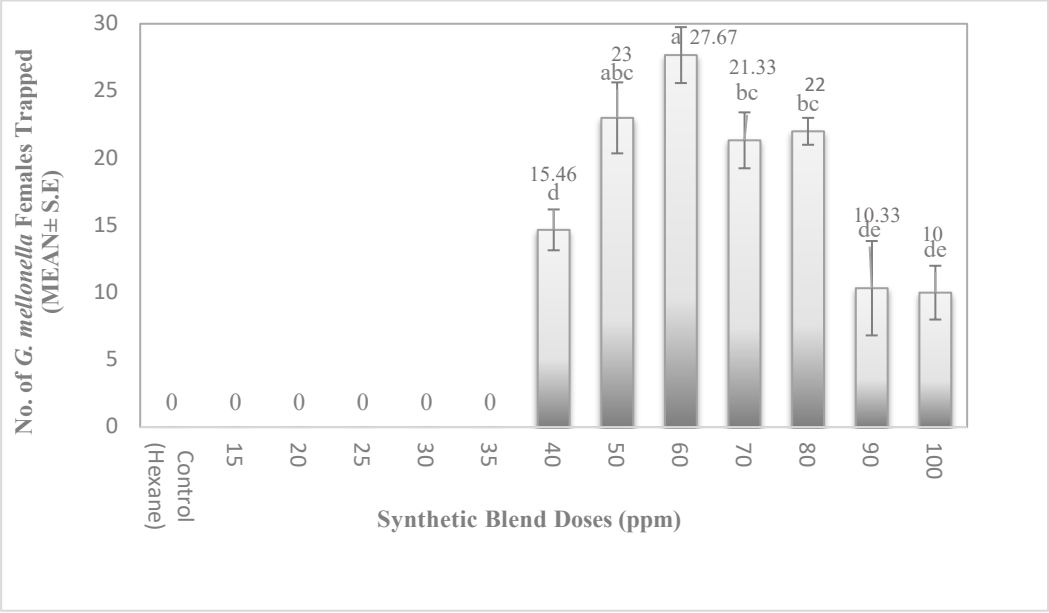


Figure 6.31: The graph illustrates the mean number of female *G. mellonella* insects trapped at various doses of a synthetic blend, measured in parts per million (ppm). The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The y-axis represents the mean number of females trapped along with the standard Error (S.E.), while the x-axis denotes the doses in (15-100) ppm. A control (solvent-only) group is also included for comparison. 35 ppm: A small number of females were trapped, marked with the letter "d", indicating a significant difference from higher doses.40 ppm to 100 ppm: Higher doses showed varying

numbers of females trapped. 40 ppm: Marked with "c", showing a moderate number of females trapped. 50 ppm: Marked with "abc", showing a higher number of females trapped.60 PPM: Marked with "a", showing the highest number of females trapped, indicating a peak in effectiveness.70 ppm: Marked with "ab", showing a slightly lower number than 60 PPM but still high.80 ppm: Marked with "bc", showing a moderate number of females trapped.90 ppm and 100 ppm: Marked with "de" and "e" respectively, showing a decrease in the number of females trapped compared to the peak doses

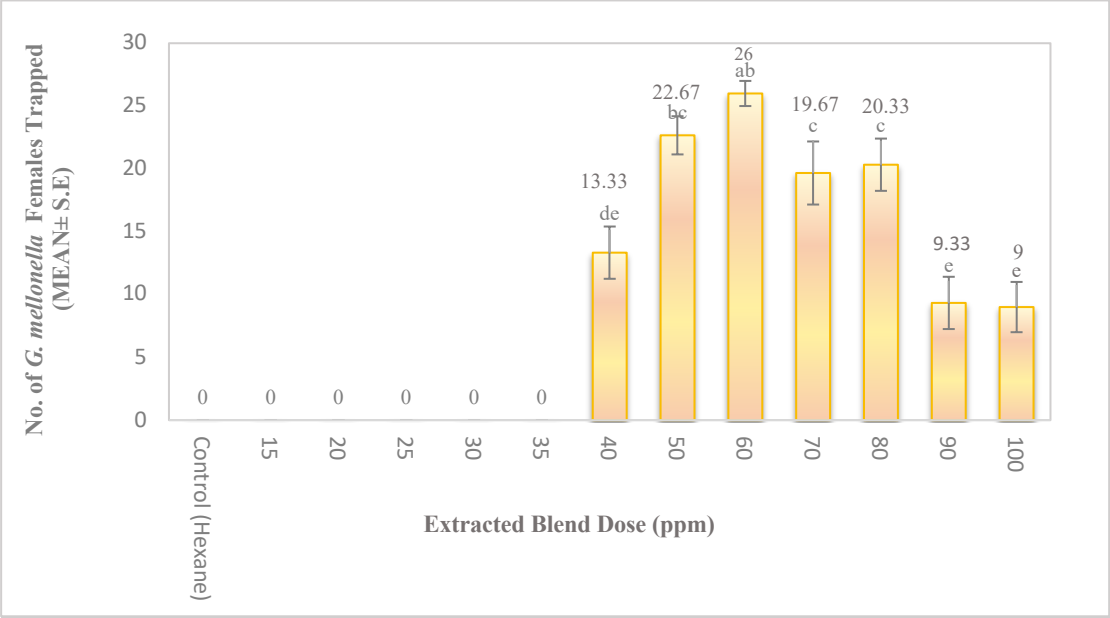


Figure 6.32: Trapping effectiveness of an extracted blend on female *G. mellonella* at different doses (ppm). The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The mean (± S.E) number of females trapped is shown, with the highest efficacy observed at 60 ppm, followed by a decline at 90 ppm and 100 ppm. The statistical significance shows that the number of trapped females increases with the dose, peaks at 60-70 ppm, and then decreases at higher doses. Control/Hexane: The control treatments (hexane) resulted in 0 females being trapped, indicating no response to these treatments. Doses (35 to 100 ppm): The response varied with different doses of the Extracted Blend: 35 ppm: Showed a lower response with fewer females trapped, marked with 'de'. 40 ppm: Slightly higher response, marked with 'd', indicating no significant difference from 35 ppm but different from higher doses. 50 ppm: Increased response, marked with 'bc', showing a statistically intermediate response. 60 ppm: Highest response, marked with 'ab', indicating a strong and significant response. 70 ppm: Also high response, marked with 'c', indicating a strong but slightly different response from 60 ppm. 80 ppm: Marked with 'f', showing a decrease in response compared to 60 and 70 ppm, statistically significant. 90 ppm and 100 ppm: Marked with 'e', indicating a lower response similar to each other and significantly different from other higher doses

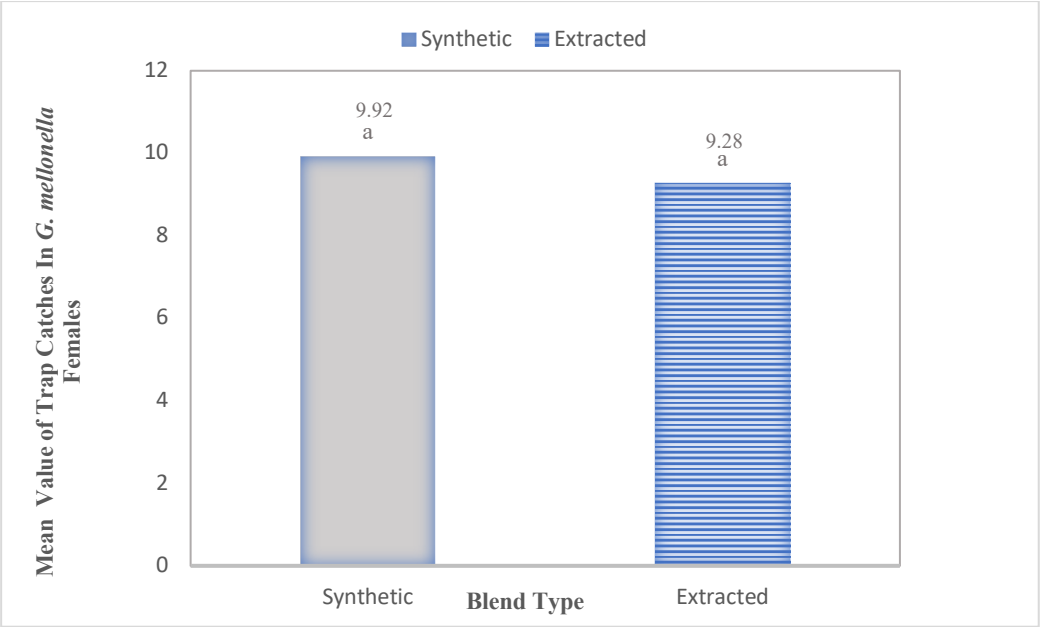


Figure 6.33: The bar graph compares the mean values of two different types of blends: synthetic and extracted. (Y-Axis) Mean value of the trap catches in *G. mellonella* (X-Axis): Different types of blends. Both synthetic and extracted blends are marked with the letter "a", indicating no significant difference between them. Tukey pairwise comparison test of trap catches for blend in *G. mellonella* with no significant pairwise differences among the means.

6.6.2 Trapping efficacy of female *A. grisella* in field conditions

6.6.2.1 Control Groups and Baseline Observations

The control group, which included hexane and blend doses from 15 ppm to 35 ppm, showed no trapping of *Achroia grisella* for both the synthetic and extracted blends. This establishes a critical baseline, indicating that the solvent (hexane 15 ppm) and lower concentrations of the blends do not possess any significant attractive properties for the moths. This lack of response underscores the necessity of higher concentrations for eliciting an attraction response.

6.6.2.2 Effective Concentrations for response initiation

Starting from a dose of 40 ppm, a noticeable difference in the trapping efficacy of both synthetic and extracted blends was observed. At this dose, the synthetic blend trapped a mean of 4.67 moths, while the extracted blend trapped a mean of 3.00 moths. Specifically, the synthetic blend trapped a mean of (4.67±1.20) moths, while the extracted blend trapped a mean of (3.00±0.58) moths. Although this level of

attractiveness is relatively low compared to higher concentrations, it marks the threshold where the blends begin to effectively attract moths. This suggests that concentrations below 40 ppm are insufficient, but starting from 40 ppm, the blends start to have a noticeable impact.

6.6.2.3 Peak Trapping Efficiency/ Optimal Dose Range

The most significant trapping efficiency was recorded within the dose range of 50 ppm to 70 ppm for both the synthetic and extracted blends. At 50 ppm, the synthetic blend exhibited a mean trapping of (19.67±3.38) moths, and the extracted blend showed a mean of (15.67±1.20) moths. The effectiveness of these concentrations is further increased at 60 ppm, where the synthetic blend achieved the highest mean trapping rate of (20.00 ±3.22) moths, while the extracted blend attracted (18.33 ±0.88) moths. Even at 70 ppm, the blends continued to perform well, with the synthetic blend trapping a mean of (16.33 ±2.96) moths and the extracted blend attracting (15.33 ±2.02) moths. These results suggest that doses within this range are optimal for attracting *A. grisella,* demonstrating the highest levels of efficacy.

6.6.2.4 Decline in Attractiveness at Higher Doses

Beyond 70 ppm, there was a notable decline in the number of moths trapped, indicating a potential saturation point or deterrence at higher concentrations. At 80 ppm, the synthetic blend trapped a mean of (11.67±0.88) moths, while the extracted blend trapped (10.33 ±1.20) moths. The reduction in trapping continued at 90 ppm, with the synthetic blend trapping (7.67±1.45) moths and the extracted blend attracting (5.33 ±1.45) moths. At the highest tested concentration of 100 ppm, the synthetic blend trapped a mean of only (5.67±0.88) moths, and the extracted blend trapped (4.33±0.88) moths. This trend suggests that extremely high doses may reduce the attractiveness of the blends, possibly due to oversaturation or repellent effects of the active compounds at high concentrations.

6.6.2.5 Comparative Efficacy and Analysis of Blends

Overall, the synthetic blend consistently showed higher trapping means compared to the extracted blend across most concentrations, particularly at the optimal range of 50

ppm to 70 ppm. This could be attributed to the purity and precise composition of synthetic blends, which might be more consistent in releasing the active attractant compounds as compared to the natural variability found in extracted blends. The synthetic blend at 60 ppm ($20.00^{a}\pm3.22$) and the extracted blend at 50 ppm (18.33^{ab} ±0.88) are both highly effective, but the differences in their statistical groupings suggest variability in moth response to different concentrations. This highlights the importance of selecting the right dose to achieve the best trapping results.

6.6.2.6 Total Mean Comparison

The total mean trapping for the synthetic blend was 6.59 moths, and for the extracted blend it was 5.57 moths, showing no significant overall difference between the two blends (Figure 6.36).

6.6.2.7 Analysis of Trap Catch in Relation to Blend, Dose, and Their Interaction in Female, *A. grisella*

This study investigated the effect of synthetic and extracted blends at various concentrations on the trapping of female, *A. grisella* (lesser wax moth) (Figure 6.34 and 6.35) in apiaries with *Apis mellifera* species. The factorial analysis of variance (ANOVA) and subsequent Tukey HSD test results are presented to elucidate the effects of blend type, dose, and their interaction on the number of moths trapped. The factorial ANOVA analysis conducted on the variable Trap catch revealed significant insights into the effects of the factor's Dose and Blend on the outcome. The results show that the Dose factor exhibited a highly significant effect on Trap catch ($f=63.35$, $p < 0.0001$), indicating that varying Dose levels had a substantial impact on the outcome. The Tukey HSD test identified distinct homogeneous groups based on Dose levels, with higher doses corresponding to higher Trap catch values. This suggests a clear dose-response relationship while the Blend factor showed a marginally significant effect on trap catch ($f=3.98$,$p = 0.0513$), implying a minor influence compared to Dose.

The Tukey HSD test for Blend did not find significant pairwise differences between the two blend levels, synthetic blend (6.59) and extracted blend (5.57), indicating that Blend variation had a limited effect on Trap catch. The interaction

between Dose and Blend was not significant (f=0.43, p = 0.94), suggesting that the combined effect of these factors on Trap catch remained consistent across different levels. This indicates that the factors did not interact in a way that significantly altered Trap catch. Additionally, the grand mean of Trap catch was calculated to be 6.077, providing a central reference point for the data. The coefficient of variation was found to be 37.36 percent, indicating a moderate level of variability in the Trap catch values. The Tukey HSD test for Dose identified 4 homogeneous groups (A, B, C, D) based on mean Trap catch values for different Dose levels. This grouping helps understand the relative impact of each Dose level on Trap catch. Doses of 60, 50, and 70 ppm form the highest group (A), while Doses of 0, 15, 20, 25, 30, and 35 ppm form the lowest group (D). The other Dose levels fall into intermediate groups.

The Tukey HSD test for blend analysis did not find any significant pairwise differences between the means of Trap catch for the two Blend levels. Both Blend levels belong to the same homogeneous group (A), indicating that the Blend factor does not have a significant effect on Trap catch. Dose*Blend Interaction- The interaction between Dose and Blend was also analyzed using Tukey's HSD test. The Tukey HSD test identified 7 homogeneous groups (A, B, C, D, E, F, and G) based on the means of Trap catch for different combinations of Dose and Blend levels. The highest mean (20.0) corresponds to Dose 60 with Synthetic Blend , while the lowest means (0.00) correspond to various combinations of low Dose levels with both Blend levels.

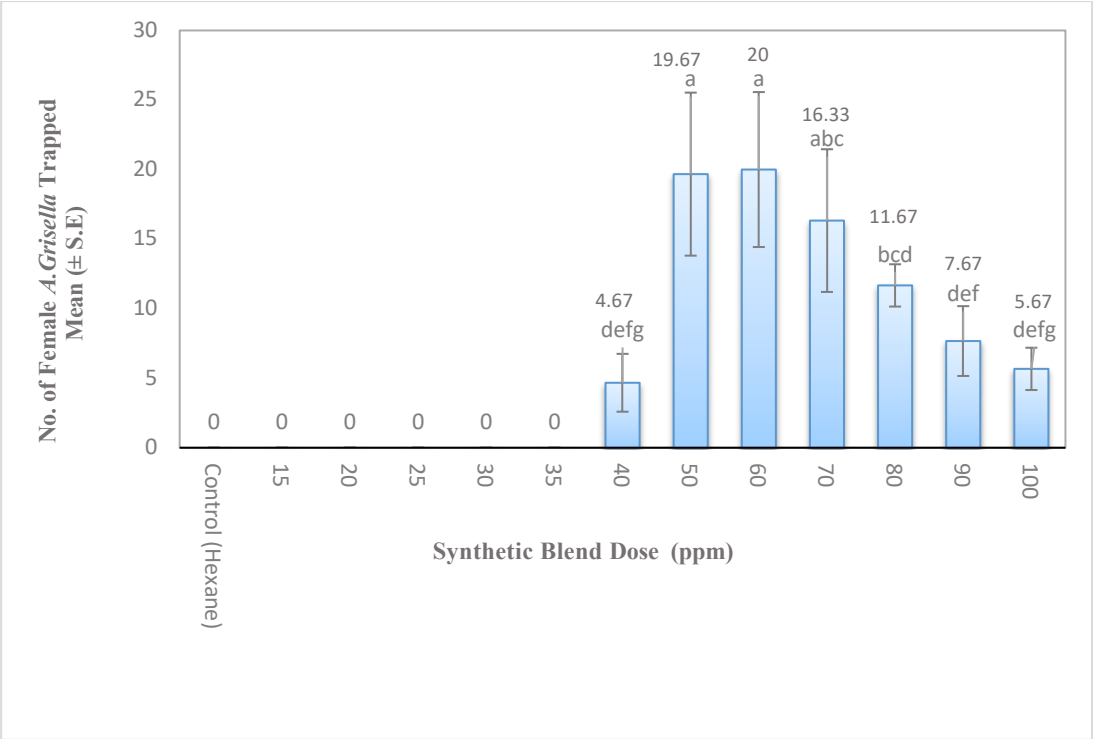


Figure 6.34: The graph represents the mean number of female *A. grisella* trapped in response to different doses of the Synthetic Blend (measured in ppm), including control treatments (Hexane). The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The y-axis represents the mean number of trapped females (Mean ± SE), and the x-axis represents the dose in ppm. Control/Hexane (0 ppm): The control treatments (Hexane) resulted in 0 females being trapped, indicating no response to these treatments. Doses (35 to 100 ppm): The response varied with different doses of the Synthetic Blend:35 ppm: Showed a low response with fewer females trapped, marked with 'defg'. 40 ppm: Slightly higher response, marked with 'def', indicating no significant difference from 35 ppm but different from higher doses. Concentration of 50 ppm and 60 ppm- The highest responses were observed at these doses, marked with 'a', indicating they are statistically similar and elicited the strongest response. Concentration of 70 ppm: This dose had a high response, marked with 'abc', indicating it is not significantly different from 50 and 60 ppm but significantly different from lower and higher doses.80 ppm: Showed a decreased response, marked with 'bcd', indicating a statistically intermediate response. 90 ppm and 100 ppm: These doses had the lowest responses among the higher doses, marked with 'defg' and 'defg', respectively, indicating no significant difference from each other but significantly different from the higher responses at 50, 60 and 70 ppm

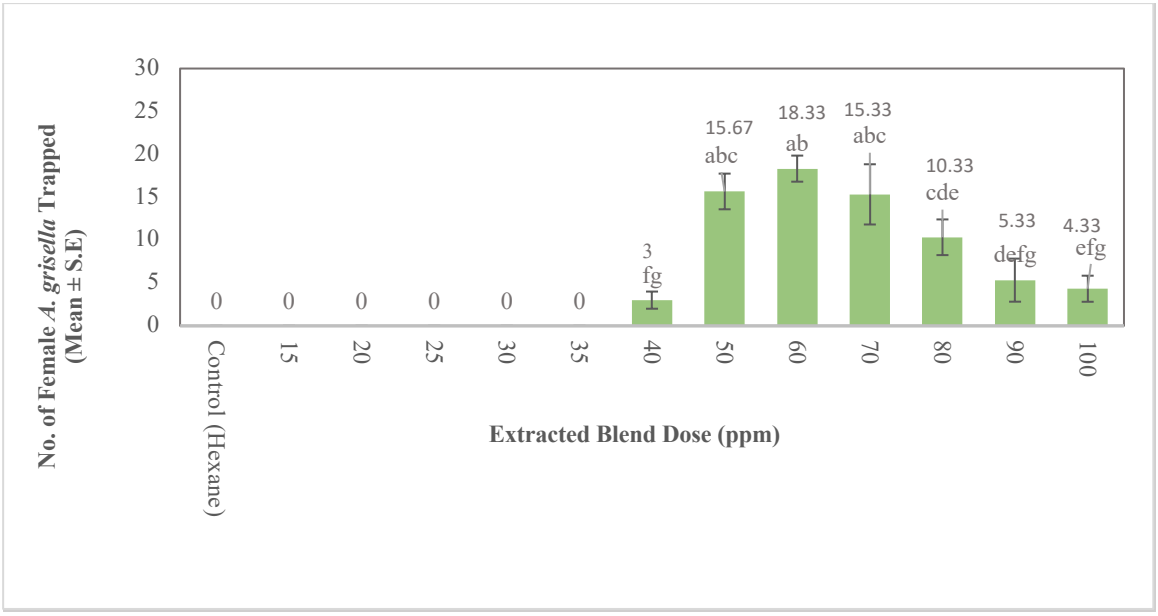


Figure 6.35: The effect of varying doses of an extracted blend on trapping female *A. grisella*. The data represents mean ± S.E. (n=3); $p<0.05$ (One-way ANOVA followed by Tukey's Significant difference test). The data shows mean (± S.E) number of trapped females, indicating the trapping efficiency peaks at 60 ppm, showing the highest mean number of trapped females, and decreases at higher concentrations. Control/Hexane: The control treatments resulted in 0 females being trapped, indicating no response to these treatments. Doses (15 to 100 ppm): The response varied with different doses of the Extracted Blend:15 to 35 ppm: These doses resulted in 0 females being trapped, indicating no response similar to the control treatments.40 ppm: This dose resulted in a low number of trapped females, marked with 'fg', indicating a significantly lower response compared to higher doses.50 ppm: This dose showed a higher response, marked with 'abc', indicating it is statistically similar to other high-response doses (60 and 70 ppm) but different from lower doses.60 ppm: This dose had the highest response, marked with 'ab', indicating a strong and statistically significant response compared to lower and higher doses.70 ppm: This dose also showed a high response, marked with 'abc', indicating no significant difference from 50 and 60 ppm.80 ppm: This dose showed a decreased response, marked with 'cde', indicating a statistically intermediate response. 90 ppm: Showed a lower response compared to peak responses, marked with 'dfg', indicating a significant difference from the highest responses.100 ppm: The lowest response among the higher doses, marked with 'efg', indicating a statistically significant lower response compared to peak responses but similar to lower response doses like 40 ppm.

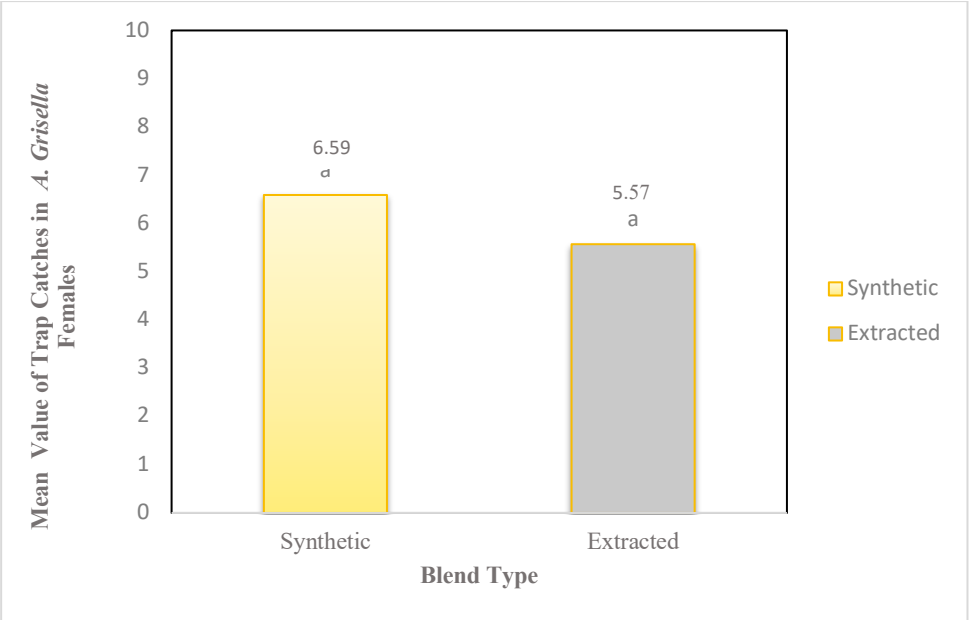


Figure 6.36: The bar graph compares the mean values of two different types of blends: synthetic and extracted. (Y-Axis) Mean value of the trap catches in *A. grisella* (X-Axis): Different types of blends. Both synthetic and extracted blends are marked with the letter "a", indicating no significant difference between them. Tukey pairwise comparison test of trap catches for blend in female *A. grisella* with no significant pairwise differences among the means.

Table 6.8: Field trapping of *G. mellonella* and *A. grisella* of female moths with different doses of synthetic and extracted blends

Dose (ppm)	No. of female *Galleria mellonella* trapped (Mean ±SE)		No. of female *Achroia grisella* trapped (Mean ±SE)	
	Synthetic blend	Extracted blend	Synthetic blend	Extracted blend
Control (15 Hexane)	0	0	0	0
15	0	0	0	0
20	0	0	0	0

25	0	0	0	0
30	0	0	0	0
35	0	0	0	0
40	15.46 ^{d} ±0.88	13.33^{de} ±1.20	4.67 ^{efg} ±1.20	3.00 ^{fg} ±0.58
50	23.00 ^{abc} ±1.53	22.67 ^{bc} ±0.88	19.67 ^{a} ±3.38	15.67 ^{ab}±1.20
60	27.67^{a} ±1.20	26.00 ^{ab}±0.58	20.00 ^{a} ±3.22	18.33 ^{ab} ±0.88
70	21.33 ^{bc} ±1.20	19.67 ^{c} ±1.45	16.33 ^{abc} ±2.96	15.33 ^{abc}±1.45
80	22.00^{bc} ±0.58	20.33^{c} ± 1.20	11.67 ^{bcd} ±0.88	10.33^{cde}±1.20
90	10.33 ^{dc} ±2.02	9.33 ^{e} ±1.20	7.67 ^{def} ±1.45	5.33 ^{defg} ±1.45
100	10.00 ^{dc} ±1.15	9.00^{c} ±1.15	5.67^{defg} ±0.88	4.33 ^{efg} ±0.88
Grand Mean	9.9231 ^{a}	9.2821 ^{a}	6.589^{a}	5.564 ^{a}

Mean values followed with different superscripts are significantly different ($p<0.05$) using Tukey's test. Statistical groupings (denoted by letters such as 'a', 'b', 'c', etc.) indicate significant differences among the mean values at various concentrations

a


b

Figure 6.37: Field trap installation in a) Alwaz Bee Farm b) Krishna Bee Farm c) *A. grisella* females captured at Alwaz Bee Farm d) *A. grisella* females captured at Vicky Bee Farm e) *A. grisella* females captured at Krishna Bee Farm f) *G. mellonella* females captured at Alwaz Bee Farm g) *G. mellonella* females captured at Vicky Bee Farm h) *G. mellonella* females captured at Krishna Bee Farm

Therefore, its necessary to discuss that economically, the profitability of beekeeping operations is negatively affected by the reduced honey yields caused by destruction of combs by the wax moths, which also incurs additional costs for hive restoration and the replacement of damaged equipment. Severe damage of combs by the wax moths also results in the loss of honey which is consumed by bees for construction of new combs in addition to undesirable wastage of working hours of honey bees in the construction and repair of comb. In terms of ecology, the reduction in pollination services that can result from the decline in bee populations as a result of wax moth infestations can have an impact on the stability of the ecosystem and the productivity of agriculture resulting in diminished colonies and an increased susceptibility to other threats (Castellanos-Potenciano et al., 2024; Parejo et al., 2024).

During the present research work, preliminary work on the Androconial gland of *G. mellonella* and *A. grisella* has been done under the stereo microscope and SEM. The study revealed the bulb-shaped structure of the androconial gland with the morphometry details and location. The length of the gland of the Greater Wax Moth is 1.33 mm, and the breadth is 4.74 mm. The length of the gland of the Lesser Wax Moth is 1.23 mm, and its breadth is 2.33 mm. The gland for both of the male species was found on the lower proximal side of the forewing (Smith,1965). **This study under stereomicroscope and SEM has not been previously reported in the scientific literature.**

For the present research work, the methodology of Lebedeva et al. (2002) was followed. 2-7-day-old males of each species (*G. mellonella* and *A. grisella*) were collected during 2-5 PM. Three replicates of six extractions were done on adult, male *G. mellonella* and *A. grisella*. The combination was quantified in a volumetric flask for analysis. All samples remained at -30°C until usage. Based on GC-MS chemicals, quantitative analysis was done. The Procurement of chemicals and comparison to the sample was done for GC-MS quantification.

The compounds quantified in the present study of male *G. mellonella* were Aldehydes namely nonanal (5.218 ppm, 6.182 min RT, 1359472 area), undecanal (7.162 ppm, 12.251 min RT, 10873560 area), heptadecane (0.203 ppm,18.005 min RT, area 3799665), heneiocosane (0.267 ppm, 22.118 min RT, area 749917) and alcohols

namely 1-nonanol (1.181ppm, 6.937 min RT, area 954113). **Heptadecane and heneiocosane, are the newly reported compounds in male, *G. mellonella* whereas Cis 9 hexadecenal was newly reported compound in *A. grisella*.** The findings can be further explored to understand their impact on moth behaviour, mating, and communication.

The isolation and identification of five compounds were the findings of the chemical analysis of the volatiles of male *G. mellonella and* two compounds of *A. grisella.* These compounds included alcohols, aldehydes, and fatty acids of undecane and nonane. Lepidopteran sex pheromones are typically composed of two to seven components, which are either non-cyclic (in the case of females) or heterocyclic (often, in the case of males), and contain functional groups such as acetates, alcohols, or aldehydes (Baker, 1989).

In the previous study, chemical analyses were performed on the pheromone-gland extracts and volatiles released by the *G. mellonella* (L.). Aldehydes, primary alcohols, and fatty acids from nonane and undecane were among the substances that could be identified and quantified in this investigation. The gland extracts of *G. mellonella* included the following average proportions of aldehydes and alcohols: 19.0% undecanal, 3.9% nonanal, 48.3% 1-undecanol, and 28.8% 1-nonanol (Romel et al., 1992). Numerous insects employ precise ratios of multi-component mixtures of sex pheromones as species-specific communication signals. Despite the fact that minor chemical components only account for a small percentage of the total volatiles, they may be essential in eliciting critical behavioural interactions (Christensen et al., 1989).

In previous work, Leyer and Monroe (1973) conducted quantification tests using Gas Liquid Chromatography (GLC) and discovered that the production of nonanal was 236 µg per male *G. mellonella* moth during the first 24-hour period and 178 µg during the second 24-hour period. The undecanal production for the same periods averaged 100 µg per male first 24 hr period and 82 µg for the second 24 hr period. Also, the studies on pheromonal volatiles of Greater Wax Moth reported two aldehydes, nonanal and decanal (Flint and Merkle, 1983). Other minor components include decanal, hexanal, heptanal, the fatty acid of nonane and undecane, and carboxylic acids like nonanoic acid and undecanoic acid, alcohols such as 1- undecanol, 1-nonanol are reported by Svensson *et al*., 2014 which supports the present study.

In the present study volatile pheromone compounds identified in the *A. grisella* includes aldehydes namely undecanal (8.745 ppm, 12.251 min RT, 13364857 area), cis- 9-hexadecenal (1.819 ppm, 20.393 min RT, 902999 area). The two compounds odour was chemically identified as a combination of undecanal and cis-11-octa decanal in *A. grisella* (Dahm et al., 1971). **The compound Cis-9-hexadecenal as a component of male *A. grisella* pheromone has not been previously reported in the literature.** Therefore, contribution of it in making pheromone trapping more effective is foreseen.

In the present section, the behavioural response of *G. mellonella* and *A. grisella* has been discussed. The higher concentrations of the synthetic and extracted blends were more effective in eliciting various behavioural responses in the moths, including upward flight, flight to the 10 cm arena area, ovipositor display, closest approach to filter paper, and orientation time on the filter paper. The extremely low p-values (P=0.00) across all measures suggest strong evidence against the null hypothesis, indicating that treatments have a significant impact on these behaviours.

The Tukey HSD test further clarifies these differences by grouping the treatments into homogeneous subsets. For all behavioural measures, the untreated control group (untreated control) and the hexane groups consistently show the nil mean values, indicating nil behavioural response. On the other hand, treatments such as extracted Blend and higher concentrations of synthetic blend consistently show the highest mean values, indicating a strong behavioural response the synthetic blends and the extracted blend had a significant impact on various behavioural responses of female moths, with the extracted blend and higher concentrations of Synthetic Blend (synthetic blend 15 ppm and synthetic blend 10 ppm) in case of *G. mellonella* and Synthetic Blend (synthetic blend 10.7 ppm and synthetic blend 5.7 ppm) in case of *A. grisella* generally showing the highest levels of upward flight, flight to the 10 cm arena, ovipositor display, closest approach to Whatman paper, and orientation time. These findings suggest that the higher concentrations of the synthetic and extracted blends were more effective in attracting and eliciting the desired behavioural responses in female moths compared to the lower concentrations. Moreover, these findings will also act as baseline data for similar laboratory studies to be conducted in the future. This present investigation provides the threshold concentration level at which the response in wax moth female moths is initiated.

Finn and Payne (1977) and Flint and Merkle (1983) have previously reported that the aldehydes are a significant factor in the attraction of female *G. mellonella*. Despite the fact that the acid and alcohol comprise a lesser proportion of volatiles than aldehyde, they may still be a significant element of the pheromonal composite. Experiments using nonanal and undecanal combinations in 7:3 intercepted males looking for females (Flint and Merkle 1983). Sweeney et al. (1990) have demonstrated that minor components of the pheromone may not be attractive on their own; however, when combined with major components, they elicit a more robust response than a major component alone. This has been demonstrated in studies of other species.

According to Cadre and Hagman, 1979, the moths show a positive response to the odour source by the flight orientation. Female greater wax moth (GWM), *G. mellonella* Linnaeus responded to different binary blends of undecanal and nonanal in the percent ratio of 95:5, 90:10, 85:15, 80:20, 60:40, 50:50, 30:70, 20:80, and 10:90 (Sangramsinh et al., 2014).

The results of the previous literature suggest that the impact of the ratios of volatile components on the behaviour of the female GWM was evaluated in windless arena bioassays suggesting that female GWMs are drawn to a diverse array of volatile combinations, which indicates a broad and adaptable response specificity. Only mixtures that release C11:AL to C9:AL in a ratio of 4:1, either in the presence of minor alcohols or with a 1:4 output of C11:AL to C9:AL, caused behaviours such as searching or circling the surface of the holding cage (Fraser, 1997). According to Svensson et al. (2014), when female wax moths were exposed to male extract, which is presumably optimal, their orientation behaviour exhibited characteristics similar to those that are typically observed when male moths are tracking suboptimal pheromone plumes. This includes extended flights back and forth across the wind direction, with limited upwind progress. Furthermore, Bhopale et al. (2016) demonstrated that in the behaviour bioassay during commencement or first quarters of the scotophase the moths of 3 to 5 days old displayed greatest responses to the best binary blend of nonanal and undecanal (3:7). With more than 60% moths displaying pheromone specific behavioural patterns, highest behavioural responses to the best pheromone mix of undecanal and nonanal (3:7). During the scotophase, the male *A. grisella* has been observed to remain

stationary and fan its wings continuously in the upper parts of the plexiglass cage (Kunike, 1930).

The study evaluated the field trapping efficacy of female *Galleria mellonella* (GWM), emphasizing the pivotal role of pheromone dose in influencing trap catch. Laboratory wind tunnel experiments initially employed concentrations of 15 ppm for both synthetic and extracted blends, but this dose failed to elicit a field response. Consequently, higher concentrations were assessed under field conditions. Responses were initiated at doses exceeding 40 ppm, with 60 ppm identified as the most effective dose for attracting GWM females. While the blend factor showed marginal significance, dose emerged as the principal determinant of trap efficacy.

High doses or suboptimal blend ratios may suppress behavioral responses or decrease bioassay sensitivity, highlighting the importance of dosage calibration (Tumlison, 1988). Similar findings were observed by Witzgall et al. (2010), who noted that pheromone dose significantly affects both attraction and orientation in lepidopteran moths. In the context of wax moths, Finn (1977) reported a 28% capture rate using a 1:1 formulated blend in field cages, while Flint and Merkle (1983) observed recapture rates of <5% in greenhouses and ~1% in apiaries using a 3:7 blend. Bhopale et al. (2016) further demonstrated that a 3:7 ratio of undecanal to nonanal was optimal for attracting GWM females in funnel traps. Moreover, Zilkowski and Cardé (2004) emphasized that supra-optimal pheromone doses may desensitize males and disrupt their ability to locate the source, aligning with the present study's observation of declining efficacy at higher concentrations.

In the case of *Achroia grisella*, both synthetic and extracted pheromone blends demonstrated efficacy in attracting females. The 50–70 ppm range was optimal, with a decline in trap catch at concentrations >70 ppm, suggesting that overdosing may reduce efficacy. Tukey HSD analysis confirmed that doses of 50, 60, and 70 ppm yielded significantly higher trap catches compared to lower doses (0–35 ppm), while doses beyond 80 ppm showed no substantial improvements, indicating a plateau effect. This dose–response behavior corresponds with work by Linn and Roelofs (1989), who found that optimal male moth attraction occurs within a narrow dose window, beyond which attraction sharply declines.

The blend factor showed no statistically significant influence on trap catch, suggesting that the observed variations were predominantly dose-dependent. These findings affirm that optimal dosing is critical in pheromone-based pest management strategies and should guide the development of pheromone formulations. Similar recommendations were made by Vickers and Rothschild (1991), who noted that even highly attractive blends could fail under field conditions if the release rates were not properly calibrated to match natural emission levels.

Pheromone traps are particularly effective for monitoring low-density or invasive populations (Liebhold & Tobin, 2008). Their role in Integrated Pest Management (IPM) is vital due to their specificity, cost-effectiveness, and minimal environmental impact. Compared to broad-spectrum insecticides, pheromone traps and other semiochemical tools enable targeted and sustainable control methods (Witzgall et al., 2008).

The theoretical foundation for female-targeted trapping is robust. According to Knipling (1966), a 10:1 trap-to-male ratio could achieve ~99% reproductive control, equivalent to a 100:1 male-destroying trap ratio, assuming females mate twice. This effect is amplified if females mate only once. However, light traps are largely ineffective for adult wax moths (Paddock, 1918), making pheromone-based approaches essential. The male moth's pheromone-mediated behaviors exhibit spatial-temporal complexity, as discussed by Cardé and Hagaman (1979), further supporting the need for refined semiochemical approaches.

Our data show a degree of correspondence between laboratory and field results, validating the use of wind tunnels to predict field performance when blend ratios and doses are appropriately matched. This correlation underscores the need for precise dose formulation in synthetic pheromone development, mirroring the emissions of natural pheromone sources (Arn, 1990).

Ultimately, 60 ppm was identified as the optimal dose for both species, with effective trapping also occurring at 50, 70, and 80 ppm. Doses outside this range—particularly those below 35 ppm or above 90 ppm—were less effective. These results contribute to the growing body of evidence supporting pheromone dose optimization as a cornerstone of effective pest monitoring and control strategies.

CHAPTER 7

SUMMARY

Beekeeping contributes to sustainable agriculture and preserves the environment. Additionally, agriculture ensures nutritional security and sustains livelihoods. Regrettably, beekeeping is adversely affected by a variety of biotic and abiotic factors. Among bee enemies, Wax moths are the major pests. Since wax moths can attack honey bee colonies in the field as well as combs in storage. They pose a significant threat to beekeeping. Wax moth larvae are destructive eaters. The Greater Wax Moth, also known as *G. mellonella*, and the Lesser Wax moth, *A. grisella* are one of the deadliest predators of honey bees due to their aggressive feeding nature.

The bee population in solid colonies is swiftly reduced, while weak bee colonies are eradicated by the severe infestation. Beekeepers have incurred significant financial losses due to the intermittent decimation of bee hives. The severity of the infestation directly results in significant economic losses.

Chemical pesticides have traditionally been employed as the prevailing approach to manage insect infestations in the agricultural sector. Unfortunately, this technique has a lot of limitations, such as crop contamination, insect resistance, and deterioration of sustainability. Insect population reduction and monitoring *via* pheromone traps is a sustainable approach to addressing these challenges. Pheromone-based traps provide targeted and environmentally favourable pest control. The aforementioned method facilitates the surveillance of the movement and activity of the targeted insects, fosters the development of beneficial insects, and eliminates undesirable insects naturally. They assist producers in the monitoring, sampling, and identification of pests in particular areas. Insect traps capture information regarding the quantity and density of insects.

Pheromone devices are frequently employed for the purpose of attracting and capturing male insects. In pest control, synthetic pheromones can be used to disrupt mating behaviours, preventing the reproduction of target pests. In addition to reducing reproduction, this practice yields significant data that can be utilized to monitor insect populations. Pheromone-based traps and dispensers are strategically placed to confuse and interfere with the mating patterns of pests.

The present investigations have filled in some of the critical knowledge gaps towards evolving improved management strategies for two species of wax moths, *G. mellonella* and *A. grisella*. The major research thrusts were improved ecological

knowledge on the pheromone isolation and identification with regard to the behavioural bioassay and field evaluation, identifying importance of pheromone compounds towards enhancing their impact potential in deployment for monitoring and mass trapping.

The primary goals of the research were to identify chemicals/ pheromones eliciting the behaviours related to mate location and courtship, as well as to design and test a synthetic pheromone lure and trapping system for use against the GWM in apiaries and/or storage facilities for beekeeping. Successfully the compounds giving the same volatile output as a critical parts of mating biology were found out. Using mass spectral library data, the compounds were identified as two aldehydes:[nonanal (9.729 min RT), undecanal (12.299 min RT)], alkane: [heptadecane (13.537min RT), heneiocosane (16.053 min (21.785 min RT)], two alcohols, [1- undecanol (13.208 min RT) and 1-nonanol (18.768 min RT)]. Heptadecane, and heneiocosane, are the compounds reported for the first time from *G. mellonella.* Aldehydes: undecanal (12.302 min RT) and Cis- 9-Hexadecenal have been identified as volatile compounds of *A. grisella* (20.393 min RT). Cis-9-hexadecenal has not previously been reported from *A. grisella* in scientific literature.

The compounds quantified in male *Galleria mellonella* were Aldehydes: nonanal (5.218 ppm, 6.182 min RT, 1359472 area), undecanal (7.162 ppm, 12.251 min RT, 10873560 area), heptadecane (0.203 ppm,18.005 min RT, area 3799665), heneiocosane (0.267 ppm, 22.118 min RT, area 749917) and alcohols: 1-nonanol (1.181ppm, 6.937 min RT, area 954113). Heptadecane and heneiocosane, are the newly reported compounds in male, *G. mellonella.* The compound cis- 9- hexadecenal has not been previously reported in literature.

Preliminary bioassay experiments in laboratory conditions prior to field trials depicts that the pheromonal compounds can attract GWM and LWM females from a significant distance. The dose-dependent behavioural responses of female moths to pheromones highlights the significance of pheromone dosage and type in eliciting specific behavioural reactions. Understanding these dynamics is essential for both ecological research and applications in pest management, as pheromones play a pivotal role in insect communication and mating behaviours. The behavioural bioassay on female *G. mellonella* and *A. grisella* moths revealed significant differences in responses

to various pheromone treatments. The present investigation evaluated the effects of extracted and synthetic pheromone blends at multiple concentrations on several behavioural parameters, including upward flight, flight to a 10 cm arena, ovipositor display, flight to the edge, closest approach to filter paper, and total orientation time. The extracted blend and the highest concentration of the synthetic blend were the most effective treatments in eliciting behavioural responses in female *Galleria mellonella* and *Achroia grisella*. As the concentration of the synthetic blend decreased, the intensity of the observed behaviours also decreased, demonstrating a clear dose-response relationship. These findings enhance our understanding of pheromone-induced behaviours in these moth species and have significant implications for developing pheromone-based pest management strategies. This pattern highlights the strong influence of specific pheromone doses on the behavioural responses of female moths, with optimal effects seen at higher doses of synthetic blends.

Additionally, the study was conducted under field conditions were necessary to confirm the relevance a blend may not be the most important aspect of product creation, choosing blends that encourage close-range behaviours like searching or circling for longer periods of time might lead to higher trapping success. The efficacy of both synthetic and extracted blends in attracting *A. grisella* and *G. mellonella*. For *A. grisella*, doses below 40 ppm showed no trapping, establishing a baseline concentrations are ineffective. At 40 ppm, the synthetic blend trapped a mean of 4.67 female moths and the extracted blend trapped a mean of 3.00 moths, indicating the starting point for effectiveness. The optimal trapping occurred between 50 ppm and 70 ppm, with peak mean catches at 60 ppm for both blends (synthetic: 20.00 moths, extracted: 18.33 moths). Beyond 70 ppm, attractiveness declined and at the highest dose of 100 ppm trapping only was 5.67 moths (synthetic) and 4.33 moths (extracted). The synthetic blend had a higher overall mean trap catch (6.59 moths)compared to the extracted blend (5.57 moths).

In conclusion, both *G. mellonella* and *A. grisella* showed optimal trapping efficiency between 50 ppm and 70 ppm for both synthetic and extracted blends. Doses higher than 70 ppm reduced attractiveness, possibly due to oversaturation or repellent effects. The synthetic blends generally showed slightly higher trapping efficiency than

the extracted blends, likely due to the purity and consistency of synthetic compounds. Dose was more critical than blend type in determining trapping success for both species.

The lack of significant differences between the two blends suggests that they can be used interchangeably without affecting Trap catch outcomes. This could have practical implications, such as allowing for the use of either blend without compromising Trap catch results. The results of this study contribute to the understanding of how Blend and Dose affect Trap catch levels, providing valuable insights for future research and practical applications in this field. Further studies could explore additional factors or interactions that may influence Trap catch levels to enhance our understanding of this phenomenon.

This research is dedicated to the advancement of pheromone formulations, with a specific emphasis on enhancing their stability and investigating novel methods of delivery. The purpose of these developments is to improve the effectiveness and feasibility of pheromone-based insect management. Pheromones are consistently being investigated by scientists in an effort to determine whether they have the capacity to regulate an even greater variety of nuisance species. This entails the identification of pheromones for historically difficult-to-manage pests. The utilization of pheromones as prospective signifies a significant progression in the domain of insect management. Their precise targeting, minimal environmental impact. Through this exploration, we aim to contribute to the resilience of these vital industries, fostering a harmonious coexistence between honeybees and their human stewards. These findings contribute significantly in the pursuit to develop pheromone based management and monitoring of *G. mellonella* and *A. grisella.* Thereby, reducing reliance on chemical use.

SOCIAL CONTRIBUTION

1. The study has identified the pheromonal components of the wax moths in existing population of Punjab and It will implicate the role of pheromones as an effective control tool and help in developing a non-chemical eco-friendly trap.
2. The development of a pheromone-based trapping system for *G. mellonella* and *A. grisella* will provide substantial benefits to beekeepers and has potential in worldwide global commercial applications.
3. The advantages of pheromones over conventional control methods include lack of toxicity, cost efficiency, and its low maintenance requirements. Moreover, it is applied to both field and storage settings.
4. It will help the new researchers/entomologists in the field of apiculture by providing them with the relevant information through patent, copyright and quality publication.

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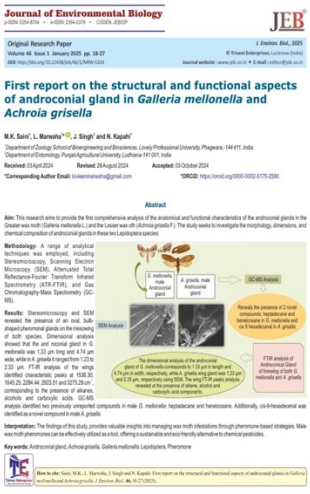
APPENDICES

Research Publications

- Manpreet Kaur, Lovleen, Jaspal Singh (2022). The Prevalence of *Galleria mellonella* and *Achroia grisella* in *Apis mellifera* Colonies in the Ludhiana District" *Res. J. Biotech.* 18(2): 97-103.
- Manpreet Kaur, Lovleen, Jaspal Singh (2025). First Time Report on the Structural and Function Aspects of Androconial Gland in the Greater Wax Moth (*Galleria mellonella*. L) and the Lesser Wax Moth (*Achroia grisella*) *Journal of Environmental Biology 46(1),*18-27.
- 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.

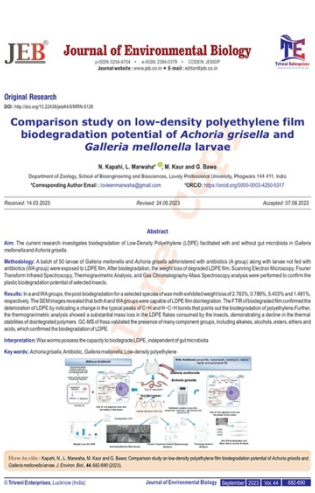

Journal of Environmental Biology

JEB

First report on the structural and functional aspects of androconial gland in *Galleria mellonella* and *Achroia grisella*


Research Journal of Biotechnology

The prevalence of *Galleria mellonella* and *Achoria grisella* in *Apis mellifera* Colonies in the Ludhiana District


JEB Journal of Environmental Biology

Comparison study on low-density polyethylene film biodegradation potential of *Achoria grisella* and *Galleria mellonella* larvae

Conferences

National:

Sr. No.	Seminar	Date	Title
1.	Participated and in 25th Punjab Science Congress "Future Endeavours of Science & Technology for Sustainable Growth" Organized by Sri Guru Teg Bahadur Khalsa College, Sri Anandpur Sahib	7-9 February, 2022	presented oral paper on the Topic *Devastating Pest of honey bee colony: Wax Moths*

International:

Sr.No.	Seminar	Date	Title of the Seminar
2.	Participated in the 6th International conference on Advances in Agriculture Technology and Allied Sciences, ICAATAS 2023 held at Loyola Academy, Secunderabad, Telangana-500010, India	19-21 June, 2023	Presented an oral presentation on the paper entitled: Chemical communication in *Galleria mellonella* and *Achroia grisella*
3.	Participated in the 6th international Conference on Strategies and Challenges in Agricultural and Life Sciences for Food Security and Sustainable Environment (SCALFE-2023) held at Himachal Pradesh University, Summer Hill, Shimla, H.P, India	28-30 April, 2023	presented an oral Presentation on the Topic "Challenges in Bee keeping Sector"
4.	Participated in 2nd International Conference on Plant Physiology and Biotechnology (ICPPB) organised by School of	20-21 April, 2023	presented poster Presentation on the Topic

	Bioengineering and Biosciences under the aegis of Lovely Professional University, Punjab		"Effective tool in Integrated Pest Management: Pheromonal Trap"
5.	Participated in an International conference organised School of Applied Sciences, REVA University, Bengaluru, India in collaboration with the Ethological Society of India	4-5 April, 2023	presented an oral Presentation on the Topic "Animal Behavior and Trends in Zoological Studies"
6.	Participated in 1st International Conference on Global Approaches in Agriculture and Allied Sciences for Sustainability, Food Security and Livelihood held at Gangadhar Shastri Bhawan, Agra College, Agra, Uttar Pradesh, India	21-23, January 2023	presented a poster on the topic entitled "Pheromonal glands in Insects"
7.	Participated in International E-conference on Recent Interdisciplinary studies in Agriculture, Forestry and Allied Sciences.	30 November, 2022	presented an oral Presentation on the Topic "Devastating Pest of Honeybee Colonies: Wax Moths
8.	5th International Conference on Advances in Smart Agriculture and Biodiversity Conservation for Sustainable Development (SABCD-2022) held at Conference Hall, Jaipur National University, Jaipur, Rajasthan, India	04-06 March, 2022	oral Presentation on the Topic Mating Behaviour in *Galleria mellonella*
9.	Emerging trends in Biotechnology and Sustainable Chemistry organised by the	27-29 April, 2022	presented oral paper in virtual International

	Department of Biotechnology and Chemistry, faculty of sciences, Baba Farid College		Conference ETBSC-2022 on the topic Damage Inflicted by Greater Wax Moth *(Galleria mellonella)* and Lesser Wax Moth *(Achroia Grisella)* in Storage in District Ludhiana

	Nptel Course	**Duration**	**Year**	**Division**
1.	Applied Entomology	11 weeks	2022	1
2.	Academic and Research Report Writing	8 weeks	2023	1
3.	Road Map for Patent Drafting	15 weeks	2023	1

Workshops

Sr. No.	Programme	Year	Organized by
1	Successfully completed online certificate course on" Amino acids, Peptides and Proteins (14 hours)	20 April to 26 April, 24	organized by Microbiologist Society, India. Reg no: MAH/4814/SAT
2	An online 14 hour certificate course on Biophysical Methods and Analytic Techniques	23 March to 29 March, 2023	organized by Microbiologists Society , India (MBSI)
3	Participated in National Workshop "Introduction to Intellectual Property Rights (IPR)/ Patent Process	8 Sept, 2020.	KRM DAV College, Nakodar in association with Rajiv Gandhi Institute of Intellectual Property Management, Nagpur

Patents Published

Sr.No.	Title	Application No.
1.	A Novel Bioassay Chamber for Behaviour Assessment of Insects	202211059647A
2.	A Novel Chamber for Collection of Insects Pheromones	202211027330 A
3.	A Device for Trapping Insects using Pheromones and Sound Frequencies	202211073978 A
4.	A Method for Stretching, Cleaning and Staining of Insect Wings for Microscopic Examinations	202211074042 A

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1. The Chemical Interactions in Insects via Receptor Organs (L-138067/2023)
2. Damage Inflicted by Wax Moths in the Bee Hives (L-148934/2024)
3. Feeding Mechanism in Greater Wax Moth (*Galleria mellonella*) (Copyright filed)
4. Pheromonal Traps: An Effective Device for Integrated Pest Management(Copyright filed)
5. Sound Production Mechanism in *Galleria mellonella*(Copyright filed)
6. Isolation and identification of the pheromonal components in the Androconical gland of *Galleria mellonella* and *Achroia grisella* (L-137907/2023)
7. Behaviour Bioassay of Female *Galleria Mellonella* and *Achroia Grisella* Towards Blends and Dosages of Male Pheromones (Copyright filed)

Appreciation Letters

Sr.No.	Agency	Date	Role
1.	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb)	23 Sept, 2022	Appreciation for judging the event Yuva Utsav District Level: Theme: Goal Developed India (Azadi ka Amrit Mahotsav)
2.	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb)	26 March, 2021	guest lecture on the Topic of "Women Empowerment "at Punjab Agricultural University, Ldh
3.	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb)	27 March, 2021	judgement of (Cultural Program) 13th tribal Youth Exchange Program at Punjab Agricultural University, Ldh
4.	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb)	23 March, 2021	Speaker of the Webinar Jal Shakti Abhyiaan under the theme "Catch The Rain, where it falls, when it falls"
5.	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb) for	30 October, 2019	Judgement of District level Declamation Contest at D.D Jain College, Ldh

Awards received

	Name/ Title of Award	Year	Contribution	Award Conferred by
1.	Young Scientist award	2023	For Strategies and challenges in Agricultural and Life Science for Food security &Sustainable Environment	Agricultural Technology Development Society, Ghaziabad
2.	Young Scientist Associate Award	2022	5thInternational Conference Advances in Agriculture Technology and Allied Sciences	Ms Swaminathan School of Agriculture, Centurion University of Technology and Management
3.	Young Research Scholar Award	2021	For contribution to the field of Zoology in Advances in Smart Agriculture and Biodiversity Conservation for Sustainable Development	Agricultural Technology Development Society, Ghaziabad
4.	Swachh Bharat Summer Internship Programme 2.0	2019	First prize in District with cash award of 30,000 for successfully completed minimum 50 hours of Swachhta	Govt. of India, Ministry of Youth Affairs and Sports, Nehru Yuva Kendra, Ldh (Pb)

Practical Manual Authorised

Sr. No.	Title	Year	Publisher	Class	University	ISBN NO/ Pages
1	New Fundamental Zoology Practical Manual	2023	Vijaya Publication	B.Sc I (Sem 1& 2)	Kurukshetra University, Kurukshetra	978-93-84004-14-9
2	New Fundamental Zoology Practical Manual	2021	Vijaya Publication	B.Sc III (Sem 5& 6)	Guru Jambeshwar University, Hisar	97893-84004-36-1
3	New Fundamental Zoology Practical Manual	2019	Vijaya Publication	B.Sc II (Sem 3& 4)	Punjab University	978-93-84004-81-1
4	New Fundamental Zoology Practical Manual	2019	Vijaya Publication	B.Sc I (Sem 1& 2)	Punjab University	978-93-84004-80-4
5	New Fundamental Zoology Practical Manual	2019	Vijaya Publication	B.Sc III (Sem 5 & 6)	Punjab University	978-93-84004-82-8
6	New Fundamental Zoology Practical Manual	2019	Vijaya Publication.	B.Sc II (Sem 3& 4)	Guru Jambeshwar University, Hisar	978-93-84004-15-6

7	New Fundamental Zoology Practical Manual	2019	Vijaya Publication	B.Sc I (Sem 1& 2),	Guru Jambeshwar University, Hisar	978-93-84004-14-9.

Other Acclamations

- Attended and participated in innotek'24: Innovation and Graduating project Expo on 26 and 27 April,24. **Title of the Proposed Idea: HARNESSING NATURE'S SIGNAL: BREAKTHROUGH DEVICE FOR WAX MOTH MONITORING AND CONTROL**
- Blood donation in recognition of Humanitarian Service as a Voluntary Blood donor to Rehras Sewa Society on 25-04-2022.

Sports Activity

- Participated in 1500 m track event Women held from 22-02-2024 to 23-02-2024 and stood **Third** in the 14th Annual Athletic Meet 2023-24 organised by School of Physical Education, Lovely Professional University, Punjab.
- Participated in Inter School Khokho Women held from 28-03-2024 to 29-03-2024 and stood **Third** in the event organised by Division of Sports, Student Welfare Wing, Lovely Professional University, Punjab.
- Participated in Inter School Volleyball Women held from 08-04-2024 to 10-04-2024 and stood **Third** in the event organised by Division of Sports, Student Welfare Wing, Lovely Professional University, Punjab