

**BIOCHEMICAL CHARACTERIZATION OF SEMIOCHEMICALS
FROM *LUPROPS TRISTIS* (COLEOPTERA: TENEBRIONIDAE)
AND THE CYTOTOXIC EVALUATION OF A SELECTED VOLATILE
COMPOUND ON RAT CELL LINES**

Thesis submitted to the
University of Calicut
for the award of the Degree of
Doctor of Philosophy in Zoology



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This is to certify that the thesis, entitled “**Biochemical characterization of semiochemicals from *Luprops tristis* (Coleoptera: Tenebrionidae) and the cytotoxic evaluation of a selected volatile compound on rat cell lines**” submitted to the University of Calicut, in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy in Zoology is a record of original work done by **Ms. Sajidha Mohammed** during the period of May 2020 - May 2025 of her research at the Department of Zoology, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree /Diploma /Associateship /Fellowship or other similar title to any candidate of any University.

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Declaration

I, Sajidha Mohammed hereby declare that the work presented in the thesis entitled "Biochemical characterization of semiochemicals from *Luprops tristis* (coleoptera: tenebrionidae) and the cytotoxic evaluation of a selected volatile compound on rat cell lines" is based on the original work done by me under the guidance of Dr. Muhammed Abdul Rafeeq, K.U, Assistant Professor, Department of Zoology, M E S Mampad College (Autonomous), Kerala and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library , University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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List of Abbreviations

CO	Carbon Monoxide
CO ₂	Carbon Dioxide
COVID	COronaVirus Disease
DMEM	Dulbecco's Modified Eagle Medium
GC-MS	Gas Chromatography-Mass Spectrometry
IEC	Intestinal Epithelial Cells
MADEP	Massachusetts Department of Environmental Protection
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NYSDOH	New York State Department of Health
OD	Optical Density
ODTS	Organic Dust Toxic Syndrome
OECD	Organisation for Economic Co-operation and Development
PEEK	Polyether Ether Ketone
PM	Particulate Matter
RAST	Radioallergosorbent Test
RAW	Ralph, rAschke, Watson
SBS	Sick Building Syndrome
SD	Standard Deviation
SO ₂	Sulphur Dioxide
SPT	Skin Prick Test
TDS	Total Defensive Substance

US EPA	United States Environmental Protection Agency
VOC	Volatile Organic Compounds
WHO	World Health Organization

Abstract

Indoor insect pests adversely affect human health and the built environment. In addition to causing bites, stings, allergic reactions, and potential disease transmission, they release volatile organic compounds (VOCs) that compromise indoor air quality. Even at low concentrations, VOCs have been linked to respiratory and allergic disorders, underscoring the importance of studying pest populations in relation to indoor system features and surrounding environments.

This study employed a questionnaire-based survey across 403 indoor spaces in Kerala to document insect prevalence, structural features, seasonal variations, and health symptoms reported by residents. Findings indicated that tropical climate alone does not fully explain pest populations; instead, anthropogenic changes such as the establishment of rubber and coconut plantations in surrounding areas significantly facilitated pest adaptation and infestations. The indoor environment was frequently cited as the second most common factor associated with illness, with residents reporting a rise in allergy and respiratory issues in spaces with higher pest prevalence.

Luprops tristis (Mupli beetle) emerged as a major nuisance species, aggregating in thousands within homes. Volatilome profiling of 500–1000 beetles detected 41 compounds, of which 18 were identified through reference comparisons. Eleven of these are compounds with reported allergenic or respiratory effects, providing a plausible explanation for unexplained allergic reactions in households. Cytotoxicity testing of heptadecane, a dominant compound, using the MTT assay on normal rat epithelial cells, revealed dose-dependent inhibition of cell growth, with the highest concentration (100 µg/ml) reducing viability by 12.89%.

This research underscores the ecological and toxicological significance of indoor insect pests in tropical environments. By linking pest ecology, environmental modifications, and health concerns, it provides a basis for integrating entomological insights into strategies for improving indoor air quality and safeguarding public health.

സംഗ്രഹം

ഇൻഡോർ കീടങ്ങൾ മനുഷ്യാരോഗ്യത്തിലും നിർമ്മിത പരിസ്ഥിതിയിലും നേരിട്ടോ അല്ലാതെയോ ദോഷകരമായ സ്വാധീനം ചെലുത്തുന്നു. ഈ കീടങ്ങൾ കടിച്ചും കുത്തിയും അലർജി പ്രതിഭാസങ്ങളും രോഗ വ്യാപനങ്ങളും ഉണ്ടാക്കുന്നതിന് പുറമെ, ചില വാതകീയവും അവാതകീയവുമായ ജൈവ സംയുക്തങ്ങളും പുറത്തുവിടുന്നു. ഈ സംയുക്തങ്ങളിൽ വാതകീയ ജൈവ സംയുക്തങ്ങൾ (VOCs) നിരവധി ആരോഗ്യ പ്രശ്നങ്ങളുമായി ബന്ധപ്പെട്ടിരിക്കുന്നതായി കണ്ടെത്തിയിട്ടുണ്ട്.

ഈ പഠനത്തിൽ, നഗരത്തിലും ഗ്രാമത്തിലും ഉൾപ്പെട്ട വീടുകളും വാണിജ്യകെട്ടിടങ്ങളും ഉൾപ്പെടുത്തി 403 ഇൻഡോർ ഇടങ്ങളിൽ, അവയുടെ ഘടനാപരമായ സവിശേഷതകളും കീടങ്ങളുടെ എണ്ണവും കണക്കാക്കാൻ ചോദ്യാവലി അടിസ്ഥാനമാക്കിയുള്ള ഒരു സമീപനം സ്വീകരിച്ചു. ഈ പഠനത്തിൽ നിന്നും മനസ്സിലാക്കിയത്, ഉഷ്ണമേഖല കാലാവസ്ഥ മാത്രമല്ല ഇപ്പോഴത്തെ ഇൻഡോർ കീടവ്യാപനത്തിലെ വർദ്ധനവിന് പിന്നിലുള്ള കാരണമെന്നും, പകരം മനുഷ്യനിർമ്മിതമായ പരിസ്ഥിതി മാറ്റങ്ങൾ, പ്രത്യേകിച്ച് റബ്ബറും തെങ്ങും ഉൾപ്പെടുന്ന കൃഷികളുടെ വർദ്ധനവും പ്രധാന പങ്ക് വഹിക്കുന്നു എന്നതാണ്. അതോടൊപ്പം തന്നെ ഈ മാറ്റങ്ങൾ കീടങ്ങളുടെ വളർച്ചക്ക് സഹായകരമാകുന്നു.

ചോദ്യാവലി വിശകലനം ചെയ്യുമ്പോൾ, ഇൻഡോർ അന്തരീക്ഷം പലപ്പോഴും പല രോഗങ്ങൾക്കുമുള്ള രണ്ടാമത്തെ പ്രധാന കാരണമായി രേഖപ്പെടുത്തിയിട്ടുണ്ട്. പല വീടുകളിലും കീടങ്ങളുടെ ബുദ്ധിമുട്ടുകൾ കണ്ടുവെങ്കിലും, അവയുടെ സാന്നിധ്യം രോഗവ്യാപനത്തിനു കാരണമാണെന്ന് കണ്ടെത്താൻ കഴിഞ്ഞില്ല. എന്നിരുന്നാലും കഴിഞ്ഞ കുറച്ച് വർഷങ്ങളിൽ കീടങ്ങളുടെ സാന്നിധ്യമുള്ള വീടുകളിൽ അലർജി രോഗികളുടെ എണ്ണവും നിരവധി ആരോഗ്യ പ്രശ്നങ്ങളും വർദ്ധിക്കുന്നത് ഇവ നമ്മുടെ അന്തരീക്ഷത്തെ ദോഷകരമായി ബാധിക്കുന്നതിനെ സൂചിപ്പിക്കുന്നു.

കീടശരീരങ്ങൾ വാതകീയ ജൈവ സംയുക്തങ്ങൾ പുറന്തള്ളുന്നു. ഇവ കീടങ്ങളുടെ രാസ ആശയവിനിമയത്തിൽ പ്രധാന പങ്ക് വഹിക്കുന്നു. ലൂപ്രോപ്സ് ട്രിസ്റ്റിസ് (മുപ്പി വണ്ട് അഥവാ കോട്ടെരുമ) എന്ന കീടം ഇൻഡോർ ഇടങ്ങളിൽ ലക്ഷക്കണക്കിന് എണ്ണമുള്ള വലിയ കൂട്ടമായി കാണപ്പെടുന്നു.

ഇവ മനുഷ്യ സമ്പർക്കം മൂലം അസ്വസ്ഥമാകുമ്പോൾ നമ്മുടെ ചർമ്മത്തിനും കണ്ണിനും ദോഷകരമായി ബാധിക്കുന്ന ചില ഫീനോളിക് സ്രവങ്ങൾ പുറത്തുവിടുന്നു.

ഈ വാതകീയ ജൈവ സംയുക്തങ്ങൾ പരിശോധിക്കാൻ, ലാബിൽ ഉപയോഗിക്കാവുന്ന ഒരു ചെലവുകുറഞ്ഞ ഗ്ലാസ് ചാമ്പർ (1000 ml) രൂപകൽപ്പന ചെയ്തു. 500-ഉം 1000-വും കീടങ്ങളിൽ നിന്ന് വാതകീയ ജൈവ സംയുക്തങ്ങൾ ശേഖരിച്ചു, തുടർന്ന് ഗ്ലാസ് ക്രോമാറ്റോഗ്രാഫി - മാസ് സ്പെക്ട്രോമെട്രി ഉപയോഗിച്ച് വിശകലനം നടത്തി.

കണ്ടെത്തിയ സംയുക്തങ്ങൾ നിലവിൽ ലഭ്യമായ ഗവേഷണ വിവരങ്ങളുമായി താരതമ്യം ചെയ്തപ്പോൾ കുറച്ച് അളവിലായാലും ആസ്തമ, ശ്വാസം മുട്ടൽ, എന്നീ രോഗങ്ങൾക്ക് കാരണമാകുന്നതും തൊലി, ശ്വാസനാളം തുടങ്ങിയവയെ ദോഷകരമായി ബാധിക്കുന്നതുമായ സംയുക്തങ്ങൾ ഉൾപ്പെടുന്നതായി കണ്ടെത്തി. ഇവയ്ക്ക് പിന്നിലുള്ള രാസ ഘടകങ്ങൾ, വീടുകളിൽ കാണപ്പെടുന്ന വിശദീകരിക്കാനാവാത്ത അലർജികൾക്ക് ഒരു കാരണമാകാം എന്ന് സൂചിപ്പിക്കുന്നു.

ഇതിലെ ഒരു പ്രധാന ഘടകമായ ഹെപ്റ്റാഡിനേൻ എന്ന സംയുക്തത്തിന്റെ കോശവിഷത്വം പരിശോധിക്കാൻ, സാധാരണ എപ്പിതീലിയൽ കോശങ്ങൾ ഉപയോഗിച്ച് MTT അസ്സേ നടത്തി. ഇത് ഡോസ് വർദ്ധിച്ചതിനൊപ്പം കോശാവരോധനം കൂടുന്നതായി തെളിയിച്ചു. ഇതുവഴി, വിവിധ അളവിലുള്ള സംയുക്തങ്ങൾക്ക് അസ്വസ്ഥതകൾ ഉണ്ടാക്കാനുള്ള ശേഷിയുണ്ടെന്നും, ഈ അദ്യശ്യ രാസ വസ്തുക്കൾ മനുഷ്യശരീരത്തിൽ ദീർഘകാല ദോഷഫലങ്ങൾ സൃഷ്ടിക്കാനുള്ള സാധ്യതയുണ്ടെന്നും കാണിക്കുന്നു.

ഈ പഠനം, ഇൻഡോർ കീടങ്ങൾ പുറന്തള്ളുന്ന വാതകീയ ജൈവ സംയുക്തങ്ങൾ, പ്രത്യേകിച്ച് ഉഷ്ണമേഖലയിലെ ജനസംഖ്യക്ക്, ദീർഘകാലാരോഗ്യ പ്രശ്നങ്ങൾ സൃഷ്ടിക്കാൻ സാധ്യതയുള്ളതാണ് എന്ന് സൂചിപ്പിക്കുന്നു, കാരണം ആളുകൾ ഇൻഡോറിൽ ചെലവഴിക്കുന്ന സമയം വർദ്ധിച്ചിട്ടുണ്ട്. ഇൻഡോർ വായുവിന്റെ ഗുണനിലവാരത്തെ ബാധിക്കുന്ന ഘടകങ്ങളെക്കുറിച്ചുള്ള കൃത്യമായ വിവരങ്ങൾ ആരോഗ്യ സംരക്ഷണത്തിനും, രോഗങ്ങൾക്കുള്ള പ്രാരംഭ ചികിത്സാ പദ്ധതി തയ്യാറാക്കുന്നതിനും സഹായകരമായിരിക്കും.

Chapter 1

INTRODUCTION

1. INTRODUCTION

Indoor air quality is a crucial determinant of human health and well-being, particularly in enclosed living environments where individuals spend a substantial portion of their time. Among the various factors that compromise indoor air quality, the presence of indoor insect pests plays a subtle yet significant role through the release of volatile organic compounds (VOCs), allergens, and other bioactive substances. *Luprops tristis* (Coleoptera: Tenebrionidae), commonly known as the Mupli beetle, is a seasonal indoor pest endemic to parts of the Western Ghats region in South India. These beetles invade residential buildings in large numbers, particularly during the post-monsoon season, often causing considerable nuisance due to their odoriferous secretions and defensive exudates. Despite their recurring infestations and the discomfort they cause, the chemical nature of the volatiles they emit and their potential impact on indoor air quality and human health remain poorly understood. This study aims to characterize the semiochemicals released by *Luprops tristis* biochemically and to evaluate the cytotoxic potential of a selected volatile compound on rat cell lines, thereby contributing to our understanding of pest-induced indoor air pollution and its possible health implications.

1.1. Indoor air quality and influence of biological contaminants

Indoor air quality is crucial for human health, as indoor pollutants often exceed outdoor levels by 2-100 times (Baek et al., 1997; Tran, 2020; US EPA, 2020). The concentration of harmful chemicals and other volatile organic compounds (VOCs) was found to be 10 times higher in Indian indoor environments (Kankaria et al., 2014). VOCs are suspected to be responsible for the death of at least 38 million people annually (WHO, 2022). Humans spend 80% to 90% of their lifetime indoors, where they may be exposed to a wide range of pollutants simply by inhaling the indoor air, resulting in negative health effects (Ahmed et al., 2019). Toxic VOCs have been associated with allergies, eye irritation, lack of concentration, nose and throat malfunction, tiredness, vascular nervous dysfunction, acute chronic health pathologies and cancer (Li et al., 2021). Higher

concentrations and long-term exposures of these were also reported to cause harm to the lungs, kidneys, liver and the central nervous system (Shuai et al., 2018). Though symptoms of most diseases caused by VOCs tend to be less severe than more acute diseases, they nonetheless need to be taken seriously as these symptoms may result in long-term effects (WHO, 2001).

The volatile organic compounds, which are usually highly reactive, include chemical substances such as benzene, toluene, ethylbenzene, m-xylene, o-xylene, p-xylene and styrene, chlorinated hydrocarbons such as dichloromethane, chloroform, methyl chloride, trichlorofluoromethane, tetrachloroethylene and organ halogens such as p-dichlorobenzene and 1,2,4-trichlorobenzene (Chao & Chan 2001; Panagiotaras, 2014). According to the World Health Organisation (WHO, 2020), volatile organic compounds are those substances that have a boiling point from approximately 50-100°C up to 240-260°C and vapour saturation pressure greater than 102 kPa at 25°C (Linak et al., 1989; Panagiotaras, 2014). Certain VOCs have been identified as toxic, genotoxic, irritant or environmentally harmful (WHO, 1989, 2002, 2005; Parra et al., 2008; Maro et al., 2012; Berenjian et al., 2012).

The major sources of indoor contamination include the occupants, inappropriate material used during building constructions, excessive use of chemical products such as pesticides and disinfectants, combustion gases, pollutants coming from outside; like carbon dioxide, carbon monoxide, nitrogen oxides, sulphur dioxide, ozone and volatile organic compounds which also form a part of the major chemical pollutants. Apart from the other gaseous or chemical contaminants, biological contaminants also affect the indoor air quality. The common biological substances include bacteria, viruses, animal dander, cat saliva, mites, cockroaches and pollen; out of which some are labelled as powerful biological allergens, especially when the damp indoor surfaces provide a breeding ground for moulds, mildews, bacteria and insects (Vermani et al., 2010; US EPA, 2019).

1.2. Volatile organic compounds and insect pests

Many studies on VOCs have proved them to be categorised as toxic chemicals, which are harmful to environmental sustainability and human health. They are also precursors of ozone and particulate matter (PM) 2.5, which cause severe air pollution (Huang et al., 2018). Certain VOCs also cause skin irritation, toxic effects, carcinogenicity, teratogenicity and mutagenicity (Woodruff et al., 1998; Morello-Frosch et al., 2000 & Huang et al., 2018). The number of allergic patients with bronchial asthma (Fleming et al., 1987; Haahtela et al., 1990; Yunginger et al., 1992; Kimber & Dearman, 2002; Gutowska-Ślesik et al., 2023) and allergic rhinitis (Aberg, 1989; Ishizaki et al., 1987; Zimmerman et al., 1988; Tosca et al., 2024) has been increasing continuously. The increase in the concentration of indoor air pollutants and the higher number of people being exposed to it, justifies the research on them (Sakai et al., 2004; Panagiotaras et al., 2014).

The indoor environment is an established source of human allergic risk factors (Gelber et al., 1883; Platts-Mills et al., 1991; Munir, 1995). The increased amount of time spent indoors by an individual and its long-term but severe effects have also helped in the establishment of certain health-based goals for different air pollutants by the WHO (1989, 2002, 2005). It is also an important health assessment as people usually take in almost 6 to 10 litres of air per minute, which amounts to about 15000 litres per day (Wood et al., 2002 & Bragoszewska et al., 2018). Thus, poor indoor air quality will harm the vulnerable groups such as children, the elderly and those suffering from respiratory and cardiac disorders (IJERPH, 2017).

The negative influence of a fungus-derived semiochemical, 1-octen-3-ol, on dopamine neuron degeneration establishes the involvement of a naturally occurring environmental agent in Parkinson's disease (Inamdar et al., 2013). Additionally, the presence of certain biological substances, mainly spores from mould and other fungi, is reported to have negative effects on air quality and human health, mostly resulting in sick building syndrome (SBS) and organic dust toxic syndrome (ODTS)

(Moldoveanu, 2015; Tran et al, 2020). It is also well established that pollen and insect-derived substances can affect respiratory health (Kausner, 2018).

India, being a tropical country with a suitable climate for most of the indoor pests, should therefore have a check on their influence on the indoor air quality. Certain volatile and non-volatile organic compounds secreted by the indoor insect pests (ants, bugs, beetles, weevils, cockroaches, houseflies, mosquitoes, moths, etc), known as semiochemicals or pheromones, are an important component of indoor air quality. As the VOCs have been associated with most of the health disorders from allergies to even cancer (Parra et al., 2008; Marc et al., 2012), elucidation of the influences of the insect semiochemicals in indoor air quality and their effect upon common diseases is to be dealt with grave concern.

Insects are constantly communicating using a bunch of chemicals. We do pass through and live in between thousands of such chemical communications of insects, particularly known as semiochemicals (Wilson & Bossert, 1963). This could also be one reason for the increasing respiratory and other health disorders. However, the mechanisms of these chemical-induced respiratory disorders, and other allergic disorders are not completely elucidated (Rothen-Rutishauser et al., 2005; Nishino et al., 2016 & Panagiotaras, 2014).

Most indoor spaces these days have their indoor environment insulated with air containing ubiquitous insect pests (Gelber et al., 1993) and their allergens (Rayapudi et al., 2010). Insect pests annoy, inflict bites and stings and even cause allergic reactions apart from transmitting numerous diseases. In India, it was observed that *Luprops tristis*, commonly known as the Mupli beetle, caused skin and eye irritations because of a phenolic secretion that the beetle releases when disturbed (Sabu et al., 2008). The cocktail of volatile organic compounds emitted by insects can be studied to receive valuable insights concerning their specific roles and impacts on human health.

1. 3. Questionnaire-based survey of indoor pests and health impacts

Semiochemicals released as a part of their communication by ants, mosquitoes and other commonly observed indoor species might have adverse effects on the inhabitants of the indoor spaces. To understand the same, this study employed a detailed questionnaire-based approach to monitor the presence and abundance of insects and the presence of health issues, including allergies. This helped evaluate the correlation between the presence of indoor insects, parameters of indoor air quality and the appearance of any allergic symptoms. Developing questionnaires and collecting information regarding the occupants' experiences and perceptions of the indoor air are useful for evaluating the results of the measures taken (Järvi et al., 2018). Asking the respondents to describe symptoms at certain time points, such as weeks or months corresponding to the occurrence or emergence of any insect group, is a common way to implement health questionnaires (Järvi et al., 2018).

The rise and fall of the insect population have always been occurring along with numerous reasons, particularly humidity and temperature (Bashir et al., 2022). Not least, the presence of surrounding vegetation, geographical features, and or any unclean or mouldy areas in the surrounding areas also favours the presence of insects. To be noted is that, type of indoor pest populations is readily controlled by the indoor characteristics of the same. The details, including seasons during which the inhabitants find them as more of a nuisance, the conditions they believe to increase their numbers and the control measures employed, could easily be gathered and analysed when working with a questionnaire. Quantifying the insect population concerning the features of the indoor systems and the features of the man-modified external surroundings is thus important in defining the cause of them being categorised as indoor pests.

1.4. Insect volatiles and health concerns

Although insect semiochemicals have been extensively studied for pest management (Cook et al., 2007; Logan et al., 2010; Brugman et al., 2018), their potential health effects on humans remain largely unexplored. These organic compounds, being intra- and interspecific signals, are crucial in animal communication (Nordlund & Lewis, 1976; Dicke & Sabelis, 1988; Landolt & Phillips, 1997). They evoke different behaviours, including stimulatory, inhibitory or deterrent in target organisms, which may also affect humans (Ali et al., 2015; El-Shafie & Faleiro, 2017).

Insect volatiles, also known as semiochemicals, can be defined as chemicals emitted by living organisms that cause a behavioural or physiological response in other individuals (Heuskin et al., 2011). These usually have between 5 and 20 atoms of carbon with molecular weights ranging from 80 to 300. Such compounds belong to either hydrocarbon, acetate ester, alcohol, acid, epoxide, ketone, isoprenoid or triacyl glyceride groups (Yew & Chung, 2015). These volatile chemical signals used for insect communication can be categorised to include intraspecific pheromones or interspecific allelochemicals. Some may also act both as a pheromone and an allelochemical signal. The qualitative and quantitative assessments of these multicomponent semiochemical volatiles to study the negative impacts of the insects can be effectively carried out using various headspace and air entrainment techniques (Blight, 1990).

Insect allergies, which occur around the areas of insect bites or stings, show symptoms of pain, itching, swelling and redness. But semiochemicals or pheromones, which are volatile and non-volatile organic compounds secreted by indoor insect pests (ants, bugs, beetles, weevils, cockroaches, houseflies, mosquitoes, etc.), may at times cause indirect allergic reactions. Airborne particles from cockroaches, cat fleas and cloth moths are a significant health concern for individuals in socioeconomically disadvantaged groups (Mathews, 1989). Allergic studies focusing on house dust mites (Calderon et al., 2015) have shown that they

can manifest in the respiratory system, causing asthma, allergic rhinitis or both. Similarly, occupational allergies (Fukutomi & Kawakami, 2021), when being exposed to a higher density of insects, were also observed in larvae of flies and moths (Siracusa et al., 1994), crickets and locusts (Linares, 2008; Lopata et al., 2005), Mediterranean flour moth (*Ephestia kuehniella*) (Moreno et al., 2014), fruit flies (Jones et al., 2017) and cockroaches (Moghtaderi et al., 2017). Such allergies included rhinitis, asthma, and immediate systemic allergic reactions. This again emphasizes the need to study the volatile compounds for negative health issues.

Respiratory issues are primarily caused by substances outdoors, but those caused as a result of cockroaches (particularly *Blattella germanica* and *Periplaneta americana*) and particles from the cat flea and the common clothes moth are also considered significant (Mathews, 1989). It has been found out that some viral infections, such as the common cold and measles, are transmitted more via indoor air (Riley et al., 1978). But, somehow, as person-to-person transmission is considered more important during viral infections, the importance and involvement of such organisms were excluded during older studies. that allergens produced by house dust mites and the dander of furred domestic animals are generally seen to be the most important cause of disease episodes in atopic individuals. For example, in a U.K. study, some 80% of asthmatic children were allergic to the house dust mite (Price et al., 1990), and a Swedish study showed that 57% of asthmatic children were allergic to at least one type of furred animal (Kjellman & Pettersson, 1983).

Even though a direct link may not be visible, certain allergic insects commonly seen around the area are also considered to be of nuisance, particularly when they come in huge aggregations like the Mupli beetle (Sabu, 2008). Such insects, which stay in millions of individuals in indoor spaces, have been found to cause skin irritations (Sabu et al., 2014) and even keratoconjunctivitis (Susan et al., 2010). Moreover, they are also suspected to be the reason for certain unexplained respiratory issues, particularly during their active season (Mohammed et al., 2023).

1.5. *Luprops tristis* Fabricius

Luprops tristis (Mupli beetle, locally known as “Mupli vandu” and “Kotteruma”) has been categorized as a nuisance pest for three decades (Narendran, 1998; Jose, 2003; Thomas & Jacob, 2005). Their massive seasonal aggregation soon after the first summer shower into residential areas in the closest vicinity of the rubber plantations, in huge numbers ranging from 0.5 to 4 million per residential building, makes them a potential nuisance pest (Sabu et al., 2008). Rather than that, they are usually considered harmless with nocturnal surface-level activity and diurnal passivity (Sabu et al., 2008). At night, they crawl and fly towards lighted surfaces of indoor structures and enter into dormancy for several months (Sabu et al., 2008). Until now, not much physical harm has been observed other than the slight burns caused by the phenolic secretion from their body released when pressed accidentally (Sabu et al., 2008). Sheds made of coconut leaf, kerosene lamps, traditionally tiled roofs, presence of haystacks, wooden blocks, coconut husks and firewood stocks are other elements attracting the pests other than the rubber plantations (Sabu et al., 2008).

No insectivores of any kind, such as wall lizards (*Hemidactylus* sp.), spiders or ants, were observed in the vicinity of the presence of studies of these beetles (Sabu et al., 2008). This indicates that these beetles might be secreting some unseen chemicals which might be deterring their colonies and other individuals from predation or other attacks.

1.6. Head space air sample collection from insects

Headspace analysis, where a volume of vapour or gas is collected above a study sample in an enclosed space, has always been a crucial part of volatile studies (Barbosa-Cornelio et al., 2019). Several techniques, such as special bags, canisters, cuvettes and modifications to these, were used for headspace volatile collection of insects. The complication of analysing such volatiles increases within the case of insects, along with their decreasing size and active nature. Since the volatiles are found in very small amounts, the analysis of them requires the development of very

sensitive techniques (Barbosa-Cornelio et al., 2019). The most efficient ones include trapping the headspace volatiles for defined periods onto a sorbent material, which will then be subjected to chemical analysis. Among this, the dynamic headspace sampling helps direct the concentration of semiochemicals readily released from insects onto the adsorbent tubes (Woolfenden, 2010).

In India, *Luprops tristis* releases phenolic secretions when disturbed, and these have been seen to cause physical irritations (Sabu et al., 2008). Inhalant allergies from insects were another kind of allergy that was noted by being exposed to the beetle, apart from having skin irritations and burns (Mohammed et al., 2023). Likewise, most of the semiochemicals released by indoor species as a part of their communication might have adverse effects on the inhabitants of the indoor spaces. Earlier, the volatiles from the Mupli beetle were trapped by generally placing adsorbent material in an earthen pot for 72 hours in a cave heavily infested by this beetle (Nirdev, 2014). To efficiently study the volatiles of such active insects and others equal to or larger than 3mm under laboratory conditions, an economically feasible glass chamber was developed during this study.

1.7. Cytotoxicity of insect volatiles on mammalian cells

Insects have been considered a constant nuisance to health due to their stings, bites (Sturm et al., 2023), allergens from their body parts, fecal matter or saliva (Kauser et al., 2022). To assess the potential toxicity of these semiochemicals, this study employs the MTT Assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay), a standard method for evaluating cell viability. Being in the indoor environment for a longer time by individuals, particularly during the COVID-19 pandemic and soon after it, along with its long-term effects, makes the investigation especially topical and important.

Living cells, mainly kidney and liver cells, when exposed to insect residues in stored grains, have been seen to have cytotoxic and genotoxic effects (Abdelfattah et al., 2022). Similar cytotoxicity was also measured while studying the total defensive substance (TDS) of a medicinal beetle, *Blaps rynchopetera*

Fairmaire (Xiao et al., 2018). These edible insects have also shown that their volatiles, when combined, exhibited even higher cytotoxicity, suggesting that they can be used for medicinal purposes (Pang X-Q et al., 2022). Similar negative changes may also be reflected in various other cell functions, including the morphology of the cells. This information is also helpful in the background of cancer therapies. A parallel antitumor effect was also seen during the study of the extracts from different ant species (Jangir & Nadumane, 2023).

Information on all the currently available research collectively points to the same conclusion. However, tropical countries like India, with a wide diversity of insects with known defensive abilities, have still to be studied in detail. One such insect, *Luprops tristis*, is the focus of this study.

1.8. Objectives

1. Conduct a field and questionnaire-based survey on common indoor insects and select one insect to act as the test organism for semiochemical analysis.
2. Compare the relationship of geographical location, structural features of indoor systems and health of inhabitants with the presence of pests.
3. Collect the test insect pest, conduct headspace semiochemical analysis to identify volatile components.
4. Investigate the cytotoxic effect of a selected semiochemical of the test insect upon normal mammalian cells using the MTT Assay

Chapter 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Indoor air quality is a growing concern due to its impact on human health. While early research primarily focused on chemical pollutants, recent studies highlight the role of volatile organic compounds (VOCs) and biological contaminants in indoor air pollution. VOCs, including emissions from fungi and insects, are linked to respiratory disorders, allergies, and other health effects. Despite increasing awareness, sources of biological VOC, particularly insect-emitted semiochemicals, remain underexplored. Insects release VOCs as semiochemicals that mediate communication, defense and environmental interactions. Yet, their impact on indoor air quality and potential cytotoxic effects requires further investigation. This chapter reviews existing literature on VOCs and biological emissions, emphasizing their role in air quality deterioration. Special attention is given to *Luprops tristis*, a species known for its semiochemical emissions. By blending previous research, this review establishes the foundation for the present study, which examines the composition and impact of *Luprops tristis* emissions on indoor environments.

2.1 Indoor volatiles

The necessity for regular monitoring of air varies according to the demanded updates in different fields, including health and the environment. One reason is the concentration of indoor air pollutants, which may stay seven times higher than the outdoor levels (Tang et al., 2002; Guo et al., 2004; Kankaria et al., 2014 & Tran et al., 2020). Brown et al (1994) have stated that humans are exposed to pollutants through indoor air inhalation and the same should be managed systematically as it remains a risk factor for numerous health conditions (Ahmed et al., 2019). Asthma is on a constant increase in its incidence and prevalence in children (Cevhertas et al., 2020), and mortality rates in adults (Dharmage et al., 2019)

The World Health Organization (WHO) in 1989 categorized the organic pollutants from indoor spaces into Very Volatile Organic Compounds (VVOCs), Volatile Organic Compounds (VOCs) and Semi-Volatile Organic Compounds (SVOCs). Among these, the volatile organic compounds have attracted significant scientific attention since then. As mentioned by Berglund et al (1992) and Baya et al (2004), this is mainly due to the diversity in the causes and effects of diseases occurring from different sources of VOCs.

In the light of such declarations, the US EPA (2011) published a work with the collection of air samples from almost 18 urban and rural settings from 1981 to 2005. Studies were further conducted by the New York State Department of Health (NYSDOH, 2006) and Zhu et al (2005) in Canada at randomly selected houses to report a baseline for indoor air quality comparisons. Two studies conducted by Rago et al (2004, 2005) also gave information about background indoor air quality in Massachusetts. Kurtz (2005), on the other hand, studied indoor air samples of 375 residences in Colorado after the installation of a sub-slab depressurization system. Similarly, Sexton et al (2004) studied 292 indoor air samples in Minneapolis/St. Paul metropolitan neighbourhoods to check the exposure of non-smoking, healthy adults to different sources of VOCs. Clayton et al (1999) analysed 396 indoor samples collected with passive air samplers (OVM 3520), which were later solvent-extracted and then analysed by Gas Chromatography/Mass Spectrometry (GC/MS) to generate percentiles. Gordon et al (1999) also conducted a study of 185 indoor air samples using a similar methodology. Other Indoor Air Quality studies include those of Kurtz and Folkes (2002) in Colorado, Van Winkle and Scheff (2001) in Chicago, Mukerjee et al (1997) in Texas, Heavner et al (1996) in New Jersey, Sheldon et al (1992) at California and by US EPA (1987, 1987a, 1987b) at California and New Jersey respectively. Although the burden of allergic diseases is increasing in India, training and studies to delve into its details are sparse (Krishna et al., 2020). Expanding such studies in the Indian context is crucial to developing effective mitigation strategies and public health policies.

The report submitted by the European Collaborative Action (1993) states that biological particles have also been implicated in sick building syndrome (SBS) (Harrison et al., 1987) and organic dust toxicity syndrome (ODTS). The allergic potential of such biological substances, as defined by Thompson and Stewart (1993), is due to their molecular complexity, concentration, solubility and stability in body fluids. Mould, pollen and animal dander are such common biological pollutants causing allergic rhinitis (Hay Fever | Rhinitis AAAAI, n.d.). Apart from this, asthma and eczema might be triggered by dust mites (Dust Allergy | Causes, Symptoms and Treatment, n.d., Dust Mite Allergy, n.d., Calderón et al., 2015). The degree of allergenicity in the work of Beaumont et al (1985) was found to be higher for house dust mites when compared to that of moulds in any indoor condition. One factor among biological agents, volatiles, was studied by Samson (1985) and recorded that fungal volatiles such as 1-octen-3-ol, 2-octen-1-ol and 1, 10-dimethyl-9-decalol (geosmin) in some people caused nausea and illness, while others rarely responded to these. Fecal allergen studies upon mites (Platts-Mills & de Weck, 1988) showed that sensitivity towards these volatiles is a severe risk factor for asthma. Severity was once again presented in the works of Platts-Mills and Chapman (1987) and Pollart et al (1988), who mentioned that even the dead and degraded body of the mite can retain its allergic properties. Poor biological air quality due to the presence of bacteria and fungi is also a trigger for infectious diseases and other digestive issues (Moldoveanu, 2015).

Allergic studies on House dust mites (Price et al., 1990 & Burr et al., 1980) and furred animals (Kjellman & Pettersson, 1983) proved that majority of the test cases were seen to be experiencing negative effects from the animals. The investigation done by Knysak in 1989 showed that other common pets like rodents, hamsters, desert rats (gerbils) and guinea pigs also contribute to allergy. It was suggested by Lowenstein et al in 1986 that pets of any kind, whether kept outside or inside, produce allergens which are quite directly inhaled. Further, rats, mice and insect vermin, such as cockroaches, were also to be considered as sources of allergens in indoor environments (Reed & Swanson, 1986). Moreover, the same

study proved that even dried saliva and urine of cats and dogs could function as a potential allergen. Other studies on cockroach allergens have shown that sensitization to the same can cause asthma (Do et al., 2016). This applies to several other insects like flies, moths, grain pests, crickets, locusts, etc. (Fukutomi & Kawakami, 2021).

Further, it was suggested that pollen or insect-derived substances may also be affecting respiratory health. A review report by Kauser (2018) suggests that wings, scales, saliva, dried faeces and venom from insects may cause allergic diseases, such as rhinitis, conjunctivitis, asthma and urticaria. The work conducted by Mathews (1989) brought out that airborne particles from cockroaches, cat fleas and cloth moths are significant to individuals in socioeconomically disadvantaged groups.

Details of animal allergens explained in the book authored by Hess-Kosa K (2002) reasoned that the kind of plantation surrounding a home is equally important. In Southern India, it was observed that rubber plantations were one reason for Mupli beetle aggregation in huge numbers (Sabu et al., 2008). Their presence in indoor spaces, in turn, resulted in the cause of a specific form of keratoconjunctivitis (Susan et al., 2010) because of a phenolic secretion they released when disturbed. It was also evident to be causing skin and eye irritations from its volatile secretions (Sabu et al., 2008). These insects are mostly attracted to tile-roofed and palm-frond thatched spaces which hold higher humidity than concrete tops (Sabu & Vinod, 2009a, b).

According to Kauser et al (2007), the presence and allergenic potential of an allergen in the patient's environment establish the clinical significance of any bioparticle. Such as the inhalation of a mycotoxin (Pitt, 2000; Rao et al., 2000a, b) resulting in acute renal failure (Di Paolo et al., 1993) and pulmonary haemorrhage in infants (Gregory et al., 2004; Yike et al., 2002; Yike et al., 2003). The review by Guillet et al (2022) additionally described in detail that insect allergies from various insect species may arise from direct contact, inhalation, or ingestion (both

accidental and deliberate), whereas the process of sensitisation usually occurs by inhalation or ingestion.

2.2. Insect semiochemicals: classifications and functions

It is estimated that there are almost 6 to 10 million insect species on Earth, and they serve as more than 90% of the life forms. Thus, humans have inescapable contact with the 33 orders of insects, which are further subdivided into 839 families (Bijlmakers insect classification, 2025). The presence of insects in indoor spaces is common and therefore, households could be classified as insect mini-ecosystems (Bertone et al., 2016; Leong et al., 2017). However, the level of infestation varies mainly with the differences in cultural practices (Schoelitz et al., 2019). The moderations of the seasonal presence of the insects are also affected by the presence of the indoor insects. (Martin et al., 2015).

The type of chemical communication that takes place between most animal organisms in the biotic community is called semiochemical communication. In the works of Regnier (1971), semiochemicals are the signal-carrying vehicles that carry information between organisms. These organic information-carrying substances of living creatures can be classified into two types. The first one includes the Pheromone. According to Karlson and Butenandt (1959), chemical communications of this kind take place between the members of the same species. This may be classified into two types, namely, releasers and primers (Reignier, 1971). The second type of communication takes place between members of different species, known as interspecific communication and includes allelochemicals. This is again divided into two types based on who favours the process of emission. As described by Brown, Eisner and Wittaker (1970), if it is the emitter who favours the chemical release process, then the semiochemical is termed as an allomone. And if it is the receiver that favours, they are termed as Kairomones (Brown et al., 1970). It is with these chemical signals that they engage and complete different needs and stages of their life cycle.

The first chemically identified pheromone was the sex pheromone of *Bombyx mori* in 1958 (Butenandt, 1959; Karlson & Lüscher, 1959). This has made insect pheromones, their nature, functions and their application a constantly researched area, particularly in the light of pest management. Insect semiochemicals favour the clustering of conspecific males and females using aggregating pheromones (Ginzel, 2010). Certain hydrocarbons found on the cuticular surfaces of insects influence mate choice to induce courtship (Ferveur, 2005; Wicker-Thomas, 2007). Selection of oviposition sites is also fixed by using similar chemicals. This scientifically proven positive relation of oviposition pheromones is released by conspecific eggs in *Culex quinquefasciatus* (Otieno et al., 1988) and by microbes for several *Aedes* species (Allan & Kline, 1995; Santana et al., 2006; Lindh et al., 2008). As mentioned by Regnier (1971), chemosensory channels with similar functions are also used for their survival through food and host selection, recruitment to food sources, alarm signalling, defence and aggregation. Arctiid moths have been seen to be using pyrrolizidine compounds, a usual toxin in some cases, to attract females and to defend themselves (Dussourd et al., 1991; Eisner et al., 2000). The hemiterpene 3-methylbut3-en-1-ol pheromone of two beetle pests (Bowers et al., 1991) also serves as an example of an aggregation pheromone. Alarm pheromones, including compounds such as sesquiterpene (E)- β -farnesene (EBF), germacrene A, and α -pinene in aphids (Vandermoten et al., 2012), oviposition deterrent pheromones of *Diachasma alloeum* (Stelinski et al., 2007), home recognition compounds in termite species (van Meer & Preston, 2008), trail detectors in hymenoptera (McPherson et al., 1997) and the sexual pheromones to complete the reproductive cycle in several insects (Baker, 2011), ascertain the influence of pheromones in all phases of an insect's life.

The semiochemicals secreted by insects may sometimes be a part of their defensive system. A study conducted by Hill and Tchinkel (1985) proved that there is a recorded difference in the secretion of semiochemicals based on the age, sex and size of beetles. It was also seen that the volume of semiochemicals collected

from the beetles varied drastically with age, supporting the fall of 64% of the total secretion between days 12 to 36.

A major part of such research complements the integrated pest management system by inducing cyclical changes to their population density (Borden et al., 2008; Jones & Evenden, 2008). This system includes monitoring, mass trapping, attract and kill and the introduction of mating disruption (El-Ghany, 2019) of insect pests.

2.3. *Luprops tristis* as a potential nuisance insect

Luprops tristis, locally known as the Mupli beetle, has been a nuisance pest for decades (Narendran, 1998; Jose, 2003; Thomas & Jacob, 2005) due to their uncontrollable seasonal aggregation. Although they negatively influence the residential areas near rubber plantations, their common host plant, they are usually harmless during the daytime (Sabu et al., 2008). But soon after sunset, these insects fly towards any lighted areas, traditionally tiled roofs, haystacks, wooden blocks, coconut husks and firewood stocks (Sabu et al., 2008). Adults of the *Luprops* beetles have three kinds of phases, including the pre-dormancy phase, dormancy phase and the post-dormancy phase. The adults of the pre-dormancy phase appear to be yellow brown. They do not start feeding until they turn dark because of an eclosion lasting for 3-4 hours, approximately (Sabu et al., 2008). Those dark beetles in the dormancy phase undergo an oligo pause for a short time from the first summer showers. They tend to move and get attracted towards dry conditions, which is one reason for their massive invasion into dry residential spaces (Watt, 1992; Endrody-Younga & Tschinkel, 1993). Soon after the leaf fall, which is a plant developed mechanism to prevent excessive water loss during the dry season (Santiago et al., 2004; Santiago & Mulkey, 2005; Sundarapandian et al., 2005), the beetles return to the thick litter spots. This thick litter base then provides suitable food and habitat for the beetles (Sabu et al., 2008). The return of the summer season after the first summer showers is working as an arousal cue for the *Luprops*. During the post-dormancy phase, the size of the residential aggregation can be seen to be decreasing

as the beetles tend to return to the rubber plantations. There, they get attracted to the falling tender leaves and water droplets, showcasing this as their intense feeding phase. Soon after their mating, the parent generation dies off, and the new generation of adults begins the cycle of dormancy.

The 3-4 layer thick, deep and strong aggregations of the *Luprops* beetle can be seen behind the crevices, attics and any dark and cozy spot. They seem to be saving energy, water and keeping themselves separated from the wet conditions present there (Wolda & Denlinger, 1984; Denlinger, 1986; Danks, 1987; Leather et al., 1995). They just depend on the fat reserves present in their body. The phenolic secretion of this beetle causes burns when pressed (Sabu et al., 2008). It has also been seen to cause keratoconjunctivitis (Susan et al., 2010).

2.4. Importance of questionnaires in indoor air quality research

In most studies regarding indoor environments, questionnaires were developed to gather foundational information prior to air sample collection. As per the one developed by Weisel et al (2006, 2008), the questionnaire is to be designed according to the types of buildings and peculiarities of both indoor and outdoor environments. Such that the questionnaire focuses on general parameters, including indoor features of the houses and products present in the house. Similarly, the indoor assessment of 100 homes (a 24-hour sample collection using Canister coupled with GC/MS analysis) was done by Weisel (2006, 2008) in New Jersey, incorporating a primary questionnaire to have an idea about the potential sources of pollution. The work by Sheldon et al (1992) was split into different stages- sampling of geographic areas in the first stage, sampling of housing units for screening in the second stage and sampling of households and persons for monitoring in the third stage. NYSDOH (2006) emphasized that systematic inspection is an important element before the sampling events to identify and minimize the factors that may interfere with the kind of test conducted.

From the questionnaire developed during the works of Cranshaw (2020) and Bertone et al (2016), it was found that the increase in the number of insect pests

was due to landscape practices, including the availability of specific host plants, lighting, features of building construction, geographic features and conditions of the internal environment.

Bennett et al (1983) reported that most residents identified ants, flies, and fleas as the predominant indoor insects, while only 3.5% considered cockroaches as a major pest. Dingha et al (2013) found that ants, mosquitoes, and flies were more prevalent than cockroaches as major indoor pests. The list of insect pests collected by the Amsterdam Municipal Health service by Buijs (2009) included grey silverfish, Indian meal moths, moth flies, clothes moths, carpet beetles, biscuit beetles, and booklice. Working questionnaires of this kind can also help establish the threshold level for pest populations to determine the acceptable population of insects (Sarisky et al., 2008). Such studies may become more informative when clubbed with both the questionnaire data and the physical data (Clayton et al., 1999). The physical conditions, including airflow and building inventory for products, may all be suitably added according to the main objectives of the study.

2.5. Analytical techniques for semiochemical characterization

Indoor volatile organic compounds are always risk factors for various health issues for them being carcinogenic and toxicants for the central nervous system (Li et al., 2021). Therefore, they require continuous monitoring. NYSDOH (2006) suggests that samples may be collected from an adequate number of houses depending on the study type, likely sources of VOCs and the potential exposure to VOCs.

The main categorization of the type of air sampling includes active sampling and passive sampling (Millar & Haynes, 1998; Tolnai et al., 2000). Huang et al (2018) have observed that most of today's analysis of the toxic VOCs, which can even deplete the ozone layer, is done using passive air sampling techniques. Passive air samplers are small, uncomplicated, easy to transport and work without the use of any external power (McLagan et al., 2016a; McLagan et al., 2016b). This method

is the most suitable for long-term monitoring work. Quality assurance, according to Parra (2008), is also to be maintained whichever method we take along with our study. The key features explained that they mainly involve the ability to accurately analyse the target compounds within detection limits, reproducibility and recovery of the analytical method, and the use of blank samples.

During their study, Panagiotaras et al (2014) found that the toxic VOCs are difficult to sample and analyse as they show great diversity in their physicochemical properties. Due to the same, different scientists have experimented using different methods, such as Kim et al (2014) with sorbent tubes, Lee et al (2002) with canisters and Yao et al (2015), Saalberg and Wolff (2016) with Tedlar bags. VOCs are also seen to be showing evident differences for different seasons in studies by Ilgen et al (2001) and Schlink et al (2004), with their concentration increasing in winter and decreasing in summer. This has also been demonstrated by Sillman (1999) as VOCs readily evaporate in summer rather than being retained in the atmosphere.

According to the works of Platts-Mills and de Weck (1988), the most practical disadvantage of airborne sampling is the long sampling periods (2-24 hours), even though the requirement of short periods of high allergen concentration remains important. The general methods for collecting volatile semiochemicals from phytophagous insects are described in the works of Blight (1990). Laboratory experiments may also be conducted to unravel the unexplained roles of insect semiochemicals. Complex semiochemical-mediated interactions have always been a subject of constant attraction (Blight, 1990). Works include those in microbe-targeting semiochemicals produced by ants to manipulate the soil environment (Vander Meer, 2012), chemically mediated defensive behaviours of ground beetles (Rork & Renner, 2018), and chemical cues that regulate/affect insect family and parental care (Steiger & Stökl, 2017).

Ponzio et al (2014) conducted studies on caterpillar-induced plant volatiles by placing the required large plants in 30 L glass jars 30 minutes before the trapping

commenced. Whereas volatiles released from non-infested wheat grist samples and faeces of *T. confusum* host larvae were elucidated by placing the sample in Erlenmeyer flasks (Fürstenau et al., 2016). Here, the collection of volatiles was static. Although the capacity seems limited, a lot of similar works have been done on the Erlenmeyer flask by bringing in modifications. According to Petroski et al (2005), the discovery of insect-borne semiochemicals becomes a tough task because of their presence in very minute quantities and being unstable at ambient temperature, thus requiring great technical skills for its work. The Volume I and II texts on chemical ecology by Haynes and Millar (1998) also give us a description of semiochemical research techniques.

During their studies on the alarm pheromone of winged dispersal morphs in aphids (Kunert et al., 2005), an apparatus which consisted of two 3L glass chambers, one containing the control and one the treatment plant, was used. Also, a volatile trapping system for plants was developed by Durenne et al (2018), introducing manual modifications to an existing gas wash Dreschel bottles to facilitate the growth of oilseed rape plantlets and the collection of volatiles.

The aphrodisiac pheromone release of small white cabbage butterflies was monitored by placing them individually or together in a clear I-chem jar (150 mL, 6 cm wide by 7 cm high) equipped with a lid containing a septum (Li & Mathews, 2016). In the experiments, a line of PEEK tubing (1/1600 OD, 0.01000 ID) from a compressed air cylinder was inserted into the septum and placed near the bottom of the jar. The second PEEK tubing (15 cm in length) was used to connect to the second jar or the cDART ion source. To identify the sex pheromone of the mealybug *Dysmicoccus grassii Leonardi*, the collected volatiles from virgin females were introduced into a 2 L round-bottom flask with its mouth fitted to a gas-washing piece with its inlet coupled to an activated charcoal-filled glass tube (Alfonso et al., 2012). The outlet of this system was then assembled into a glass tube filled with 250 mg of preconditioned type Q, 80–100 mesh Porapak (Sigma-Aldrich). The “purge and trap” approach used by Schoelitz et al (2020) also

adsorbed the semiochemicals of mosquitoes onto another adsorbent material, Tenax TA 20/35, to remove the background volatiles; they made use of a carbon filter.

2.6. Influences of biological volatiles on human health

The number of allergic patients with bronchial asthma (Fleming et al., 1987; Haahtela et al., 1990; Yunginger et al., 1992) and allergic rhinitis (Aberg, 1989; Ishizaki et al., 1987; Zimmerman et al., 1988) has been increasing continuously. Since many studies on volatile organic compounds (VOCs) have proved them to be categorised as toxic chemicals which are harmful to environmental sustainability and human health, such areas need to be researched. Volatile organic compounds have been associated with most health disorders like allergies, eye irritation, vascular nervous dysfunction, acute chronic health pathologies and even cancer (Parra et al., 2008; Marc et al., 2012). The increased amount of time spent indoors by an individual and its long-term but severe effects have also helped in the establishment of certain health-based goals for different air pollutants by the World Health Organization (WHO 1989, 2002 & 2005). One study conducted by the WHO (2002) states that indoor air pollution mostly brings in acute respiratory infections in children and chronic lung diseases in adults.

In a work conducted by Young et al (2015), it was seen that certain human semiochemicals and human sweat compounds attracted major vectors of Onchocerciasis in causing diseases. Therefore, the elucidation of the influences of insect semiochemicals on indoor air quality and their effect upon common diseases is to be dealt with grave concern.

The usual diseases and symptoms of these diseases caused by VOCs occur with less severity because, from the public health point of view, the total impact of air pollution was always dominated by the less severe health effects, such as subclinical and symptomatic events (WHO, 2001). But this is to be considered with mere seriousness as these symptoms may result in long-term effects (Mangotha and Singh, 2024). The press of British Columbia did release a few health concerns of

VOCs, which included eye, nose and throat irritation, shortness of breath, headaches, fatigue, nausea, dizziness and skin problems.

The presence of certain other biological substances also imparts their negative effects on air quality and human health, like those in fungi as studied by Burge (2004), Institute of Medicine (2004), Straus (2009) and WHO (2009). Most of the diseases which appeared since the appearance of humans have been dealt with by great scientific technologies and theories, but most of them regarding Parkinson's disease (Inamdar et al., 2013) and others have just focused on synthetic chemicals like insecticides and pesticides (Sarwar, 2016). Salama and Arias-Carrión (2011), who worked on the environmental factors that influenced Parkinsonism, stated that this was something that began appearing even before the industrial revolution and the synthesis of chemicals. The presence of certain biological substances like fungi (Empting, 2009; Kilburn, 2009) has always been linked to certain neurologic and neuropsychiatric signs and symptoms, including movement disorders and loss of balance and coordination. As per the reports of the Institute of Medicine, the VOCs and SVOCs produced by various moulds and bacteria are found to be causing irritation and inflammation of mucous membranes, respiratory effects, immunotoxicity, neurotoxicity, sensory irritation, dermatotoxicity, along with some carcinogenic effects. The negative influence of the fungus-derived semiochemical, 1-octen-3-ol, on dopamine neuron degeneration, however, establishes the involvement of a naturally occurring environmental agent in parkinsonism (Inamdar et al., 2013).

2.7. Experimental models to study the health influences of insects

Insects in indoor spaces can be mostly classified as nuisance insects. The common colonising indoor insects like cockroaches and silverfish have their bodily modification resembling the cave-dwelling insects, such as flattened bodies, long antennae, or active dispersal capabilities (Martin et al., 2015). Nonetheless, there are other insects still capable of infesting indoor environments (Schoelitz, 2019). The local availability of certain plant species was the most

common landscape feature related to the presence of such nuisance invaders (Cranshaw, 2020), which concluded that the effect of the presence of plants on insects may sometimes be indirect (for them providing honeydew to the invader species). It is seen that lighting and certain planting practices around the building have contributed to the increase in the number and effect of nuisance invaders (Cranshaw, 2020). However, the temperature and humidity differences from room to room in every house were seen to be tolerable by such insect communities (Frankie & Ehler, 1978).

All body matters (Katial & Waibel, 2003; Cavazos et al., 2008), and the chemicals of insects can be categorized as insect allergens. These allergens can be recognized as inhalants, ingested and injectable allergens depending on the route of sensitization (Elshabrawy et al., 2014). The studies conducted by Kauser et al (2007) proved the existence of species-specific mosquito-derived allergens in the air. They have also conducted skin prick test (SPT), RAST, and immunoblot experiments to bring out the fact that it was important in causing type 1 allergic respiratory disorders. Will and his co-workers have mentioned that allergic diseases caused by insects are caused by the scales and toxic fluids of adult moths and butterflies, chironomid bodies and scales allergy by mayflies causes allergic rhinitis and asthma (Figley, 1929; Mazur et al., 1988). The review works of Kauser et al (2007) mention that the individual chemicals of the stinging insects were seen to be mild by nature, but when combined, they became stronger. Similar conditions can also be expected in the case of semiochemicals.

The World Allergy Organization has brought out reports of deaths from insect allergies. It can be observed that it is 40 deaths per year in the US, 11.5% greater than that of the US is in the UK (Turner et al., 2015), 15-25% of the total population in Australia (Hedde & Brown, 2004) and more than half of the total population in Japan (Tang et al., 2009). 28% of the total population in Africa and one-third of Indians from the total population (Kauser, 2018) are also affected by the same. Natural, domestic, hobby-related and occupational kinds of allergies may sometimes be observed from exposure to insects and their materials. High incidence

of skin positivity to insect extracts was reported to cause asthma, hay fever, atopic dermatitis, and conjunctivitis (Feinberg et al., 1956, Nabavizadeh et al., 2025), bronchial asthma or allergic rhinitis (Wiseman et al., 1959; Hellreich, 1962; Hosen, 1970), respiratory diseases (Sethi et al., 1989; Chaudhry et al., 1990; Gupta et al., 1990; Jhamb et al., 1991; Jhamb et al., 1992; Jhamb et al., 1995; Jhamb et al., 1994(a); Jhamb et al., 1994(b)) and type I allergic respiratory disorders (Jhamb et al., 1992).

Skin tests with an accuracy of 90-95% remain among the most common diagnostic tests (Golden, 2011). To have a much clearer elucidation, certain other sensitivity tests may also be performed. Hirvonen (1997) studied them using certain gram-positive and gram-negative bacteria, among which endotoxin LPS and *Pseudomonas fluorescens* showed a decrease in the viability of cells after 4 hours.

Inflammatory response studies include RAW 264.7 (Receptor Activation With 264.7 cell line) macrophages to three mycobacteria by Huttenen et al (2000), RAW 264.7 macrophages to *Streptomyces annulatus* spores by Hirvonen et al (2001) and effects of three moulds (*Stachybotrys chartarum*, *Aspergillus versicolor*, and *Penicillium spinulosum*) and one gram-positive bacterium (*Streptomyces californicus*) isolated from water-damaged buildings were conducted by Murtoniemi et al. (2002). The cytotoxicity of 20 *Stachybotrys* isolates was also studied by Neilsen (2001) to study their ability to induce inflammatory responses in RAW 264.7 macrophages. Other studies conducted in mice include inflammatory responses to a single intratracheal instillation of three doses of *Streptomyces californicus* spore isolates by Jussila et al. (2001), inflammatory and toxic responses to the bacteria *S. californicus* (Jussila et al., 2001, 2003) and *Mycobacterium terrae* (Jussila et al., 2002a) and the fungi *Aspergillus versicolor* (Jussila et al., 2002b) and *Penicillium spinulosum* (Jussila et al., 2002c). A few studies were also conducted to study the respiratory effects. Pang et al (1987) studied a single nebulized dose of T-2 toxin at 9 mg/kg on the lung tissues of young pigs (9–11 weeks old). Whereas Nikulin et al.

(1996) studied the effect of two strains of *Stachybotrys atra*, using a group of four 5-week-old mice. Mason et al. (1998) similarly examined the effect of *Stachybotrys chartarum* conidia and isosatratoxin-F, from rabbit lung, and their effects in mice.

The study conducted by Flemming et al (2004) elucidated clear-cut differences in vascular, inflammatory, and cytotoxic lung responses in mice intratracheally exposed to *Cladosporium cladosporioides* or *Stachybotrys chartarum* spores, even at low spore doses. Similar important results were also brought out by Rand et al (2002,2003) in lung cytology and histopathology associated with exposures to spores of these two species. Most of the works, including animal models like that of Jussila et al (2003) and Rudnicka et al. (2019) had models that were exposed via intratracheal instillation to a specific number of doses (with day/hour intervals) of the test microbe or chemical. Further, after almost 24 hours, they investigated changes in cell populations in the lungs, lymph nodes, and spleen. The changes in the number of neutrophils, macrophages and lymphocytes suggested that most of the exposures provoked a dose-dependent inflammatory cell response. An increase in T lymphocytes and biochemical markers like albumin, total protein, and lactate dehydrogenase was also observed. Such skin sensitisation tests of laboratory animals are to be done following the OECD guidelines (2015).

Insects, however, have been considered a constant nuisance to health mostly due to their stings, bites (Sturm et al., 2023), allergens from their body parts, fecal matter or saliva (Kauser et al., 2022). One way to elucidate their probable negative impact on the human body is to conduct cell cytotoxicity assays.

Abdelfattah et al (2022) performed a cytotoxicity assay by exposing living cells, mainly kidney and liver cells, to insect residues in stored grains and found that these had notable cytotoxic and genotoxic effects. Similarly, the cytotoxicity of the total defensive substance (TDS) of a medicinal beetle *Blaps rynchopetera* Fairmaire, was also measured by Xiao et al (2017). Further research on this insect by Pang et al (2022) has shown their potential usages in cancer studies

due to their cytotoxic properties. It was seen that their volatiles, when used in different combinations, exhibited even higher cytotoxicity. Similar works of Jangir and Nadumane (2023) also showed a parallel antitumor effect with the volatile extracts from different ant species. With a place of rich insect diversity, more scientific understanding regarding their mode of action and the involvement of various other insects calls for more work on the same.

2.8. Research gaps and future

Systematic indoor environmental studies consider different air monitoring techniques, sources of contamination, study samples, analytical methods for air toxins, quality assurances, data evaluation procedures and health risk assessments. These can also be done following the guidelines of MADEP (2002). The importance of such works increases when the concentration of the VOCs detected stays above the health-based screening levels mentioned in the reports of the US EPA (1995, 2002) and the New Jersey Department of Environmental Protection (2005). According to Weisel et al (2008), one of the major limitations is that some of the VOCs may have their detection limits above the health-based indoor screening levels, so the need to implement effective remediation strategies remains high. The reported works of the European Collaborative Action (1993) clearly define that the detailed investigation into the understanding of biological samples in air and dust demands skill and experience. To provide standardization in the future, these kinds of work require international collaborative studies, particularly on sampling and analytical methods.

Chapter 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

This chapter outlines the materials and methods employed to investigate semiochemicals from *Luprops tristis* and their influence on cell lines. The first part of the work used a detailed questionnaire, which helped understand pests found in indoor spaces and resulted in identifying the *Luprops tristis* as the study organism. This was followed by the collection of this pest to subject them to volatile organic compound analysis. A volatile collection chamber was specifically designed to conduct this procedure. After analysing the volatiles, an MTT assay was conducted to understand the cytotoxic effects of a selected volatile compound.

3.1. Survey and analysis

3.1.1. Questionnaire design

A detailed questionnaire consisting of 40 questions was prepared, referring to various published surveys related to indoor air quality measurement, the presence of pests and allergies. The questions were categorised in the order of general information, starting from the respondent's name, contact number, place, number of occupants, noting children under 12 and adults above 60. Age of the building, structural type (house, apartment or commercial space), ventilation status and roof type were to be considered before noting the values of general air quality parameters, including temperature, humidity, SO₂, CO, PM 2.5 and PM 10. Information from the surrounding vegetation and common biological contaminants, including animal dander, pollen, moulds and mites, was also to be recorded. The survey also had questions related to entomological information, including insect pests' occurrence, kinds of pests, signs of infestation, possible reasons for their presence, control measures used, and any allergy-related issues connected with the presence of the insects. Detailed health-related information regarding the number of individuals with an illness, their diagnosis, and any medications they were taking.

Before data collection, population figures, geographical position, surrounding vegetation and pollution sources were also considered to ensure representation from diverse environmental contexts.

3.1.2. Survey data collection

A detailed survey was carried out in 403 randomly selected indoor settings for 5 months (from 23rd September 2020 to 17th March 2021). The survey covered 322 indoor spaces across 56 rural villages and 81 indoor spaces in four municipalities within Malappuram district (Coordinates: 11°N 76°E), Kerala, India (Figure 3.1). Three methods were deployed to collect the information - direct interview, telephonic interview and self-administered questionnaires. During the process, information was also collected using a number of other questions (as detailed in the appendix).

The 5-month dataset was aggregated for statistical analysis using R (version 4.0.5) (R Core Team, 2021). R Studio has a user-friendly interface with the availability of using different packages. The raw data was imported using the “readr” package after converting it into a comma-delimited file. The packages including “dplyr”, “tidyr” and “janitor” were used for handling missing values, converting the types of variables, renaming the columns and filtering the rows. The chi-square test was used to find the significance of different variables using the package ‘chisq.test()’.

Additionally, data manipulation and cleaning packages including “dplyr” - to filter, select and summarise datasets, “stringr”- to match patterns and replacements, “tidyverse”- a metapackage including several other packages, “tidyr”- to reshape the data, “tibble”- for better printing and subsetting, “magrittr”- to provide pipe operator and “readxl”- to read Excel files were used.

Data visualisation packages used include “ggplot2”/“ggplot” - core package to create graphical visualisations, “RColorBrewer”- to apply color to charts, “patchwork” -to display 2 charts together, “hrbrthemes”- to apply themes to charts, “ggpubr”- to enhance the ggplots, “scales”- to provide formation of axis and its labels, “ggtext”- to add in text elements, “box”- to introduce reusability of data, “ggsci”- to introduce sci-fi inspired colour palettes and “ggthemes”- to add extra themes. The other statistical analysis packages used include “car” to conduct

regression analysis, “lsr” for basic statistical analysis, and “MASS” to use functions and data sets.

As a result of the analysis, it was concluded that *Luprops tristis* was an insect that was found to be in huge numbers in indoor spaces, causing unexplained health issues, even without being an insect that stings or bites directly. Hence, luprops was selected as the test organism for further studies.

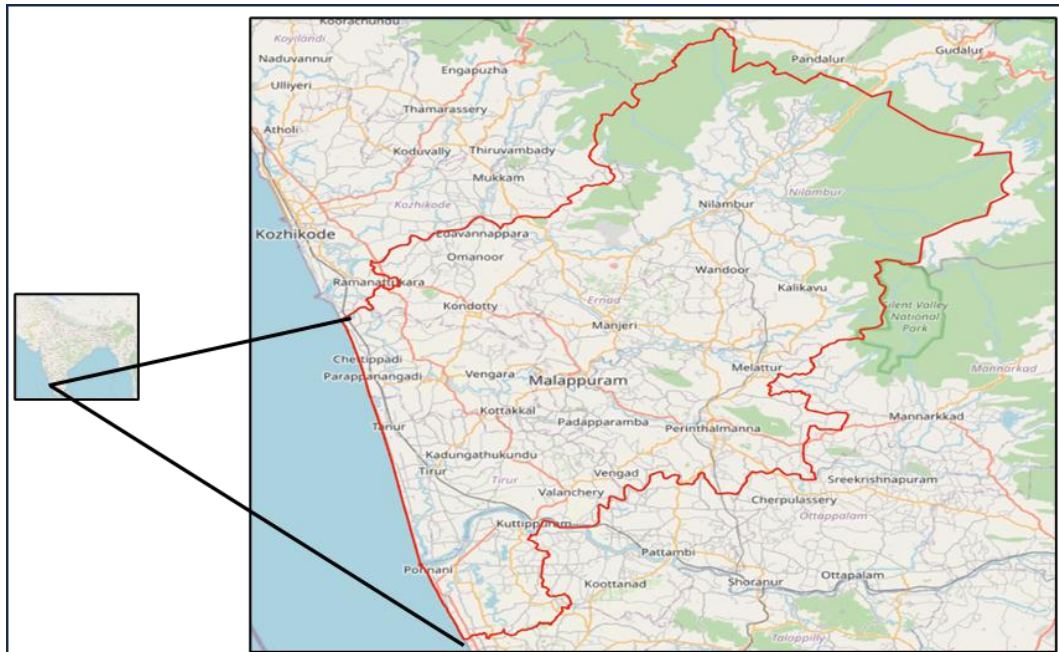


Figure 3.1. Geographical map of the study area (11°N, 76°E, Malappuram, a District in the State of Kerala, India, with an area of 3,554 km²)

3.2. Volatile analysis

3.2.1. Insect collection for semiochemical analysis

Luprops tristis (Figure 3.2 and 3.3) were collected from an indoor system for nearly 2 months (April to May 2022) from Malappuram (Coordinates: 11°N 76°E), Kerala, India. Suitable collection methods, including hand-picking and brushing, were used to collect the insects in a glass jar from the sampling site. The lid of the glass jar was perforated to allow the required amount of gas exchange. The insects collected were transported to the laboratory. After which they were transferred into a headspace volatile collection chamber for direct volatile organic compound analysis. General parameters such as Temperature, Humidity, HCHO, TVOC, Particulate matter 2.5 and 10 from the site of collection and the lab were also collected using air quality monitors.



Figure 3.2. Dorsal view of adult *Luprops tristis*.



Figure 3.3. Indoor aggregation of Mupli beetles (*L. tristis*) observed in a household in Malappuram.

3.2.2. Experimental design of VOC collection chamber

To facilitate the profiling of the whole body volatilome of *Luprops tristis*, an economically feasible sampling chamber was engineered to collect the headspace volatiles from the entire insect body in laboratory conditions.

A glass chamber with a total volume of approximately 1000 ml was designed with 2 inlets and 2 outlets of 5mm in diameter each on its cap (Figure 3.4-A). Almost halfway through the chamber is a slight constriction to rest a removable glass disc with perforations measuring 1 mm in diameter. This is used to restrict the insects placed at the bottom of the chamber from moving upwards towards the inlets and outlets, thus blocking the pathway of the air passage. Air at a controlled flow rate (1000 ml/m) was allowed to pass through silicon tubes to activated charcoal (200mg, 40-80 mesh size) embedded in a glass tube to remove contaminants. The filtered air then enters through a silicon tube to the inlet of the chamber. The volatiles released by the beetles, which are collected at the headspace portion of the glass chamber, is carried along with the filtered air through another silicon tube connected at the outlet into a different glass tube embedded with activated charcoal (200mg, 40-80 mesh size), now acting as adsorbent material. Whole-body VOCs were analysed from freshly collected batches of 500 and 1000 beetles at different time intervals (2hr, 6hr, 12hr and 24hr period) (Figure 3.4-B). The activated charcoal was subjected to solvent-assisted desorption using n-hexane (Emplura-Merck) for 24 hours and subsequently analysed using gas chromatography/mass spectrometry (GC-MS) to characterize the compounds present in the whole-body headspace sample. A control experiment, replicating the same procedure but devoid of beetles, was also maintained for each period.

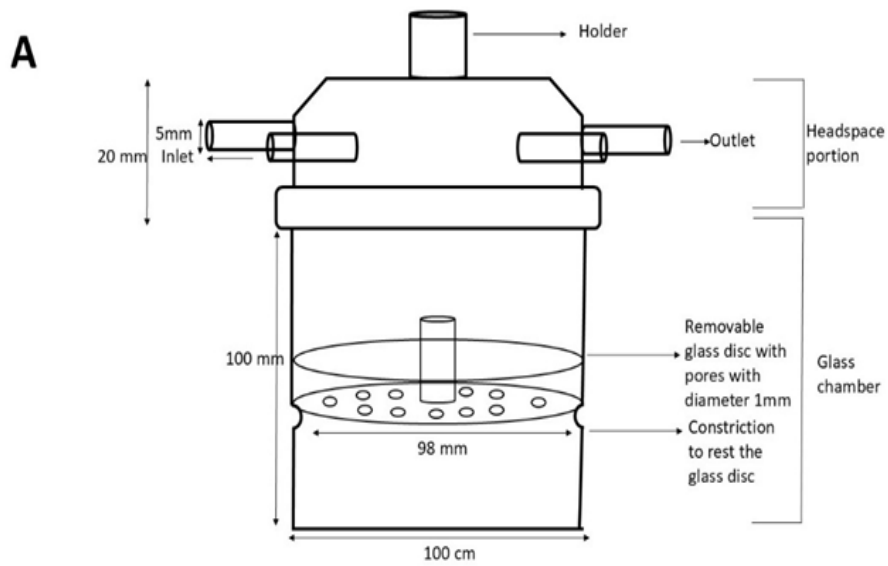


Figure 3.4. A- The picture depicts an economically feasible glass chamber designed to conduct the whole body volatilome of insects. This chamber has 2 inlets and 2 outlets to facilitate the movement of volatile compounds along with air pushed through the system. The separation disc prevents the unnecessary movements of the beetle through the chamber. **B-** Figure represents the experimental setup. Air was pumped after filtering through activated charcoal to the inlet, which collects the air containing volatiles from beetles and pushes them to the activated charcoal adsorbent attached to the outlet.

3.2.3. Gas Chromatography- Mass Spectrometry

A Shimadzu GC-MS system with model number QP2010S was used for the volatile compound analysis. The column used was ELITE-5MS with a 30-meter length, 0.25 mm internal diameter and 0.25 μm thickness. Data acquisition and analysis were performed using GC-MS Solutions software, utilising the NIST 11 and WILEY 8 spectral libraries for compound identification.

During the analysis, the column oven temperature was maintained at 70°C and the injection temperature at 250°C. The injection was carried out in splitless mode. The initial hold time was 2.00 minutes and the flow control mode was set to linear velocity. The pressure was adjusted to 61.5 kPa, the total flow to 54 mL/min, the column flow to 1 mL/min, the linear velocity to 36.7 cm/sec, the purge flow to 3 mL/min and the split ratio to 50

The ion source temperature was maintained at 200°C, the interface temperature at 280°C and the solvent cut time at 4 minutes. The detector gain mode was relative detector gain at 1.12 kV +0.20 kV and threshold at 1000. The start time was 4.10 minutes and the end time was 35.75 minutes. The ACQ mode was scanned with the event time for 0.30 sec and scan speed at 1666.

3.3. Measurement of irritational potential of volatiles

3.3.1. Selection of heptadecane volatile for MTT assay

The GC-MS profiles of the compounds identified from the *Luprops tristis* in the glass chamber with 500 and 1000 individuals were identified to be of different chemical nature. Hydrocarbons, Alcohols, Esters, Aldehyde and Ether being the major classifications found.

Among them, Heptadecane was selected due to its known aspirational hazard. Heptadecane was procured from Sigma Aldrich (Linear Formula: $\text{CH}_3(\text{CH}_2)_{15}\text{CH}_3$, CAS No.: 629-78-7, Molecular Weight: 240.47, Pack size: 25g, EC No.: 211-108-4 and Beilstein No.: 1738898)

3.3.2. Cell line for MTT assay

Test material: Heptadecane (Sigma Aldrich)

Cell line used: Rat intestinal epithelial cells (IEC 6)

Cell culture: Cell line (IEC-6) was procured from the National Centre for Cell Sciences, Pune, India and maintained in DMEM (Dulbecco's Modified Eagle Medium) media, supplemented with 10% fetal bovine serum, 100 µg/mL penicillin and 100 µg/mL streptomycin and kept at 37°C in an incubator with 5% CO₂. The cells were passed at 80-90% confluence and the medium were changed every third day.

3.3.3. MTT assay procedure

Cytotoxicity was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)- 2,5-Diphenyltetrazolium Bromide) assay (Mosmann, 1983). Approximately 1x10⁵ cells/mL were seeded in a 24-well plate, with a complete growth medium named Dulbecco's Modified Eagle Medium (DMEM) and allowed to attach and grow. At 80% confluency, the medium was replaced with fresh medium containing different concentrations of samples (6.25-100 µg/mL) and incubated for 48 hrs. At the end of the incubation period, the spent medium was removed and washed with PBS. Thereafter, the cells were overlaid with fresh DMEM containing 40 µL/mL (5mg/mL) MTT and incubated for another 4 hrs. The formazan crystals formed were dissolved in dimethyl sulfoxide and the absorbance was measured at 570 nm in a microplate reader (BioTek, USA). A control experiment, without any treatment and a vehicle control with the presence of the agent used to dissolve the test material was also maintained. It is similar to control tubes.

The percentage cell viability was calculated using the formula:

$$\% \text{ viability} = (\text{OD of control} - \text{OD of Treated}) / (\text{OD of control}) \times 100$$

3.3.4. Statistical analysis

The cytotoxicity assay results were aggregated for statistical analysis using R (version 4.0.5) (R Core Team, 2021). The raw data was imported using the “readr” package after converting it into a comma-delimited file. The chi-square test was used to find the significance by using the dosage of heptadecane as the first variable and the percentage of cell inhibition as the second variable.

Other packages used for data manipulation and cleaning include “dplyr” to filter, select and summarise datasets, “janitor” to clean the data, “stringr” to match patterns and replacements, “tidyverse” a metapackage including several other packages, “tidyr” to reshape the data, “tibble” for better printing and subsetting, “magrittr” to provide pipe operator and “readxl” to read Excel files.

Data visualisation packages used include “ggplot2”/“ggplot” core package to create graphical visualisations, “RColorBrewer” to apply color to charts, “patchwork” to display 2 charts together, “hrbrthemes” to apply themes to charts, “ggpubr” to enhance the ggplots, “scales” to provide formation of axis and its labels, “ggtext” to add in text elements, “box” to introduce reusability of data, “ggsci” to introduce sci-fi inspired colour palettes and “ggthemes” to add extra themes.

The packages used for statistical analysis include “chisq.test()” to perform a chi-squared test, “car” to conduct regression analysis, “lsr” for basic statistical analysis, and “MASS” to use functions and data sets. The results obtained were further compared with previous literature works to reach conclusions as discussed in the upcoming chapter.

Chapter 4

RESULTS

4.RESULTS

This section provides the results of the work done investigating the impact of volatiles on rat cell lines. Here, the results are divided into sections and subsections, where the images are displayed as graphs and tables with detailed descriptions and statistical inferences to aid the interpretations. The first section of the results deals with the information obtained during the data collection using the detailed questionnaire. This is the part which allowed the research to understand, identify and select *Luprops tristis* as the test insect. The second section deals with the results of the volatile analysis, where a detailed understanding of the chemical composition of the volatiles released by the beetle was obtained. This also resulted in the selection of a compound to conduct the cytotoxicity assay. The last section describes the results of the cytotoxicity assay using heptadecane as the test compound.

4.1. Survey of indoor insects for the selection of a test species

4.1.1. Distribution of common indoor insects based on building characteristics

Numerous pests were reported to be present in the indoor spaces surveyed (Figure 4.1). Although most were insects, some non-insect pests, such as lizards, spiders, and mice, were also recorded. Mupli beetles, mosquitoes, ants, cockroaches, termites and house flies were the most common insects. A few uncommon ones like cicadas, centipedes and fall webworms were also observed in some indoor spaces. Mupli beetles, mosquitoes and ants were found in more than 15% of the indoor spaces surveyed, while cockroaches and termites were observed in more than 10% of the sites. House flies were recorded at over 5% of the sites surveyed, while other pests were found in less than 5% of the indoor spaces.

A comparison between the presence of insects and the type of surveyed locations (categorised as rural and urban) (Figure 4.2) showed that some insects were recorded more frequently from certain places. There was a significant increase in the number of Mupli beetles recorded in rural areas than the urban spaces ($\chi^2 = 14.3$, $df = 1$, $p = 0.0001$). 84% of the indoor spaces in rural areas had Mupli beetles,

while it was only 65% in the urban areas. Whereas, less and non-significant difference was found between rural and urban areas for the presence of other most common insects such as ants ($\chi^2 = 0.03$, $df = 1$, $p = 0.85$), cockroaches ($\chi^2 = 1.52$, $df = 1$, $p = 0.21$) and mosquitoes ($\chi^2 = 0.0002$, $df = 1$, $p = 0.98$).

Figure 4.3 shows a comparison between the presence of insects and the age of the building showed a significant difference in the case of Mupli beetles, as their number remained high in indoor spaces constructed less than 2 years of age and those over 10 years ($\chi^2 = 7.29$, $df = 2$, $p = 0.02$). Whereas the percentage of beetles recorded at the building over 5 years of age but less than 10 years of age was recorded lesser. The presence of all the other insects such as ants ($\chi^2 = 0.80$, $df = 2$, $p = 0.66$), cockroaches ($\chi^2 = 0.33$, $df = 2$, $p = 0.84$) and mosquitoes ($\chi^2 = 0.08$, $df = 2$, $p = 0.95$) was found to be consistent irrespective of the age of the buildings. These insects were present a higher numbers at all the buildings irrespective of the age of their construction.

A comparison of the insect presence and the ventilation status of the buildings shows that most insects were not significantly affected by changes in indoor ventilation. There was no significant increase in the number of Mupli beetles ($\chi^2 = 0.18$, $df = 2$, $p = 0.91$), ants ($\chi^2 = 0.51$, $df = 2$, $p = 0.77$), cockroaches ($\chi^2 = 1.05$, $df = 2$, $p = 0.59$) and mosquitoes ($\chi^2 = 0.61$, $df = 2$, $p = 0.74$). All the insects were found in consistent numbers despite the level of ventilation being classified into bad, average and good as seen in Figure 4.4.

The comparison between the presence of insects and the type of roofs of buildings showed a significant difference in the case of Mupli beetles as seen in Figure 4.5 ($\chi^2 = 15.75$, $df = 2$, $p = 0.0003$), where they were seen mostly (91%) in indoor spaces in buildings with mud tile traditional roofing systems. The type of roofing systems had a significant impact on mosquitoes ($\chi^2 = 9.40$, $df = 2$, $p = 0.009$) and cockroaches ($\chi^2 = 40.658$, $df = 2$, $p = 1.483e-09$), whereas ants were seen equally at all places ($\chi^2 = 0.40$, $df = 2$, $p = 0.818$).

Numerous trees were reported to be present surrounding the indoor spaces at the surveyed sites. Coconut, rubber, mango and teak were the most common trees as visualized in Figure 4.6. A few uncommon ones like sandalwood, neem, Indian rosewood and gulmohar were also observed. Coconut was found in more than 30% of the indoor sites, while rubber was seen in more than 20% of the places. The other trees were only recorded from less than 10% of the indoor spaces.

A comparison between the presence of insects and the two major kinds of trees recorded during the survey shows these certain insects were found more during the presence of trees (Figure 4.7). There was a significant increase in the number of insects with the presence of coconut trees. This was considered significant in the case of Mupli beetles ($\chi^2 = 90.31$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 263.5$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 259.68$, $df = 1$, $p < 0.005$) and cockroaches ($\chi^2 = 223.03$, $df = 1$, $p < 0.005$). The presence of rubber plantations nearby was also a significant reason for increasing the number of beetles ($\chi^2 = 150.94$, $df = 1$, $p < 0.005$). The same remains significant for ants ($\chi^2 = 180.33$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 184.18$, $df = 1$, $p < 0.005$) and cockroaches ($\chi^2 = 150.94$, $df = 1$, $p < 0.005$).

The presence of forests surrounding the indoor spaces surveyed was recorded to consider them to be a reason for the increasing number of pests in indoor places (Figure 4.8). Forests were recorded only in approximately 10% of the overall sites surveyed.

Different types of water bodies (Figure 4.9) were reported to be present surrounding the indoor spaces surveyed, and this was gathered to consider them to be a reason for the increasing number of pests in indoor places. More than half of the surveyed sites had no water bodies near them. Pond, river and brook are the most common ones found from more than 10% of the surveyed sites, whereas the rest were found from only 5% of the sites.

According to Figure 4.10, various types of industrial establishments were recorded in the vicinity of the indoor spaces surveyed, as part of assessing their potential role in increasing pest presence indoors. More than 80% of the surveyed sites had no industries nearby. Among the sites where industries were present, rice flour mills and wood mills were the most commonly recorded.

The inhabitants reported different reasons (Figure 4.11) for the increase in the number of indoor pests. More than 25% of the surveyed sites mentioned the presence of rubber plantations as one of the most important reasons. Whereas, the presence of farms or natural fertilizers and the storage of firewood was also recorded as a reason for more than 20% of the sites. The rest of the reasons were recorded from less than 10% of the indoor spaces surveyed.

A comparison between the presence of insects and the four major reasons the inhabitants suggested for the increase in the number of pests indoors (Figure 4.12) shows that certain insects were found more often during certain circumstances. There was a significant increase in the number of insects with the presence of farms or natural fertilizers. This was considered significant in the case of mupli beetles ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 85.04$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$) and cockroaches ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$). The presence of rubber plantations nearby was also a significant reason for increasing the number of beetles ($\chi^2 = 88.16$, $df = 1$, $p < 0.005$). The same remains significant for ants ($\chi^2 = 98.84$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 114.04$, $df = 1$, $p < 0.005$) and cockroaches ($\chi^2 = 88.16$, $df = 1$, $p < 0.005$). However, the presence of storage of firewood at all the recorded homes had no instances of insects being absent, proving their evident significant role in being a habitat for insects. Nonetheless, for the case of unhygienic areas the comparison was again significant for mupli beetles ($\chi^2 = 27.12$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 23.51$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 23.51$, $df = 1$, $p < 0.005$) and cockroaches ($\chi^2 = 17.06$, $df = 1$, $p < 0.005$).

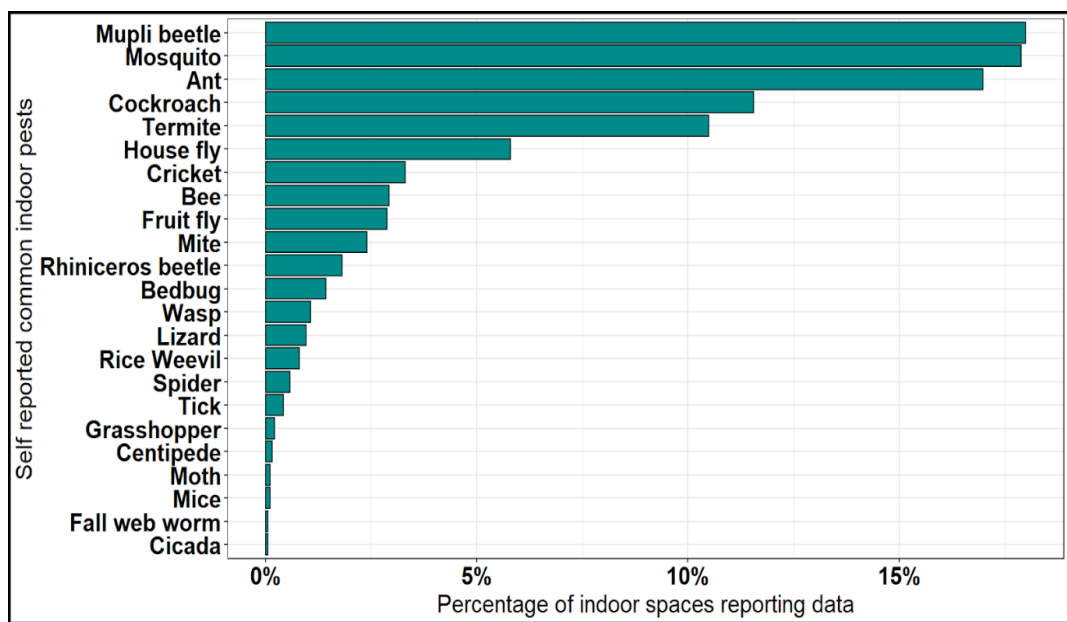


Figure 4.1. Relative abundance of indoor pests based on survey responses (n=403 spaces, 1972 observations)

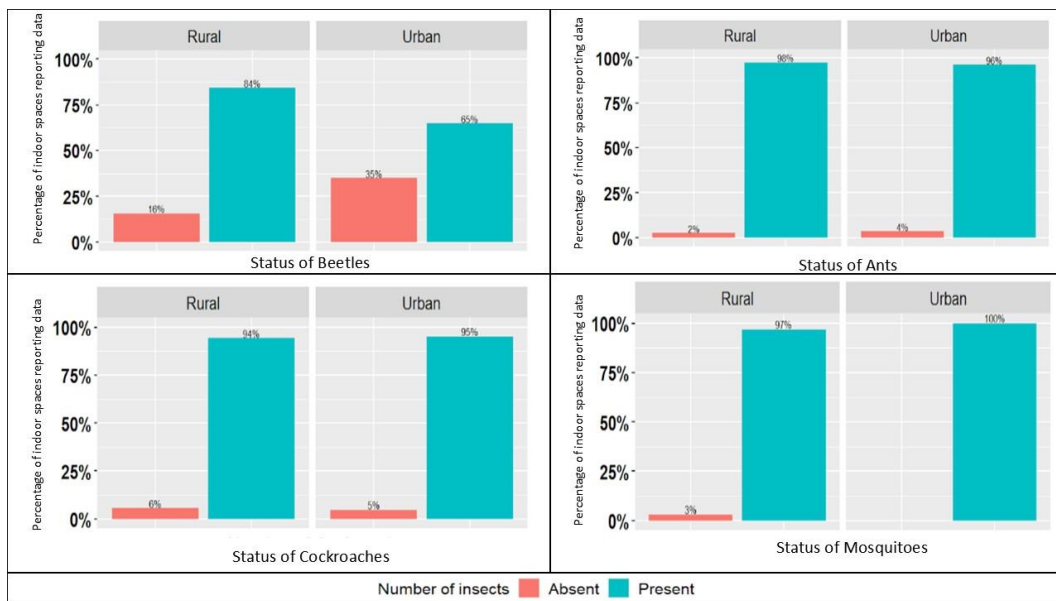


Figure 4.2. Comparison of insect prevalence between rural and urban indoor spaces (n=403)

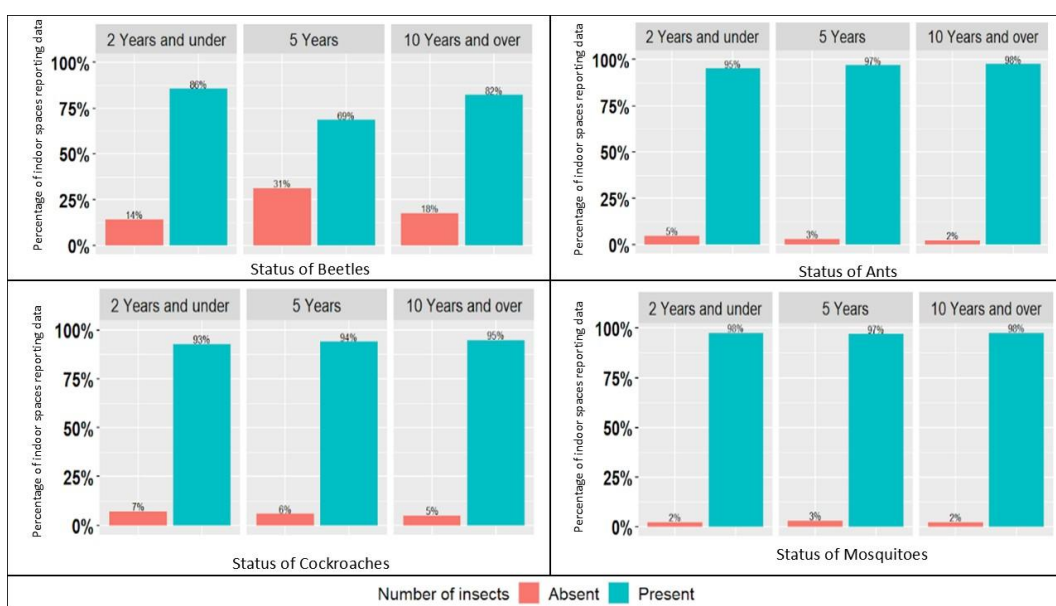


Figure 4.3. Distribution of indoor insects by building age (n=403)

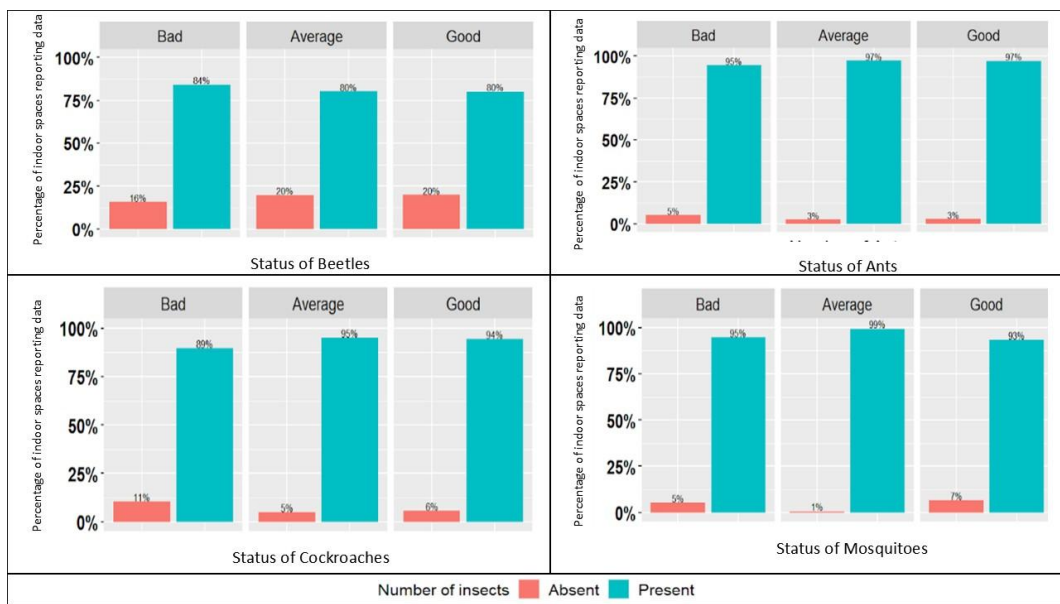


Figure 4.4. Effect of ventilation on the presence of indoor insects (n=403)

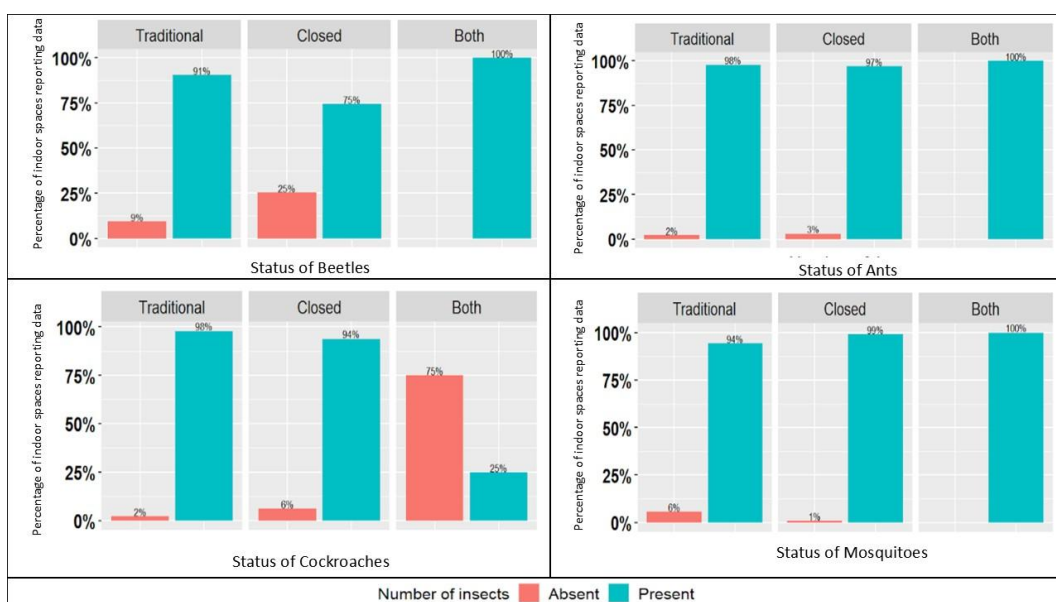


Figure 4.5. Association between roof type and indoor insect presence(n=403)

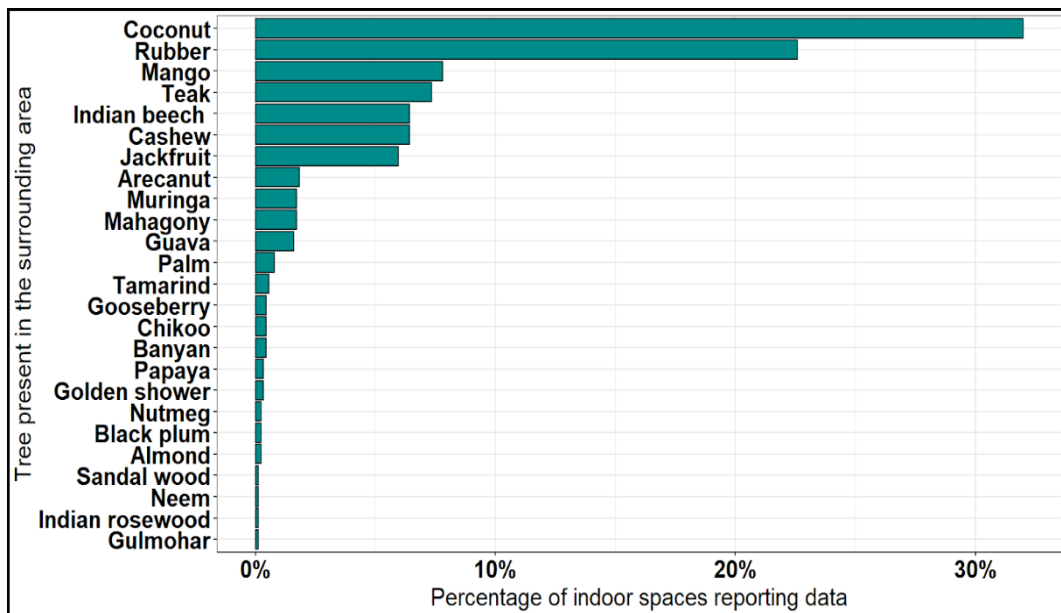


Figure 4.6. Types of trees reported from the surrounding vegetation area of the surveyed indoor spaces (n=403, 873 observations)

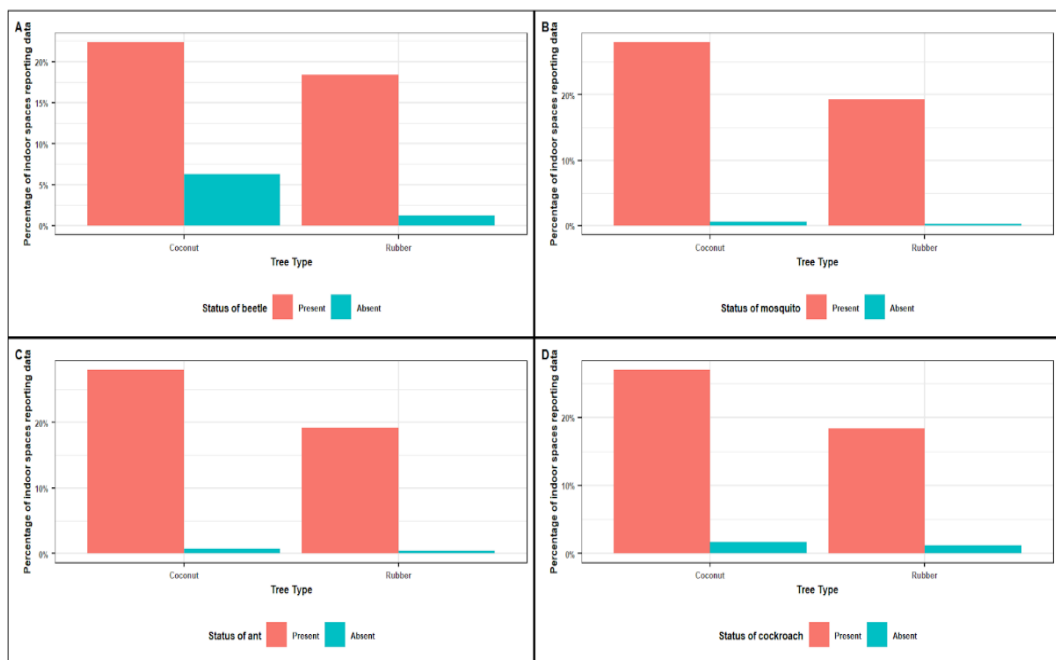


Figure 4.7. Comparison of insect's prevalence by presence of coconut and rubber trees (n= 403, 484 responses)

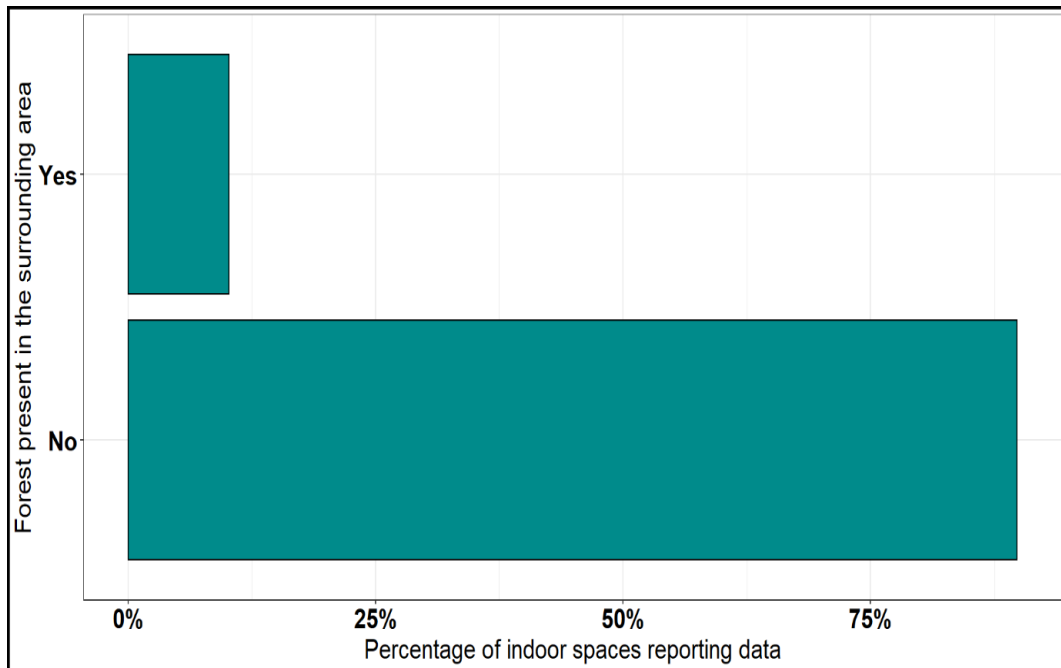


Figure 4.8. The percentage of forests surrounding the surveyed indoor space (n = 403)

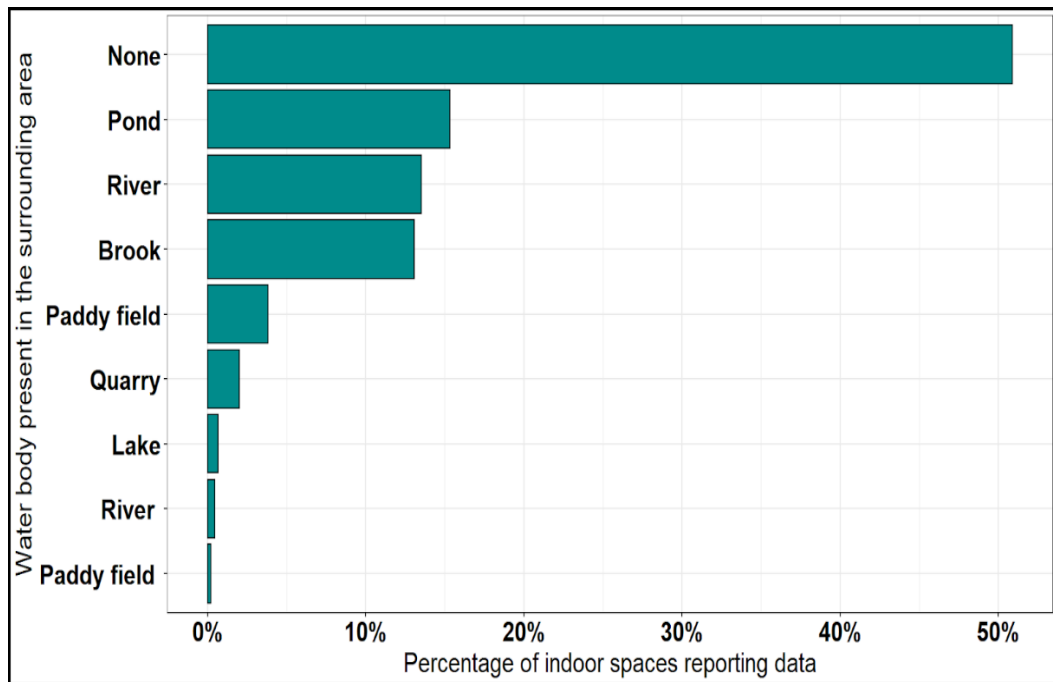


Figure 4.9. The presence of waterbody (n = 403, 445 observations)

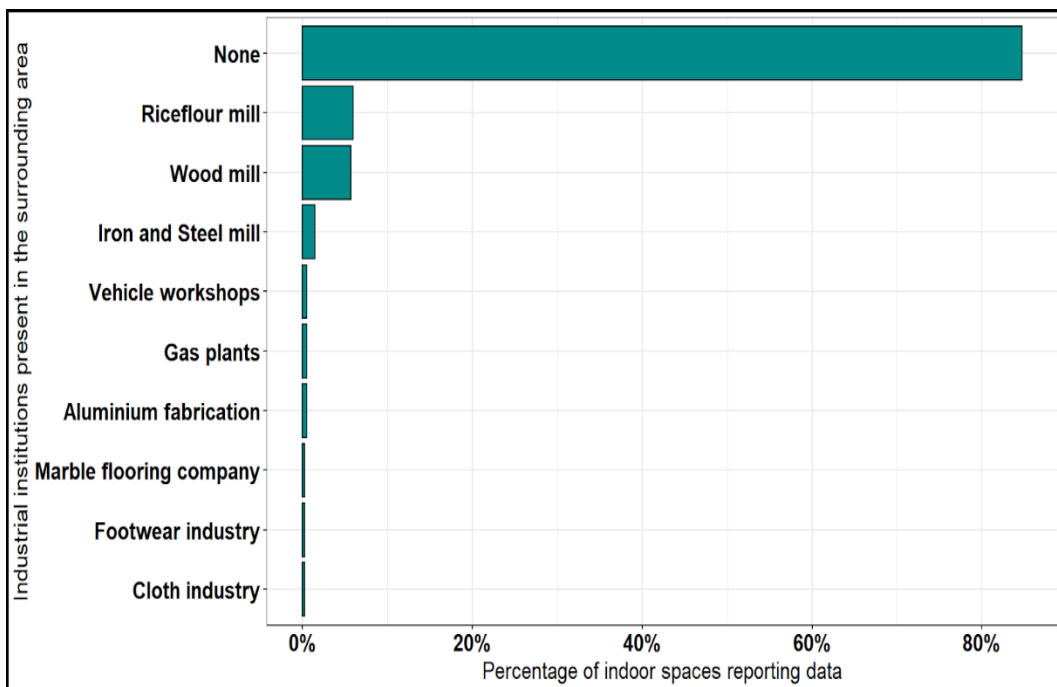


Figure 4.10. The presence of industrial institutions surrounding the indoor spaces (n = 403, 406 observations)

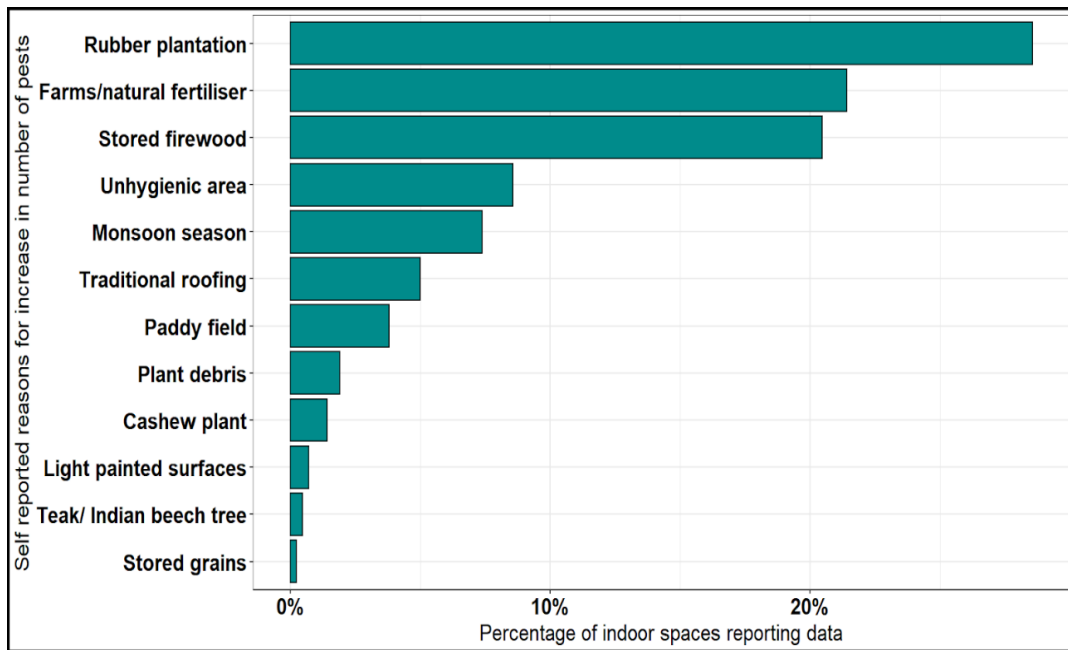


Figure 4.11. The self-experienced reasons for the increase in the number of indoor pests as described by the respondents in the survey (n = 403, 421 observations)

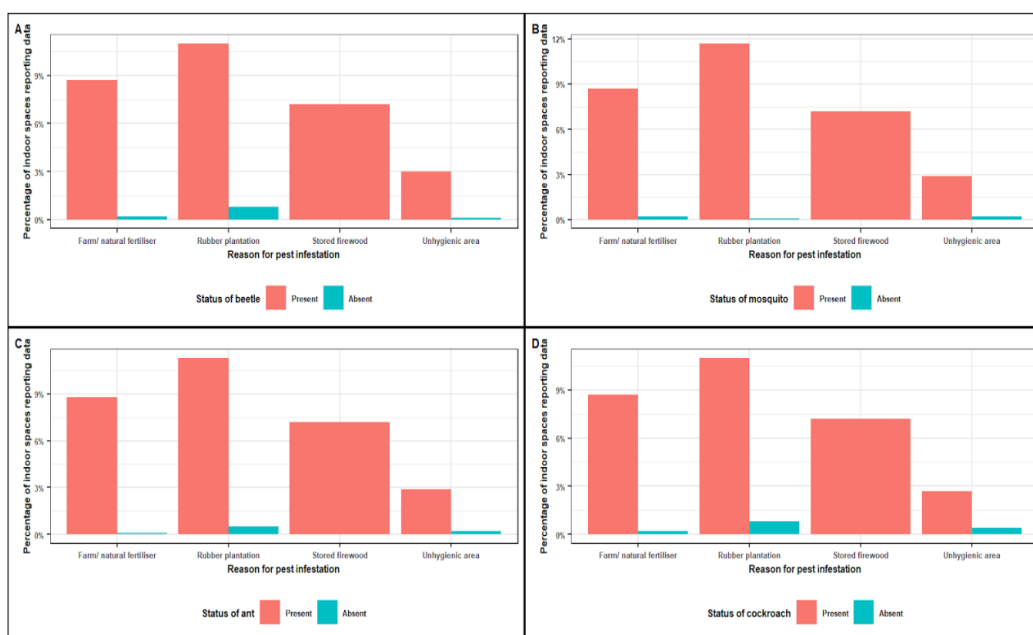


Figure 4.12. Comparison of reasons for the increase in the number of insect pests and the presence or absence of specific insects (n=403, 311 responses)

4.1.2. Distribution of indoor insects in relation to reported health issues

Numerous symptoms and disease conditions were reported by indoor inhabitants, including lifestyle diseases such as diabetes, high cholesterol and high blood pressure as presented in Figure 4.13. Genetic disorders, such as Parkinson's disease, were also reported. The most commonly reported symptoms included headaches, multiple colds, allergic rhinitis and fatigue. Most of the symptoms were related to the respiratory system, including frequent coughing, wheezing, shortness of breath and asthma. Interestingly, no instances of COVID were reported by any of the households, but that might be due to limited availability of testing and a general reluctance to admit a positive test.

The climatic seasons during which the indoor-related symptoms first appeared or became worse were looked into (Figure 4.14). Most of the respondents reported that their symptoms were independent of the climatic conditions. Furthermore, the second most common factor identified was the indoor environment with pollutants not specifically identified. The third most common reason was the onset of rainfall. This study, which focused on the allergic issues with insects, surveyed inhabitants of indoor spaces who mostly did not consider insects to be a major source. Less than 15% of respondents identified insects as the cause of an allergy. Although five insects were nonetheless considered to be causing one or more types of allergic reactions (Figure 4.14) in various indoor environments. Furthermore, the most common allergic response caused by these insects was coughing and sneezing, which would not result from coming into direct contact with most of the allergy-causing insects observed (Figure 4.14). Allergic reactions caused by direct contact with insects (predominantly swelling and a burning sensation) were relatively rare.

Figure 4.15 depicts the number of beetles reported from indoor spaces with ill inhabitants was 96% which did not significantly differ from the 95% of beetles recorded at those spaces without any illness ($\chi^2 = 0.003$, $df = 1$, $p = 0.951$). With only a one-digit increase observed in the presence of mosquitoes in households with ill inhabitants (97%), these insects were observed to have a non-significant relation with the number of inhabitants being ill ($\chi^2 = 0.088$, $df = 1$, $p = 0.76$). A similar trend was observed for the number of ants. The number of indoor spaces with no illnesses recorded the ant's presence at 93% and those with illnesses recorded 98%. This difference was not statistically significant ($\chi^2 = 2.639$, $df = 1$, $p = 0.104$). In the case of biological contaminants, mites and mould were observed slightly more frequently than in indoor spaces with no inhabitants with an illness, while the opposite was true for pollen. There is a significant relation between the presence of biological contaminants and with number of inhabitants being ill ($\chi^2 = 6.32$, $df = 2$, $p = 0.04$).

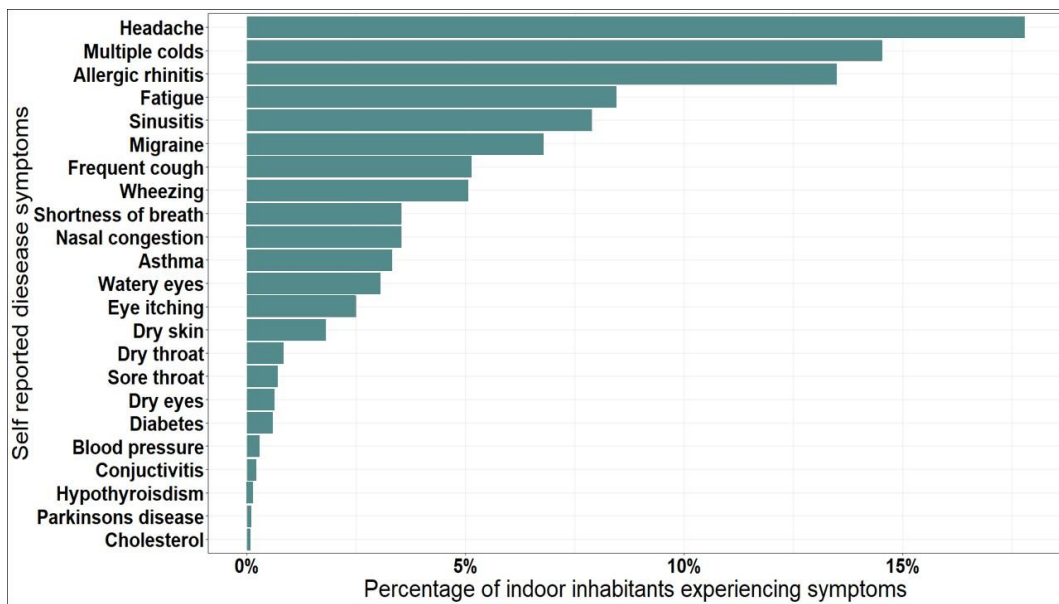


Figure 4.13. Self-reported health symptoms among indoor inhabitants (n=403, 1147 observations)

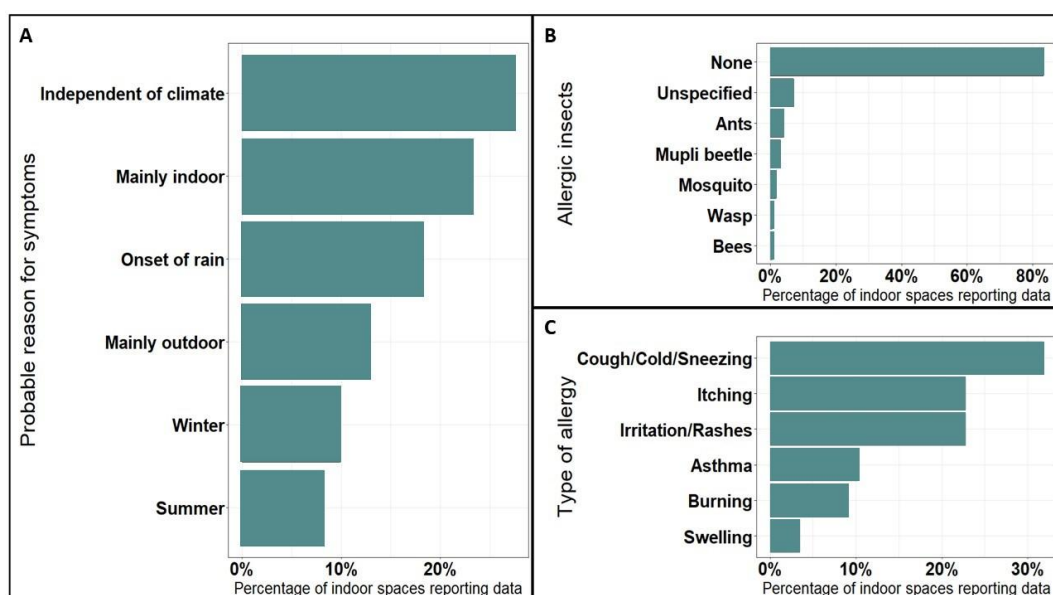


Figure 4.14. Inhabitants perception of health symptoms and allergic reactions related to indoor insects. A- Perceived causes of symptoms (n=403, 698 responses). B- Insects linked to allergic reactions (n=403, 417 responses). C- Health effects from insect contact (n=67, 88 responses).

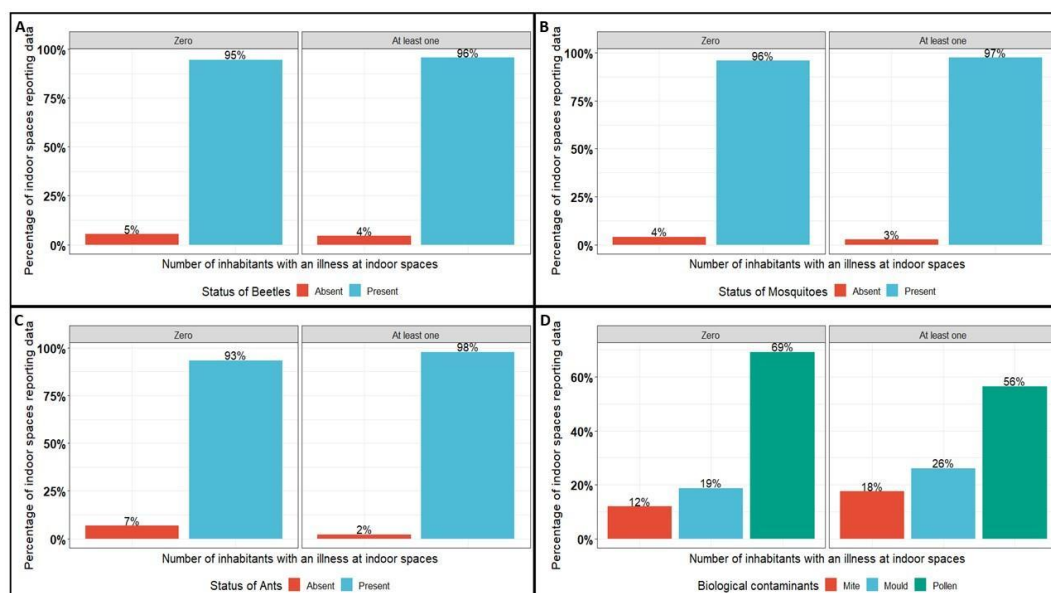


Figure 4.15. Presence of specific insects and biological contaminants in households with (n=403, 314 observations) and without illness (n=403, 89 observations) (A, B and C - insects, D- biological contaminants (n=403, 563 observations))

4.2 Semiochemical profiling of the test insect using GC- MS

The number of compounds detected during the headspace volatile analysis experiment conducted with 500 and 1000 beetles for 12, 24, 48 and 72 hours, respectively, was recorded as shown in Figure 4.16. For 500 beetles, the increase in the number of compounds was not significantly steady with the increasing period. The number of volatiles released by these was also found to be more than that of 1000 beetles for 12 hours. However, a slight steady increase was observed with 1000 beetles after 24 hours. While the number of compounds detected stayed high during the shortest period (12 hours), this number decreased during 24 hours for both groups. After which, this increased for later periods, particularly for the experiments with 1000 beetles. Although there have been slight differences in the numbers of compounds detected during different periods, the change is not significant ($\chi^2 = 0$, $df = 3$, $p\text{-value} = 1$). The beetles were freshly collected for every experiment. A control devoid of the beetles was maintained for every period.

The 12 hydrocarbon compounds identified are provided in Table 4.1 along with their retention time, peak area percentage and function. 4 of them were identified as cuticular hydrocarbons, 5 defensive compounds and 3 attractants. Amongst these, the most common and abundant compounds detected in the whole-body assay were 2-methyloctacosane. The retention time of the compounds identified was between 10 to 31 minutes. The 12 unidentified hydrocarbon compounds are provided in Table 4.2 along with their retention time and peak area percentage. Amongst these, the most common and abundant compounds detected in the whole-body assay were Heptadecane, 2,6,10,15-tetramethyl-. The retention time of the compounds detected was between 18 to 33 minutes.

The 4 alcohol compounds identified are provided in Table 4.3 along with their retention time, peak area percentage and function. 2 of them were identified as defensive compounds and the remaining 2 as sex pheromones. All the compounds detected in the whole-body assay were detected only for a single time.

The retention time of the compounds identified was between 18 to 25 minutes. The 3 unidentified alcohol compounds are provided in Table 4.4 along with their retention time and peak area percentage. All the compounds detected in the whole-body assay were detected only for a single time. The retention times of the compounds detected were 12 and 23 minutes.

The aldehyde compound identified is provided in Table 4.5 along with its retention time, peak area percentage and function. The function of the same was identified to be as of sex pheromone and the compound was detected in the whole-body assay was only detected for a single time. The retention time of the compounds was 16 minutes.

The ester compound identified is provided in Table 4.6 along with its retention time, peak area percentage and function. This was identified as a component with a defensive function. It was detected in the whole-body assay for a single time. The retention time of the compound identified was 24 minutes.

The 7 unidentified ester compounds are provided in Table 4.7 along with their retention time and peak area percentage. All the compounds detected in the whole-body assay were detected only for a single time. The retention time of the compounds detected was between 21 and 28 minutes.

The unidentified ether compound is provided in Table 4.8 along with its retention time and peak area percentage. This compound was detected in the whole-body assay for a single time. The retention time of the compounds detected was 16 minutes.

The 11 hazardous volatile compounds are provided in Table 4.9 along with their chemical nature and effects caused due to their exposure. Among them were 6 hydrocarbon compounds, 4 alcohol compounds and 1 ester compound.

Analysis of the volatiles released by the Mupli beetle placed in the glass chamber revealed 41 different compounds with retention times between 10 and 31 minutes. These compounds belonged to different chemical natures, including hydrocarbons, alcohols, aldehyde, ester and an ether compound. Identification of the 18 pheromones was possible by referring to previous literature on various other insects. The identified volatiles included 8 defensive pheromones, 6 sex pheromones and 4 cuticular hydrocarbons. Among the unidentified chemical volatiles were 12 hydrocarbons, 3 alcohols, 7 esters, and 1 ether compound.

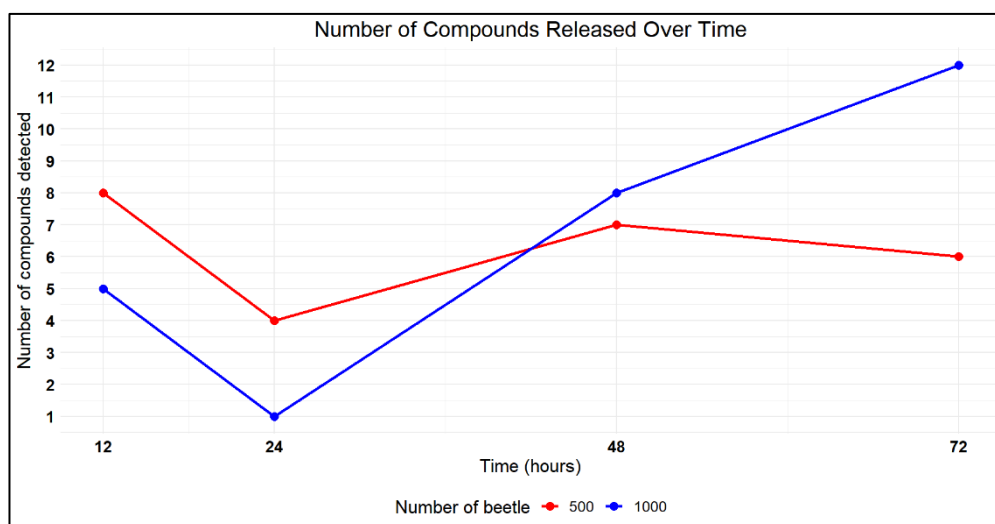


Figure 4.16. Number of compounds detected from the adsorbent material in the headspace volatile collection chamber with 500 and 1000 number of *L. tristis*.

Table 4.1. Identified hydrocarbon compounds with known functions in *L. tristis* headspace volatiles

Sl.NO	Retention time (minutes)	Compound Name	Area%	Frequency	Function	Reference
1	10.43	Nonadecane, 2-methyl-	19.17	1	Cuticular Hydrocarbon	Vanícková et al 2012
2	21.320	Eicosane	3.57	2	Defensive	Sarkar et al 2013
3	23.473	Heptadecane	9.94	1	Defensive	Geiselhardt et al 2009b, Moore 1979
4	24.515	Octane, 3,4,5,6-tetramethyl-	2.05	1	Defensive	Bagnères 1991
5	24.520	Eicosane, 10-methyl-	13.89	1	Sex Pheromone	Romero-López et al 2019
6	25.740	2-methyloctacosane	14.76	3	Cuticular Hydrocarbon	Lockey 1979
7	27.024	Eicosane, 2-methyl-	10.57	2	Sex Pheromone	Geiselhardt et al 2011
8	27.031	Heptadecane, 9-octyl-	4.28	2	Defensive	Drilling and Dettner 2010
9	27.067	Tritetracontane	11.05	2	Cuticular Hydrocarbon	Jackson 1970
10	27.087	Heneicosane	13.48	2	Sex Pheromone	Bennett and Shotwell 1976
11	28.465	Tetracosane, 11-decyl-	11.41	1	Cuticular Hydrocarbon	Geiselhardt et al 2011
12	31.320	Undecane, 2,7-dimethyl	5.76	1	Defensive	Geiselhardt et al 2009

Table 4.2. Hydrocarbon compounds with unidentified function detected in *L. tristis* volatiles

Sl.No	Retention time (minutes)	Compound	Area%	Frequency
1	18.685	1,3-Dioxolane, 2-(2-propenyl)-	1.01	1
2	19.656	2-Thiophenepropanamine, N, N-dimethyl-	13.27	1
3	23.490	Di-n-decylsulfone	1.49	1
4	25.724	Heptadecane, 2,6,10,15-tetramethyl-	11.38	2
5	29.848	2-Bromotetradecane	9.01	1
6	29.880	Decane, 1,1'-oxybis-	4	1
7	31.341	Dodecane, 1-fluoro-	4.46	1
8	31.357	Nonadecane, 1-bromo-	8.87	1
9	32.130	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	4.35	1
10	32.540	2-Methyl-5-t-butyl-1,3-oxathiane	4.29	1
11	33.071	1-Octadecanesulphonyl chloride	6.62	1
12	33.631	2,3-O-Benzal-d-mannosan	8.05	1

Table 4.3. Identified alcohol compounds with known pheromone or defensive role detected from the volatiles of *L. tristis*

Sl.No	Retention time (minutes)	Compound	Area%	Frequency	Function	Reference
1	18.969	1-Heneicosanol	5.71	1	Defensive	Burger et al 1999
2	22.453	1-Hexanol, 5-methyl-2-(1methylethyl)-	1.36	1	Defensive Allomone	Tanaka et al 1986
3	24.531	2-Nonen-1-ol, (E)	2.01	1	Sex Pheromone	Francke & Schul 1999
4	25.627	n-Heptadecanol-1-	3.11	1	Sex Pheromone	Tsoukatou et al 2001

Table 4.4. Alcohol compounds with unidentified function detected in *L. tristis* volatiles

Sl.No	Retention time (minutes)	Compound	Area%	Frequency
1	12.607	Phenol, 2,4-bis(1,1-dimethylethyl)-	10.18	1
2	21.370	Melochinin	2.06	1
3	23.384	n-Nonadecanol-1	6.63	1

Table 4.5. Identified aldehyde compounds from *L. tristis* volatiles

Sl.No	Retention time (minutes)	Compound	Area%	Frequency	Function	Reference
1	16.449	E-15-Heptadecenal	2.67	1	Sex Pheromone	Greenblatt 1977

Table 4.6. Ester compound identified with known defensive function from *L. tristis*

Sl.No	Retention time (minutes)	Compound	Area%	Frequency	Function	Reference
1	24.510	Sulfurous acid, 2-propyl tridecyl ester	9.99	1	Defensive pheromone	Prasanna et al 2016

Table 4.7. Ester compounds with unidentified function detected from the volatiles of *L. tristis*

Sl.No	Retention time (minutes)	Compound	Area%	Frequency
1	21.268	Trifluoroacetic acid, pentadecyl ester	7.73	1
2	22.394	Oxalic acid, allyl decyl ester	2.33	1
3	22.443	Oxalic acid, decyl propyl ester	6.12	1
4	25.695	Oxalic acid, 6-ethyloct-3-yl heptyl ester	2.94	1
5	27.070	Stearic acid, 3-(octadecyloxy)propyl ester	3.27	1
6	28.427	Methoxyacetic acid, 3-tetradecyl ester	10.43	1
7	28.436	Sulfurous acid, decyl 2-propyl ester	3.62	1

Table 4.8. Ether compound with identified function detected in *L. tristis* volatiles

Sl.No	Retention time (minutes)	Compound	Area%	Frequency
1	16.770	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether	4.82	1

Table 4.9. Potentially hazardous volatile compounds identified in *L. tristis*

Sl.No	Compound Name	Chemical Class	Reported Effect
1	Heneicosane	Hydrocarbon	Skin & Eye Irritations
2	1-Octadecanesulphonyl chloride	Hydrocarbon	Skin corrosion/irritation
3	Heptadecane	Hydrocarbon	Aspiration hazard
4	Eicosane	Hydrocarbon	Aspiration hazard
5	1-Heneicosanol	Alcohol	Slight Hazard to aquatic environment
6	Sulfurous acid, decyl 2-propyl ester	Ester	Long term Hazard to aquatic environment
7	Dodecane, 1-fluoro-	Hydrocarbon	Acute Oral Toxicity
8	1-Hexanol, 5-methyl-2-(1-methylethyl)-	Alcohol	Acute Allergic Effects
9	Decane, 1,1'-oxybis-	Hydrocarbon	Irritant
10	n-Nonadecanol-1	Alcohol	Irritant
11	n-Heptadecanol-1	Alcohol	Irritant
Total	11 Compounds (6 hydrocarbons, 4 alcohols,1 ester)		

4.3. Cytotoxicity assay of heptadecane on normal intestinal epithelial cells

Sample - Heptadecane

Cell line - IEC 6

Vehicle - Ethanol

The absorbance value and the percentage of their inhibition obtained with the experiments using different dosages of heptadecane, including 6.25, 12.5, 25, 50 and 100 $\mu\text{g/ml}$, are tabulated above. The percentage inhibition is calculated for each dosage and absorbance value. The percentage of inhibition was seen to be 9.88 ± 0.52 for 6.25 $\mu\text{g/ml}$, 9.60 ± 0.04 for 12.5 $\mu\text{g/ml}$, 11.72 ± 0.92 for 25 $\mu\text{g/ml}$, 13.08 ± 0.23 for 50 $\mu\text{g/ml}$ and 12.89 ± 3.09 for 100 $\mu\text{g/ml}$. The percentage of cell death was not seen to be increasing with an increase in dosage. A control without any treatment and a vehicle control with the agent used to dissolve the test material were also maintained during the experiment.

Data in Figure 4.17 is representative of the cytotoxicity experiments and values are given as mean \pm SD. No significant difference was observed in cell viability among the different dose groups. The cytotoxic effect of heptadecane on cell culture was analysed for different doses. The control had a low level of cytotoxicity (4.72%), while the percentage of cell inhibition increased along with increasing doses of the chemical compound. The mean cell inhibition was noted to be 9.88% for 6.25 μg , 9.60% for 12.5 μg , 11.73% for 25 μg , 13.08% for 50 μg , and 12.89% for 100 μg . The MTT assay indicated an approximately straight-line increase in the percentage of cell inhibition with increasing dosage.

Figure 4.18 depicts the changes in the intestinal epithelial cells observed. Cells were treated with 6.25 μg , 12.5 μg , 25 μg , 50 μg and 100 μg of Heptadecane for 48 hours. A Control was also maintained for the same. Heptadecane on cell numbers was recorded by taking images. The control, which had a low level of cytotoxicity, has a greater number of viable cells, which is clear from the image. Whereas the number of cell deaths increased with increasing dosage. This was observed under an inverted light microscope (20 \times magnification).

Table 4. 10. Data of cytotoxicity assay using heptadecane

Concentration ($\mu\text{g/ml}$)	Absorption	Percentage of Inhibition
Control	0.884 \pm 0.09	
Vehicle Control	0.843 \pm 0.06	4.71 \pm 2.62
6.25	0.801 \pm 0.07	9.88 \pm 0.52
12.5	0.804 \pm 0.07	9.60 \pm 0.04
25	0.785 \pm 0.06	11.72 \pm 0.92
50	0.774 \pm 0.07	13.08 \pm 0.23
100	0.779 \pm 0.09	12.89 \pm 3.09

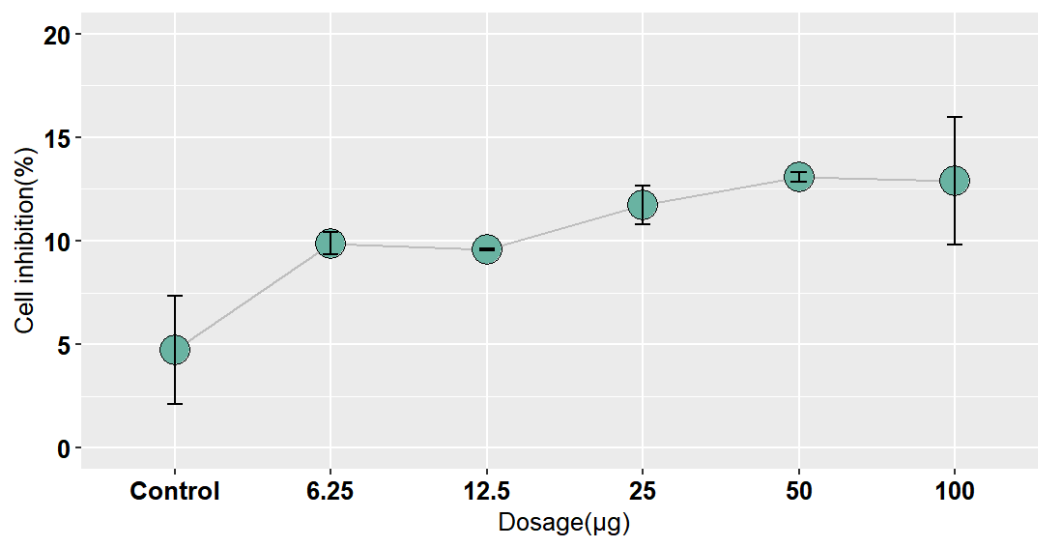


Figure 4.17. Cytotoxic effects of Heptadecane on IEC- 6 cells over a 48-hour time-course

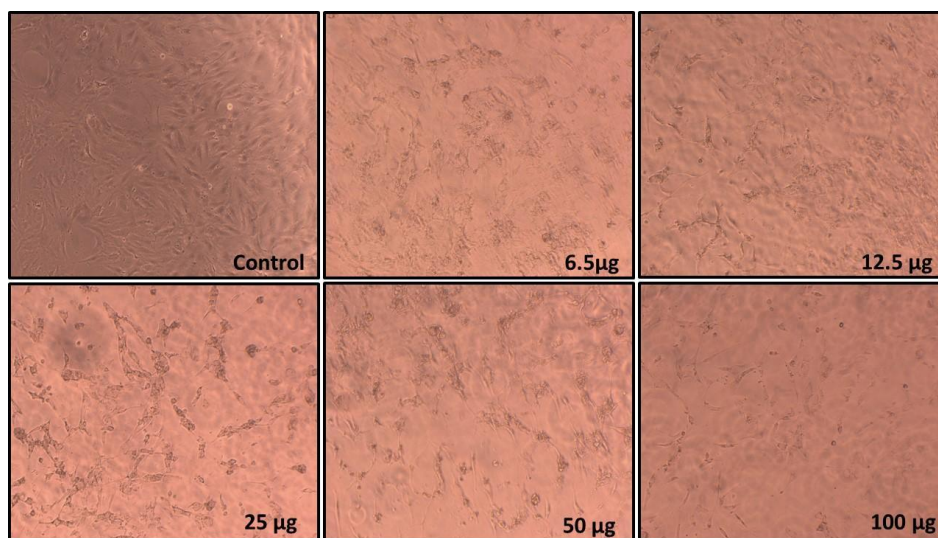


Figure 4.18. Morphological changes in IEC-6 cells after heptadecane exposure

Chapter 5

DISCUSSION

5.DISCUSSION

Indoor insect pests are prevalent across most regions of the world, posing a persistent challenge to public health and hygiene. As individuals spend approximately 80–90% of their lifetime indoors, exposure to volatile compounds, known as semiochemicals, released by insects for communication, may contribute significantly to the rising incidence of respiratory and other health disorders.

This study investigated the occurrence and abundance of indoor pests in relation to various environmental and structural factors and their potential link to human health issues. A field-based and questionnaire-supported survey was conducted across multiple sites to identify the most common nuisance insect pests and to assess the influence of factors such as building design, ventilation, roofing type, and building age. The tropical climate of India, with its consistently warm and humid conditions, is a key factor promoting the persistence and diversity of insect pests in indoor environments (Gullan & Cranston, 2014). In the present study, this climatic influence was evident in the widespread presence of nuisance pests across surveyed households. However, environmental conditions alone do not explain their distribution. Anthropogenic modifications such as the establishment of tree plantations (Scherer-Lorenzen et al., 2005), unhygienic conditions in nearby barren lands (Robinson, 2020), and the use of chemicals in building construction and maintenance (Reinprecht, 2016) were also found to create conducive microhabitats that support pest proliferation. Furthermore, specific structural characteristics of indoor environments, including ventilation quality, roofing material, and building age, significantly influenced pest occurrence patterns.

Based on survey results, *Luprops tristis* (Mupli beetle) was selected for further investigation due to its frequency and perceived nuisance level. Headspace semiochemical profiling was employed to identify volatile compounds released by the beetle, followed by a cytotoxicity assay on rat intestinal epithelial cells to evaluate the potential health impact of one selected compound. This study provides novel insights into how semiochemicals released by indoor insect pests, particularly

non-biting species, may act as hidden biological pollutants, with potential implications for respiratory health and indoor air quality.

5.1 Survey of indoor insect diversity and selection of test species

During the survey, detailed data were collected on the abundance and diversity of indoor insects. Twenty-three species of pests, irrespective of their season, were recorded during the survey (Figure 4.1). Although most of them were insects, a few non-insect pests were also observed. However, being a tropical country, pests were found to be present in most of the surveyed spaces, although some species were recorded in low numbers.

After identifying the type of pests, the presence or absence of particular indoor features was evaluated as a potential factor for influencing their abundance and diversity. Likewise, comparisons for the presence of common insects like Mupli beetle, ants, mosquitoes and cockroaches with various features such as location type (rural/ urban), roof type, ventilation status and age of building showed fluctuations in their populations. The location type (rural/ urban) had a significant impact on the Mupli beetle ($\chi^2 = 14.3$, $df = 1$, $p = 0.0001$) (Figure 4.2). Their higher prevalence in rural areas may be due to the presence of an increased number of rubber plantations and several other vegetations which serve as their primary habitat. These findings are in agreement with Panda & Sarkar (2020), who reported that these plantation areas affect the presence of pests. The abundance of pests in vegetation areas may also vary seasonally, as demonstrated by the findings of John et al. (2011). Urbanized areas with a decreased number of host plantations of Mupli beetles have therefore shown a decrease in the number of this pest. Ants had an insignificant ($\chi^2 = 0.03$, $df = 1$, $p = 0.85$) increase in their numbers in rural areas (98%) compared to that of urban spaces (96%). A similar small rise in the number of cockroaches and mosquitoes in urban areas than rural areas may be attributed to their widespread abundance irrespective of habitat selection as discussed in previous studies (Memon et al, 2017; Hawkes & Hopkins, 2022).

Since this work investigates insects found in indoor spaces, the age of the buildings was also considered along with their other features to see if their diversity and abundance vary with any of these factors. While looking into the effect of the age of the building on insect presence (Figure 4.3), there was a small dependency in the case of Mupli beetle ($\chi^2 = 7.29$, $df = 2$, $p = 0.02$) towards the oldest and newest houses constructed. Their presence in houses over 10 years old may be attributed to the tendency of these insects to use long-standing structures as aggregation sites, a finding that aligns with the observations of Sabu et al. (2008). Whereas their presence in the recently constructed houses may be due to the fact that these reconstructions or renovations are occurring in areas already known to have beetle infestations. Nonetheless, their overall reduction in numbers during this survey is relatable to their seasonal presence, also noted by Sabu et al. (2008). Other insects like ants ($\chi^2 = 0.80$, $df = 2$, $p = 0.66$), cockroaches ($\chi^2 = 0.33$, $df = 2$, $p = 0.84$) and mosquitoes ($\chi^2 = 0.08$, $df = 2$, $p = 0.95$) showed their presence in a higher number in much older indoor spaces with their values of lesser significance. These insects, on the other hand, may have already adapted to their respective environments and found them to be nurturing them through the pressure from abiotic factors and natural enemies (Sruthi & Deepthy, 2024).

Many insects have been a constant presence in unclean environments (Olagunju, 2023). The lack of proper air circulation, thereby reducing the exchange of air in and around a particular area, also increases humidity, which can promote the growth of biological contaminants such as mould (Rawat & Kumar, 2023). This again increases the chances of retaining allergens released by pests (Kumar et al., 2021). For the same reason, an understanding of the ventilation of the buildings was also included as a parameter in the survey. However, the impact of this on the presence or absence of insects was found to be less diverse depending upon the quality of ventilation systems (Figure 4.4). In the case of Mupli beetles, although their number decreased with better ventilation, the difference was not statistically significant ($\chi^2 = 0.18$, $df = 2$, $p = 0.91$). A similar pattern was observed for ants ($\chi^2 = 0.51$, $df = 2$, $p = 0.77$), and cockroaches ($\chi^2 = 1.05$, $df = 2$, $p = 0.59$). Mosquitoes,

on the other hand, increased with increasing ventilation quality ($\chi^2 = 11.61$, $df = 2$, $p = 0.03$). Furthermore, it is noteworthy that these insects have a higher likelihood of entering indoor spaces when there are increased openings to the external environment, potentially being carried in by the wind, as explained by Snehalatha et al (2003).

One can easily observe that houses and commercial buildings in Kerala have various types of roofing systems, which may regulate the temperature and humidity of the internal environment as described by Rawat & Singh (2022). Since insects are highly sensitive to temperature and humidity (Skendžić et al., 2021), roofing types were also considered as a parameter in the present study. Mud tile roofing systems favoured Mupli beetles ($\chi^2 = 15.75$, $df = 2$, $p = 0.0003$) (Figure 4.5). This type of roofing is the oldest kind to be seen, which makes the environment humid, cool and supports the diapause of the beetles as understood from the works of Sabu et al (2008). These insects were observed in 91% of such indoor spaces. Mosquitoes ($\chi^2 = 9.40$, $df = 2$, $p = 0.009$), on the other hand, were more commonly found in closed indoor systems, probably due to the reduced level of sanitation (Olagunju, 2023) and ventilation, which this study noted earlier. Cockroaches ($\chi^2 = 40.658$, $df = 2$, $p < 0.05$) showed a similar trend to Mupli beetles, with their presence more in indoor spaces with mud tile roofing systems. Like Mupli beetles being attracted to the dark nooks and corners (Raju & Radhika, 2023) of such roofing systems, cockroaches also regard these environments as favourable hiding habitats (Manyullei et al., 2022). In contrast, ant populations did not show any significant association with roofing types ($\chi^2 = 0.40$, $df = 2$, $p = 0.818$), likely due to their ecological adaptability. Ants are known to inhabit a wide range of terrestrial environments worldwide (Fittkau and Klinge, 1973), which may explain their relatively uniform presence across different indoor settings in the present study.

Apart from collecting data from the indoor structural and internal features, the surrounding environment was also considered. Almost 25 varieties of trees were reported as a whole (Figure 4.6). Coconut trees were recorded in more than 30% of the sites surveyed. Owing to the fact that these are one of the earliest domesticated

plants (Chowdappa et al., 2017) extensively succeeding in numbers due to favourable soil texture and improved crop management facilities (Mohan & Kunhamu, 2022). Additionally, the cultivation of rubber plantations has been increasing in recent years. This trend is in line with the observations made by Vijayan et al. (2024), who discussed the socio-economic drivers behind the expansion of rubber cultivation. Correspondingly, rubber trees were recorded in more than 20% of the indoor study sites (Figure 4.6), suggesting that land-use changes in the surrounding environment may influence indoor ecological conditions.

A comparison regarding the number of coconut and rubber trees, as they were seen to be the most common kinds, and the abundance of beetles, ants, mosquitoes and cockroaches was also conducted (Figure 4.7). The presence of both these trees is highly statistically significant for increasing the number of common insects including Mupli beetle ($\chi^2 = 90.31$, $df = 1$, $p < 0.005$ for coconut trees and $\chi^2 = 150.94$, $df = 1$, $p < 0.005$ for rubber trees), ants ($\chi^2 = 259.68$, $df = 1$, $p < 0.005$ for coconut trees and $\chi^2 = 180.33$, $df = 1$, $p < 0.005$ for rubber trees), mosquitoes ($\chi^2 = 263.5$, $df = 1$, $p < 0.005$ for coconut trees and $\chi^2 = 184.18$, $df = 1$, $p < 0.005$ for rubber trees) and cockroaches ($\chi^2 = 223.03$, $df = 1$, $p < 0.005$ for coconut trees and $\chi^2 = 150.94$, $df = 1$, $p < 0.005$ for rubber trees). These situations mostly occur when insects are associated with the tree in terms of being their pests (Gougherty & Davies, 2022) or being a part of their ecological interactions (Whitehill et al., 2023). These findings highlight how the immediate outdoor vegetation can influence indoor insect populations through ecological connectivity.

Further, the presence of forests was recorded and analysed to assess their potential role in increasing the number of pests (Figure 4.8). However, in this study, more than 75% of the surveyed sites did not have forests nearby. The presence of water bodies, which was also recorded (Figure 4.9), owing to the fact that improper management of these may increase the number of pests (Lin et al., 2023; UC IPM, 2025). However, more than half of the surveyed sites in the current study did not

record the presence of a water body, and hence, this was not further considered in relation to the occurrence of insect pests.

Another parameter recorded was regarding the presence of industrial establishments near the surveyed sites (Figure 4.10). This was considered due to the changes that industries bring in; including, increase in the production of waste, to whose decomposition, insects may be attracted as outlined by Siddiqui et al. (2024). More than 80% of the surveyed sites did not have any industries surrounding them and hence, further evaluation using this parameter was not conducted.

The respondents were also asked to provide information regarding what they experienced and believed to be the reason for the increasing number of insect pests in their indoor environments (Figure 4.11). The results showed that the presence of farms or any kind of natural fertilizer, rubber plantations, and the storage of firewood were believed to be a reason by more than 20% of the inhabitants. The presence of unhygienic areas, the onset of monsoon season and traditional roofing using mud tiles were a few other reasons mentioned. Four major reasons among these were subjected to comparison with the presence and absence of insects. The results revealed a statistically significant association between the presence of farms and natural fertilizers and increased indoor occurrence of all four major insect groups studied, Mupli beetles ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 85.04$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$), and cockroaches ($\chi^2 = 81.18$, $df = 1$, $p < 0.005$) (Figure 4.12). These findings align with previous studies that suggest certain insects may be beneficial to farmers due to their ecological roles around farm environments (Reddy et al., 2022). Furthermore, Singh and Sarkar (2021) reported that many insect species are attracted to areas with high nitrogen content, such as those enriched by natural fertilizers, which may explain their increased abundance in such settings.

The presence of rubber plantations was found to significantly influence the abundance of indoor insect pests. Statistically significant associations were

observed between rubber plantations and the presence of Mupli beetles ($\chi^2 = 88.16$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 98.84$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 114.04$, $df = 1$, $p < 0.005$), and cockroaches ($\chi^2 = 88.16$, $df = 1$, $p < 0.005$). Mupli beetles are known to feed on rubber leaves, as documented by Sabu et al. (2014), which may explain their higher occurrence near such plantations. In addition, the leafy microenvironment and nitrogen-rich soils of rubber plantations may support other insect groups, either as symbionts or as part of broader ecological interactions (Ren et al., 2022). Storage of firewood was also found to be significantly associated with increased insect presence ($p < 0.05$). This observation is supported by the findings of Haack et al. (2010), who noted that firewood serves as a major medium for the spread of wood-boring insects and other pest species from collection sites to storage areas and eventually to indoor environments. Moreover, the presence of unhygienic surroundings showed a statistically significant relationship with the increased occurrence of all major insect groups, Mupli beetles ($\chi^2 = 27.12$, $df = 1$, $p < 0.005$), ants ($\chi^2 = 23.51$, $df = 1$, $p < 0.005$), mosquitoes ($\chi^2 = 23.51$, $df = 1$, $p < 0.005$), and cockroaches ($\chi^2 = 17.06$, $df = 1$, $p < 0.005$). Such areas, especially those with decomposing organic matter, serve as suitable oviposition sites and breeding grounds for disease-carrying insects (Spindola et al., 2017; Kotzé & Tomberlin, 2020), thereby posing potential public health concerns.

5.2. Association between indoor insects and health issues among occupants

The number of allergic patients with bronchial asthma (Bhalla et al., 2018; Dharmage et al., 2019; Cevhertas et al., 2020) and allergic rhinitis (Wheatley & Togias, 2015; Krishna et al., 2020; Scadding et al., 2021) is currently increasing globally. The survey, which was mostly carried out during the COVID-19 pandemic, when inhabitants primarily stayed indoors, reported headaches, frequent colds and allergic rhinitis as the most common symptoms (Figure 4.13). The respondents partly ascribed these symptoms to indoor air quality (Figure 4.14-A). However, a higher number of people responded that their symptoms were independent of the climatic conditions and many were also unsure about blaming the most easily agreeable reason for their allergic issues – outdoor pollution,

possibly because they were in lockdown at the time of responding to the questionnaire. Therefore, irrespective of climatic changes, the indoor environment is suspected to be a significant contributing factor. Less than 20% of the respondents also suspected the onset of rain as a reason for their deteriorating health conditions (Figure 4.14-A). Such monsoon-related illnesses may occur due to the synchronized activity of the insect life cycle in response to temperature changes, also noted during the works of Chowell (2019). Insect pests like the Mupli beetle have their life cycles synchronised with climatic conditions to wake from the 11-month diapause at the onset of rain (Vinod & Sabu, 2009). They escape from the wet floors covered with rubber leaves to drier indoor spaces where they form huge aggregations. Vinod & Sabu (2009) have shown that Mupli beetles are mostly attracted to tile-roofed and palm-frond-thatched spaces which retain higher humidity than concrete roof tops. This connects the dampness with the presence of beetles in indoor spaces.

Natural, domestic, hobby-related and occupational exposure to invisible insect volatiles can negatively affect human health. Several studies have documented asthma and allergic rhinitis resulting from exposure to insect allergens originating from species such as mayflies, aphids, caddisflies, houseflies, beetles, and others (Parlato, 1929; Jamieson, 1938; Gaillard, 1950; Feinberg et al., 1956; Ganseman et al., 2022). Patients' positive responses to skin tests using insect extracts indicated insects to be an important cause of inhalant allergies (Do DC et al., 2016). Occupational exposure to grasshoppers (*Melunoplus sanguinipes*) also resulted in a high prevalence of atopic sensitization (Soparkar et al., 1993).

Ants, which were the most common allergy-inducing insects in this study (Figure 4.14- B), usually inflict painful stings by injecting formic acid into the skin (Bagnères et al., 1991; Touchard et al., 2016). Although our data showed a higher presence of ants in households reporting illnesses, this association was not statistically significant (Figure 4.15-C). Mosquitoes, the third most common insect pest in this study, can cause a nuisance by biting but also cause serious illness and even death in humans (Prudêncio, 2020) and some animals (IDPH, 2017) by vectoring pathogens. However, in our study, we found no significant impact of

mosquitoes on the level of household illnesses. This is probably because they mainly cause itching or acute allergic reactions to their bites, but no serious health issues were observed (Figure 4.15-B). These insects also had a considerable negative impact with their constant presence, resulting in more inhabitants with an illness. The fourth and fifth most common allergy-inducing insects- bees (Wehbe et al., 2019) and wasps (Lee et al., 2016), respectively- are known to sting and inject a chemical cocktail when disturbed. Nevertheless, the second most allergy-inducing insect, the Mupli beetle (Figure 4.14-B), which neither stings nor bites, was the only insect that seemed to be causing indirect irritation through its defensive phenolic secretions. When disturbed, it releases an odoriferous volatile substance that causes skin irritation (Sabu et al., 2008) and keratoconjunctivitis (Susan et al., 2010). Similarly, VOCs from other biological contaminants have also been associated with various health disorders. One such volatile semiochemical produced by a fungus, 1-octen-3-ol, affects the Dopamine Neuron Degeneration and forms a significant risk factor for Parkinson's disease (Inamdar et al., 2013).

Nearly 78% of the indoor spaces surveyed recorded the presence of beetles. Though free from direct stings and bites, the Mupli beetle was still reported as an allergy-inducing insect and, although not statistically significant, they were found to be slightly more common in indoor spaces with occupants reporting allergy symptoms, which could suggest a minor impact of the chemicals they release (Figure 4.14-B). Such an increase in the population of a particular insect and their influence on the outbreaks of infectious fevers has been studied in tiger moths (Wills et al., 2016). Although the lack of a significant effect of beetles in this study can be related to the fact that they are only found around certain types of vegetation and during particular seasons, outside of which their impact is likely to be negligible. This survey, conducted from September 2020 to March 2021, collected data mostly during the inactive season of these nuisance pests, whose massive seasonal aggregation occurs after nine months in an inactive dormancy phase following summer rains, usually towards the end of March, as explained in the works of Sabu et al (2008). Large aggregations occur near indoor spaces in rubber

plantations, where these beetles (particularly larval instars, pupae and teneral adults) feed on tender rubber leaves rich in nitrogen and moisture content, which is a crucial factor for the growth and survival of their next generation (Sabu & Vinod, 2009a, b). Mosquitoes, on the other hand, are active flying nuisance pests present throughout the year, particularly in the tropics (Reinhold et al., 2018). Similarly, ants are also abundant in the terrestrial ecosystems throughout the year in tropical regions (Touchard et al., 2016). An increase in the number of indoor spaces reporting moulds synchronizing with a rise in insect pests, should also be taken into consideration (Figure 4.15-D). Considering the above-mentioned circumstances, the most common indoor allergy symptoms such as cold, cough, sneezing etc recorded as a part of this work (Figure 4.14-C) may be categorised as insect-induced allergic reactions as detailed in AAFA (2022) and ACAAI (2022). Although not clinically proven, headaches, watery eyes and exhaustion, and other usual hay fever symptoms (Figure 4.13) as seen in AAAAI (2022) may also be influenced by the presence of insects.

Indoor air pollution is influenced by several factors, including occupant density, use of inappropriate construction materials, excessive use of chemical products such as pesticides and disinfectants, combustion gases and pollutants entering from outside. Biological pollutants include bacteria, viruses, animal dander, cat saliva, mites, cockroaches, and pollen (US EPA, 2019). Some of these are classified as highly potent biological allergens. Among biological contaminants (which also played a statistically significant role in causing indoor illnesses) mould and pollen were reported at higher concentrations (Figure 4.15-D). Damp indoor surfaces provide a breeding ground for mould, mildew, bacteria and insects (US EPA, 2019). Damp conditions support mould growth, which attracts insects that feed on it (WHO, 2009). This behaviour has also been observed in foreign grain beetles and booklice (Jacobs, 2017). Humidity also serves as an attractive microclimate for various indoor pests, including rodents, woodlice, insects and even some birds (Pinniger, 2021).

5.3. Semiochemical profiling of the test insect using headspace GC-MS analysis

The glass chamber, designed to replicate the natural environment to study the volatiles released by Mupli beetles in indoor spaces using headspace semiochemical analysis, proved successful. This method facilitates standardized and quantitative analytical profiling of the insect's whole-body volatilome. Using this glass chamber during the whole-body headspace collection, a range of compounds from different chemical families were trapped, detected, and identified. Analysis of the volatiles adsorbed onto activated charcoal released by Mupli beetles placed in the glass chamber revealed 41 distinct compounds with retention times between 10 and 31 minutes. This closely resembles the analysis of defensive gland secretions in adult beetles of the same species conducted by Nridev (2016), which also identified three compounds, as observed in the present study. Among the 18 known pheromones (Table 4.1), there were eight defensive pheromones, six sex pheromones and four cuticular hydrocarbons. Whereas among the unidentified chemical volatiles (Table 4.2), there were twelve hydrocarbons, three alcohols, seven esters and one ether compound.

Luprops tristis is a nocturnal insect, and each set of experiments in this study was initiated during its peak activity period in the evening. As a result (Figure 4.16), the 12-hour experiment recorded a higher number of volatiles, as the entire sampling period coincided with the beetles' most active phase. In contrast, the 24-hour experiment showed a reduction in the total volatiles detected, as the latter half of the sampling period coincided with their inactive daytime phase of approximately 12 hours. Moreover, the volatile nature of the compounds increases the likelihood of losses from the first 12 hours during the extended collection period. Experiments exceeding 24 hours encompassed more than one day–night cycle of the beetle. Consequently, a slightly higher number of volatile compounds were obtained when a greater number of beetles were placed in the glass chamber for longer durations. The present experiment, conducted from April to May 2022, recorded data primarily during the active season of the Mupli beetle, whose amazingly massive seasonal aggregation in each generation occurs after a nine-

month dormant phase as reported by Sabu et al (2008). Their mass aggregation after the summer showers, usually during the end of March, occurs at indoor spaces near rubber plantations which allows them to feed on tender rubber leaves rich in nitrogen and moisture content, which is a crucial factor for the survival of the next generation (Sabu et al., 2014).

Although insect semiochemical systems are considered potentially important for intra-specific communication (Tumlinson, 1988), such chemicals may also be a component of their irritant secretions. This study investigates the nature of the volatiles detected during the headspace collection and the possible negative impacts they might cause. Out of the 41 components detected during the experiment, 11 have been categorised as hazardous chemicals (Table 4.9). These included two hydrocarbons with defensive function, two alcohols with defensive function, one hydrocarbon and one alcohol each acting as sex pheromones and the remaining ones with unidentified functions. The nature of their negative effects on human health varied, although most were associated with respiratory issues.

Kauser (2018) explained that the presence of insects has at times been associated with the onset of certain allergies. The Mupli beetle has also been shown to cause similar effects through its volatile secretions (Sabu et al., 2014), sometimes resulting in keratoconjunctivitis (Susan et al., 2010). The probable reason for noticing the actions of the Mupli beetle is due to its large-scale aggregation and nuisance when in direct contact. Inhalant allergies caused by such allergens are mostly concentrated in the air surrounding the allergic insects and have been observed during the mass emergence metamorphosis of chironomid midges in Japan (Hemmer, 2010), which usually results in asthma (Baur, 1992; Hirabayashi, 1997, Yi et al., 2019). A similar case of inhalant allergy was observed in ladybird beetles, although they are considered to be more beneficial than ones causing a health issue (Guillet et al., 2022). These beetles also exhibit a seasonal appearance pattern as that of the Mupli beetle, although during winter and have been seen to cause asthma, allergic rhinitis, and angioedema, due to the presence of proteins such as Har a 1 and Har a 2 (Nakazawa et al., 2007; Davis et al., 2006; Goetz, 2009).

Similar is the case of cockroach allergies in children, especially in inner cities, being exposed to 12 allergens from cockroach feces or various body parts (Milligan et al., 2016; Pomes et al., 2017).

The survey conducted during the pandemic reported that the Mupli beetle was an allergic insect irrespective of the fact that it neither stings nor bites (Mohammed et al., 2023), supporting the statement that the volatiles released by the same might be the culprit. A similar observation of atopic sensitization was reported in another study. During this program, the researcher's asthma and allergy worsened upon exposure to grasshoppers (Soparker et al., 1993). Chironomid larvae also do not sting or bite and do not directly transmit diseases of any kind (Guillet et al., 2022), but are still seen to be causing allergic issues. This study focused on the indoor pests from which no human can escape, making things more complicated. Therefore, the hazardous individual components of the beetle's volatile emissions were examined in detail by comparing them with existing literature to understand more about their nature and possible implications on human health upon exposure.

As a result of this investigation, Heneicosane (Table 4.3), an identified hydrocarbon during the assay, was found to be a skin and eye irritant (NCBI, 2023), while another hydrocarbon, 1-Octadecanesulphonyl chloride, may sometimes result in being a corrosion compound (NCBI, 2023) apart from already being an established irritant. Heptadecane and Eicosane (Table 4.1) are other identified hydrocarbons known to be causing aspiration hazards (NCBI, 2023). Another hydrocarbon, Dodecane, 1-fluoro- (Table 4.2), on the other hand, is known to cause acute oral toxicity (NCBI, 2023). Whereas Decane, 1,1'-oxybis- (Table 4.2), is an irritant (NCBI, 2023) along with alcohols n-Nonadecanol-1 (Table 4.4), (NCBI, 2023) and n-Heptadecanol-1 (Table 4.3) (NCBI, 2023).

The alcohol compound 1-Hexanol, 5-methyl-2-(1-methylethyl)- has also been seen to be causing acute allergic effects (NCBI, 2023) while 1-Heneicosanol causes slight hazard to the aquatic environment (Table 4.3) (NCBI, 2023). The only ester compound that seemed to be hazardous among the detected ones was

Sulfurous acid, decyl 2-propyl ester (Table 4.7). This compound was also found to pose long-term hazards to the aquatic environment (Table 4.9) (NCBI, 2023).

5.4. In vitro evaluation of heptadecane-induced cytotoxicity in intestinal epithelial cells

Insects are recognized as a persistent health nuisance due to their stings and bites (Sturm et al., 2023), allergens from their body parts, fecal matter or saliva (Kausar et al., 2022). Living cells, when exposed to insect residues, also have cytotoxic and genotoxic effects (Abdelfattah et al., 2022). This study further investigated a single volatile from *Luprops tristis* to gain a novel understanding of the potential negative impact on the human body after prolonged exposure to insect volatiles in indoor spaces. Heptadecane, a compound with a proven track of aspiration hazard, is considered fatal if ingested (NCBI, 2024). With various structural modifications of heptadecane repeatedly identified as volatile during this study, the compound was subjected to cytotoxicity assays.

The cytotoxicity assay revealed a very low rate of cell inhibition at lower concentrations, with 9.88% inhibition at 6.25 μ g/ml (Table 4.10). However, there is a visible increase with the increase in dosage (Figure 4.17). In the background of cancer therapies, this dose-dependent pattern is consistent with the general toxicological principle of concentration-driven effects and aligns with findings from Xiao et al (2018), who reported high cytotoxicity in cell lines exposed to the total defensive secretion (TDS) of a medicinal insect, *Blaps rynchopetera*. Similar negative changes may also be reflected in various other cell functions, including the alterations in cell morphology (Figure 4.18). A parallel effect, which was also seen during the study of the effect of extracts from different ant species (Jangir & Nadumane, 2023), provided detrimental results.

Therefore, cellular exposure to heptadecane may also induce several physiological and molecular changes. Although this study did not directly assess toxicity, the observed cytotoxic effects raise the possibility that heptadecane may contribute to DNA damage, including both single and double-stranded breaks, as

reported in earlier toxicological studies (Crespo RM, 2011). This study, which moves through the effects of just one compound, suggests a glance into the extrapolatory effects of the same kind in much higher dosages and also when combined with other volatile components from the defensive secretions of the same beetle.

Here, the dose dependency of damage to the cells was comparatively non-significant ($p=0.2243$), possibly due to the study being conducted using a single compound. As the compound was tested alone and not in mixed combinations with other hazardous compounds identified during the study, the percentage of cell inhibition stays below 50; thus, this chemical, as of now, may be considered a non-irritant (EU Science Hub, 2024). Whereas this chemical, when combined with other volatiles identified, may be an evident cytotoxic compound. Further evaluations into combinations of compounds from TDS of the same beetle, in particular, are warranted.

Chapter 6

CONCLUSION

6.CONCLUSION

The primary purpose of this study was to investigate the diversity and health implications of indoor insect pests in different environments across Malappuram. The research aimed to identify commonly occurring indoor insects through field surveys and resident-based questionnaires, with a specific focus on selecting one pest species for in-depth semiochemical and toxicological analysis. Further, the study aimed to investigate how the structural elements of indoor spaces, geographical location, and nearby vegetation affect the presence of insect pests, and to assess whether there is any association between pest prevalence and reported health concerns among inhabitants. A central goal was to isolate and identify volatile compounds released by the selected test insect and to evaluate their cytotoxic effects on normal mammalian cells using the MTT assay. Through this multidisciplinary approach, the study sought to establish a foundational understanding of the ecological, chemical, and health-related dimensions of indoor insect infestations.

Field and questionnaire-based surveys confirmed a diverse range of indoor insect pests, with *Luprops tristis* (Mupli beetle) emerging as a significant nuisance species across multiple sites. The data showed clear variation in pest occurrence depending on structural features, especially roofing materials, and the quality of surrounding vegetation. The presence of pests and other biological contaminants was also higher in locations where residents reported allergies or respiratory symptoms. Semiochemical analysis of *Luprops tristis* revealed the presence of volatile compounds, notably heptadecane. Cytotoxicity testing through the MTT assay demonstrated that heptadecane negatively affected normal mammalian cell viability, indicating a potential health hazard. Although a direct clinical correlation between pest presence and specific health conditions could not be conclusively established, the results support the hypothesis that certain indoor insects contribute to deteriorating air quality and related health concerns.

This research advances the understanding of indoor pest ecology by demonstrating how architectural and environmental factors influence pest distribution and diversity. It also provides novel toxicological evidence linking indoor insect emissions to adverse cellular effects, thereby suggesting a potentially underrecognized public health concern. These findings create a basis for interdisciplinary exploration at the interface of entomology, environmental science, and health studies in tropical indoor environments.

The study was geographically limited to a selected region in Kerala, and therefore, the results may not be generalizable to all settings. Furthermore, the study did not incorporate clinical testing or immunological profiling of affected individuals, which restricts the strength of its conclusions regarding health outcomes. In addition, volatile profiling focused on a limited number of compounds from a single species, leaving scope for broader chemical and species-level analysis.

Future studies should expand to include more diverse geographical areas and utilize molecular tools for precise species identification. Collaborative clinical research is also needed to explore allergic and toxic responses in exposed individuals through immunoglobulin and skin prick tests. Broader chemical analyses of insect-emitted volatiles and exploration of their long-term impacts on indoor air quality will strengthen the scientific understanding of this domain. The integration of entomological insights into urban planning and indoor environment design may further aid in creating pest-resistant and health-conscious living spaces.

This study brings to light the subtle but significant role of indoor insect pests in shaping environmental health, establishing an important foundation for future interdisciplinary research and informed public health interventions.

Chapter 7

RECOMMENDATIONS

7. RECOMMENDATIONS

Future research in this area should aim to broaden its scope by encompassing a wider range of geographical regions, particularly those with contrasting climatic conditions, building structures, and pest prevalence, to capture the diversity of human-insect interactions across varied settings. Also, incorporating molecular tools such as DNA barcoding and high-throughput sequencing will enable precise species identification, which is crucial for distinguishing cryptic or morphologically similar taxa that may differ significantly in their allergenic or toxic potential. These molecular approaches can also shed light on population genetics and adaptive traits of pest species, thereby enriching both ecological and biomedical perspectives.

In parallel, collaborative clinical research will be essential to link entomological and chemical findings with tangible health outcomes. Systematic assessment of allergic sensitization through immunoglobulin profiling, skin prick tests, and longitudinal epidemiological studies will help establish causal connections between insect-emitted volatiles and human health. Such integrative efforts could uncover not only acute allergic responses but also the potential for chronic effects, such as respiratory disorders, neurological impacts, or immune dysregulation, arising from prolonged exposure to indoor environments.

Equally important is the need for broader chemical analyses of volatile organic compounds (VOCs) released by insects, using advanced techniques such as GC-MS, and real-time air monitoring. These analyses should aim to characterize both the diversity and concentration dynamics of insect-derived emissions under different ecological and environmental conditions.

Overall, this study highlights the subtle yet consequential role of indoor insect pests in shaping environmental health. By bringing together the disciplines of entomology, toxicology, immunology, chemistry, and urban planning, it establishes a critical foundation for interdisciplinary research.

Chapter 8

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8. REFERENCES

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APPENDIX

QUESTIONNAIRE

Name: _____ Contact : _____

1. Place :
2. Village/Municipality :
3. Age of building : 2yrs 5yrs 10yrs
4. Structure of building : House Apartment Commercial
5. Ventilation Status : Good Average Bad
6. Roof Type : Traditional Closed
7. Number of Members :
8. Number of children below 12 years :
9. Number of adults above 60 years :
10. General Parameters :

a) Temperature :	c) SO ₂ :
b) Humidity :	d) CO :
	e) PM 2.5 :
	f) PM 10 :
11. Number of diseased ones :
12. Disease diagnosis :

<input type="checkbox"/> Allergic rhinitis <input type="checkbox"/> Asthma <input type="checkbox"/> Conjunctivitis <input type="checkbox"/> Sinusitis <input type="checkbox"/> Migraine <input type="checkbox"/> Frequent cough <input type="checkbox"/> Dry skin <input type="checkbox"/> Dry eyes <input type="checkbox"/> Exhaustion <input type="checkbox"/> Others _____	<input type="checkbox"/> Wheezing <input type="checkbox"/> Dry throat <input type="checkbox"/> Shortness of breath <input type="checkbox"/> Nasal congestion <input type="checkbox"/> Sore throat <input type="checkbox"/> Eye itching <input type="checkbox"/> Watery eyes <input type="checkbox"/> Headache <input type="checkbox"/> Multiple colds
--	---
13. If you suffered from any of the above,
 Did these symptoms occur :

<input type="checkbox"/> Mainly outdoor <input type="checkbox"/> Mainly indoor <input type="checkbox"/> Onset of Rain <input type="checkbox"/> Summer <input type="checkbox"/> Winter	<input type="checkbox"/> Independent of environmental conditions
---	--
14. Do you experience any problems occurring frequently during any specific season of the year
 - a) Problem :

b) Season :

15. Qualitative survey-Surrounding plot :

Tree _____

Shrub _____

Herb _____

Forest _____

Waterbody _____

Houses _____

Industrial
area _____

Others _____

16. Biological contaminants observed :

Animal Dander

Moulds

Pollen

Mites

Entomological Survey

17. Common insects observed Indoor :

18. Quantitative survey - kinds of insects :

Sl.No	Insect	5-10	10-15	Above 15
1	Flies			
2	Mupli beetle			
3	Mosquito			
4	Cockroaches			
5	Ants			
6	Other _____ _____			

19. Signs of infestation -

Swarming

Frass

Others _____

Noises

Nuisance

20. Do you think any particular landscape /

Practices causes an increase in their

Number

:

Yes

No

21. If Yes, provide details : _____
22. Are the pests at your place controllable : Yes No
23. Control measures taken :
 Sprayed _____
 Dusted _____
 Others _____
 Non-Chemicals _____
 Baits _____
24. Do these insects or infestations cause any allergy related issues : Yes No
25. If yes, mention the type of allergy : _____
26. Are you allergic to insect bite/insect Sting : Yes No
27. If yes, give details : _____
28. Have you been medically evaluated For Allergies : Yes No
29. Are you currently taking medicines : Yes No
30. If Yes, provide details : _____
31. Do you have a history of having asthma : Yes No
32. If Yes, Age of onset (in years) : 1-2 3-5 5-10 > 10
33. Frequency of attacks :
 2 or less times a week
 3-6 times a week
 3-4 times a month
 Throughout the day
 Occasionally Similarly, most of the semiochemicals released

34. Details of Medicines to control asthma : _____

35. Do you experience night cough : Yes No

36. Season it gets worse in : _____

37. Do you experience difficulties when you
wake up in the morning : Yes No

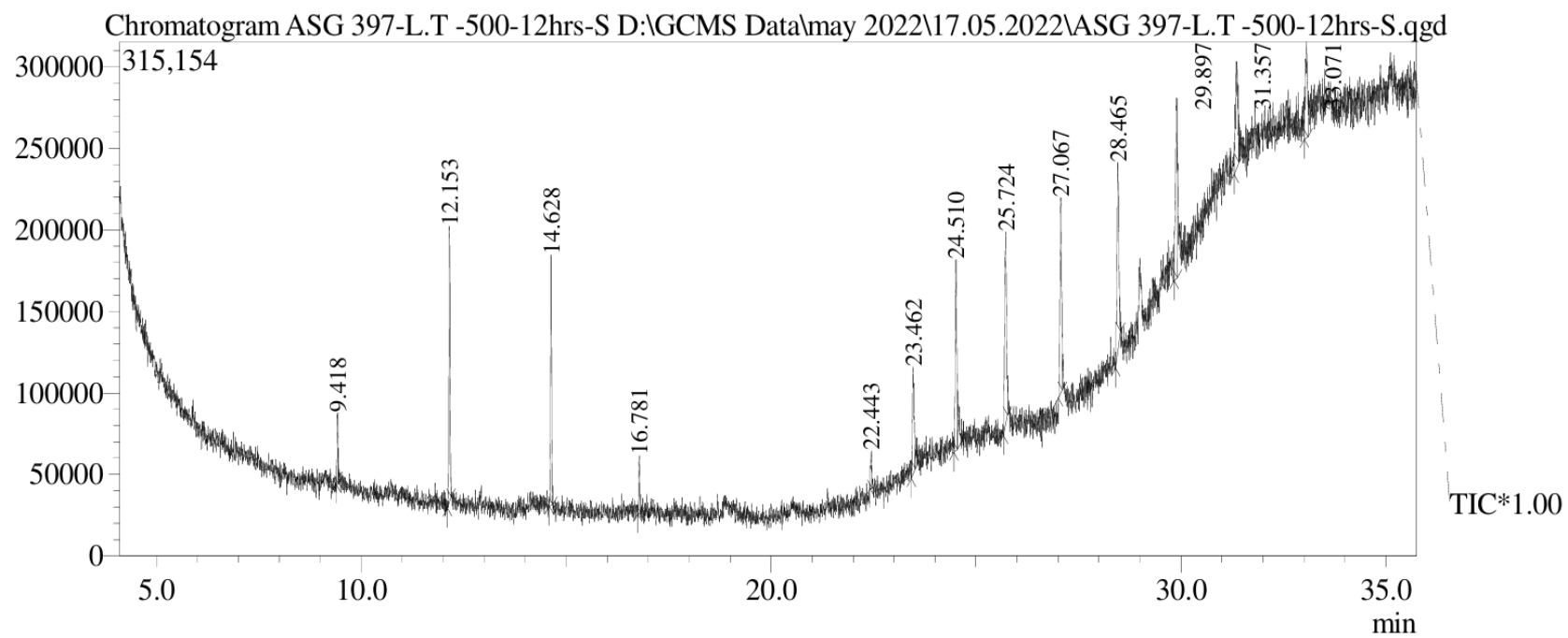
38. Season it gets worse in : _____

39. Surgery histories of sick ones: _____

40. Immunizations of sick ones up to date : Yes No

Comments/ Suggestions:

Chromatogram of Volatile Compounds Emitted by 500 Beetles After 12 Hours of Aggregation

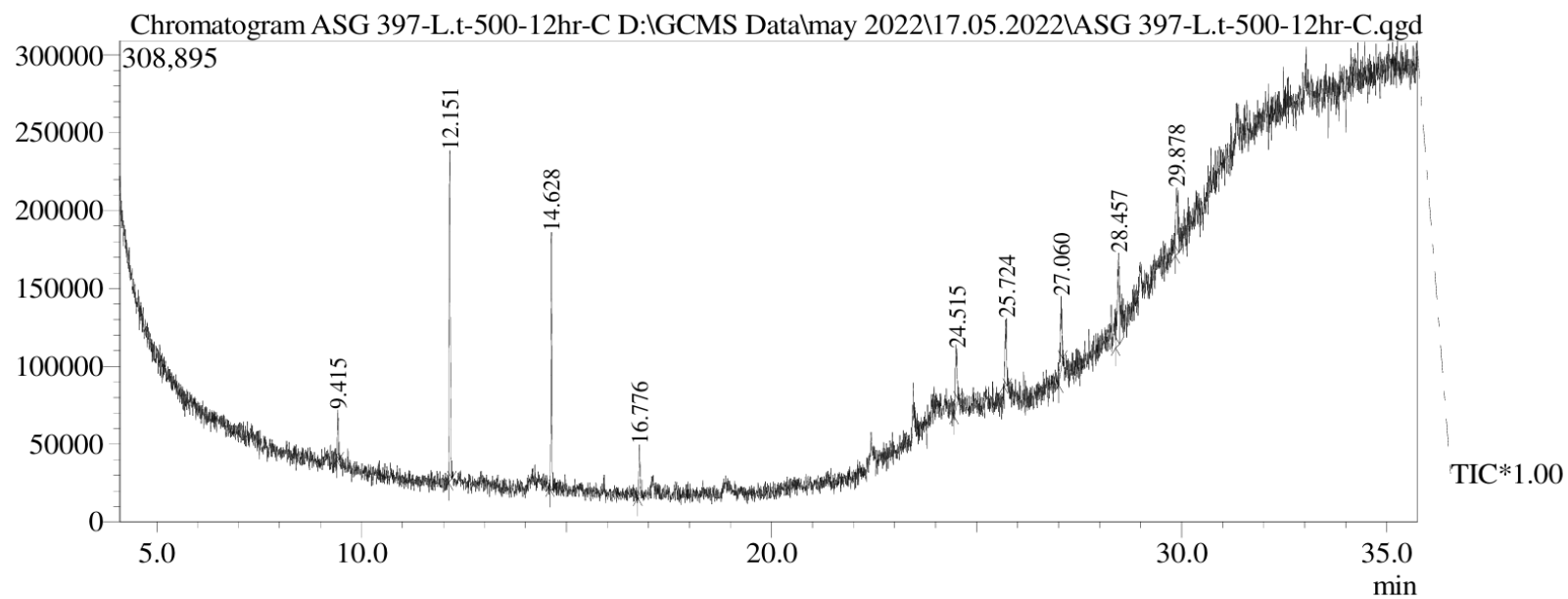


Peak Report of Volatile Compounds Emitted by 500 Beetles After 12 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.418	83346	2.68	43579	3.64	Cyclohexasiloxane, dodecamethyl-	73.10
2	12.153	316031	10.15	168583	14.10	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
3	14.628	256423	8.23	153567	12.84	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.05
4	16.781	60014	1.93	35625	2.98	1,1,1,5,7,7,7-Heptamethyl-3,3,5-tris(trimethylsiloxy)tetrasiloxane	73.05
5	22.443	42252	1.36	23186	1.94	1-Hexanol, 5-methyl-2-(1-methylethyl)-	57.10
6	23.462	196732	6.32	66276	5.54	Methoxyacetic acid, 3-tetradecyl ester	57.10
7	24.510	312329	10.03	117068	9.79	2-methyloctacosane	57.10
8	25.724	354521	11.38	118041	9.87	Heptadecane, 2,6,10,15-tetramethyl-	57.10
9	27.067	344356	11.05	120248	10.06	Tritetracontane	57.10
10	28.465	355480	11.41	114595	9.58	Tetracosane, 11-decyl-	57.05
11	29.897	311121	9.99	110552	9.24	Sulfurous acid, 2-propyl tridecyl ester	57.10
12	31.357	276262	8.87	65881	5.51	Nonadecane, 1-bromo-	57.10
13	33.071	206140	6.62	58616	4.90	1-Octadecanesulphonyl chloride	57.05
		3115007	100.00	1195817	100.00		

Chromatogram of Control Sample Collected Over a 12-Hour Period

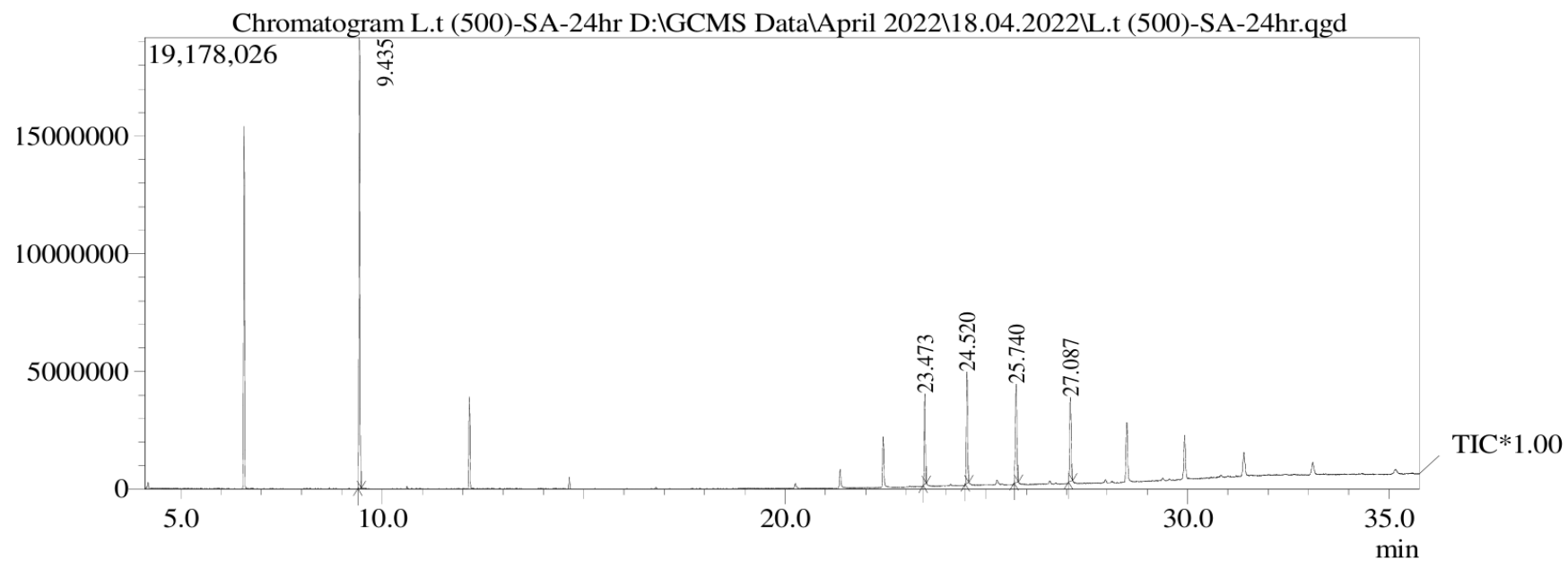


Peak Report of Control Sample Collected Over a 12-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.415	49616	3.46	32222	4.79	Cyclohexasiloxane, dodecamethyl-	73.10
2	12.151	378644	26.41	212226	31.58	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
3	14.628	270632	18.88	165203	24.58	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.05
4	16.776	68834	4.80	33856	5.04	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
5	24.515	108810	7.59	35911	5.34	Octane, 3,4,5,6-tetramethyl-	57.10
6	25.724	95839	6.69	43417	6.46	2-methyltetracosane	57.05
7	27.060	128583	8.97	47063	7.00	Methoxyacetic acid, 4-tridecyl ester	57.05
8	28.457	193722	13.51	59218	8.81	2-Bromo dodecane	57.05
9	29.878	138948	9.69	42886	6.38	Triacotane, 1-bromo-	57.10
		1433628	100.00	672002	100.00		

Chromatogram of Volatile Compounds Emitted by 500 Beetles After 24 Hours of Aggregation

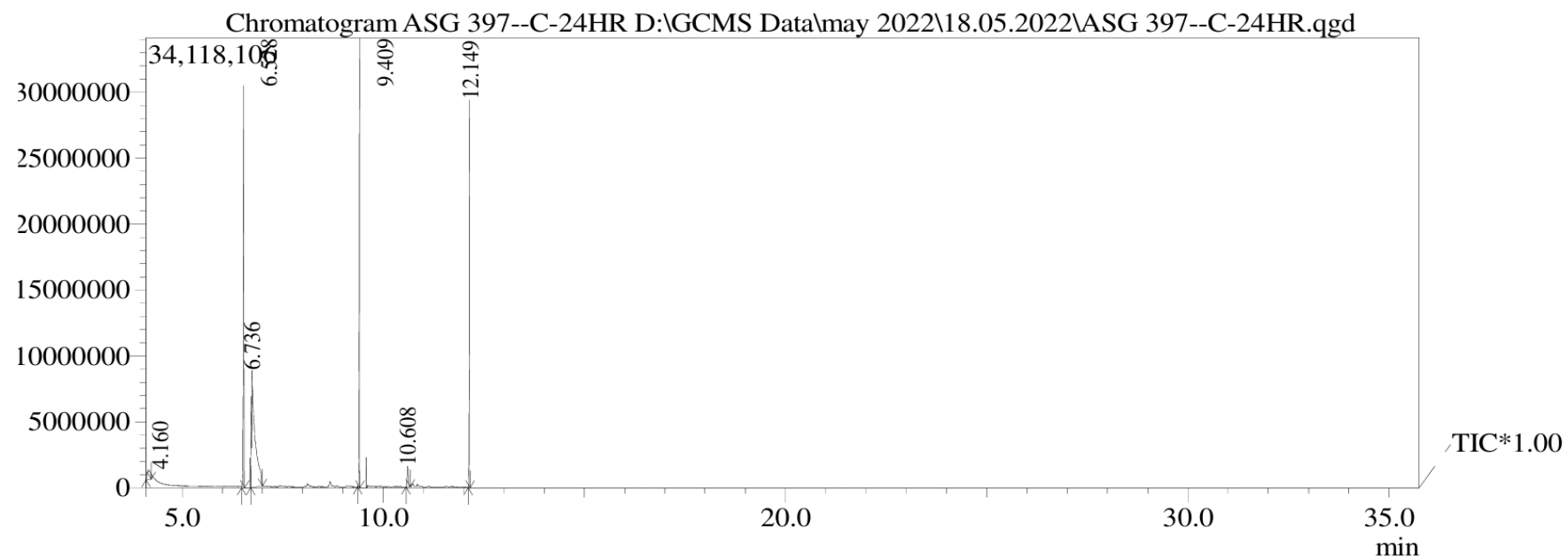


Peak Report of Volatile Compounds Emitted by 500 Beetles After 24 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.435	33938071	47.93	19129814	53.96	Cyclohexasiloxane, dodecamethyl-	73.10
2	23.473	7036759	9.94	3841678	10.84	Heptadecane	57.10
3	24.520	9835418	13.89	4737585	13.36	Eicosane, 10-methyl-	57.10
4	25.740	10449770	14.76	4181899	11.80	2-methyloctacosane	57.10
5	27.087	9541022	13.48	3561956	10.05	Heneicosane	57.10
		70801040	100.00	35452932	100.00		

Chromatogram of Control Sample Collected Over a 24-Hour Period

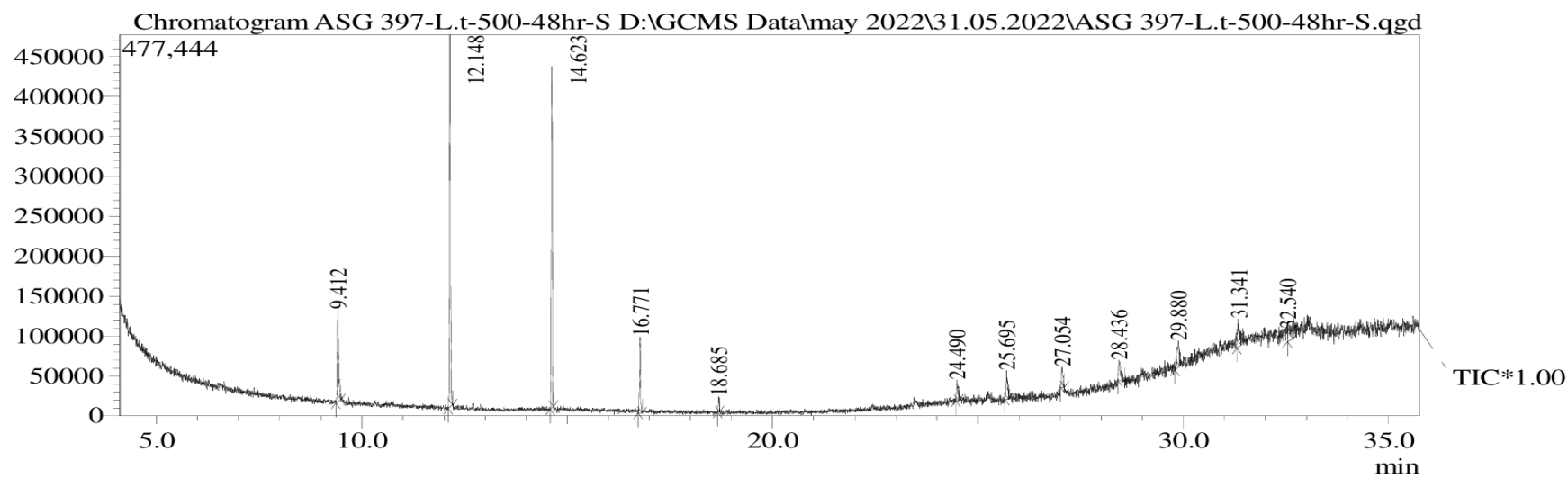


Peak Report of Control Sample Collected Over a 24-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	4.160	3997141	3.04	724110	0.69	Cyclotetrasiloxane, octamethyl-	280.80
2	6.528	25029672	19.03	30506338	28.99	Cyclopentasiloxane, decamethyl-	73.00
3	6.736	53648203	40.78	8887403	8.45	N-(Trifluoroacetyl)-N,O,O'-tetrakis(trimethylsilyl)norepinephrine	73.00
4	9.409	25331820	19.25	34101307	32.41	Cyclohexasiloxane, dodecamethyl-	73.00
5	10.608	3037626	2.31	1573635	1.50	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethylsilyloxyethyloxy)phenyl]propane	73.00
6	12.149	20517209	15.60	29422115	27.96	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
		131561671	100.00	105214908	100.00		

Chromatogram of Volatile Compounds Emitted by 500 Beetles After 48 Hours of Aggregation

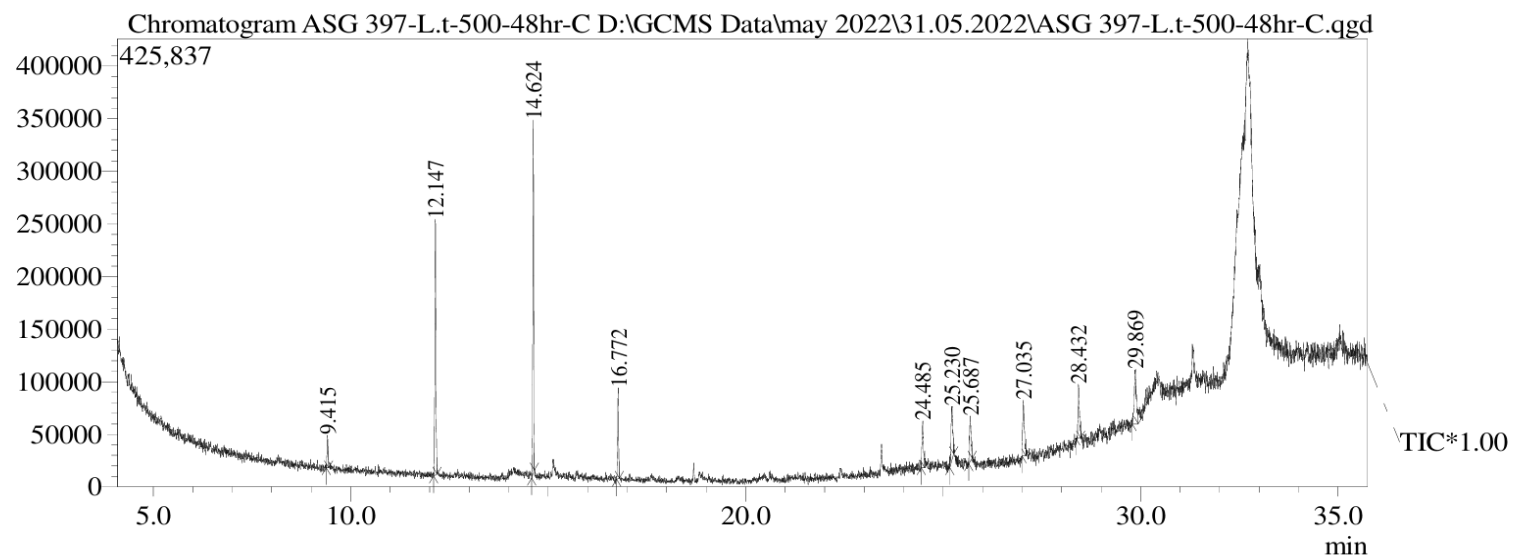


Peak Report of Volatile Compounds Emitted by 500 Beetles After 48 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.412	280254	10.51	115186	8.73	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.148	846994	31.76	466030	35.34	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
3	14.623	713301	26.75	427315	32.40	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
4	16.771	173490	6.51	95395	7.23	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
5	18.685	26849	1.01	19249	1.46	1,3-Dioxolane, 2-(2-propenyl)-	73.05
6	24.490	55964	2.10	26205	1.99	Sulfurous acid, 2-propyl undecyl ester	57.05
7	25.695	78771	2.95	35644	2.70	Oxalic acid, 6-ethyloct-3-yl heptyl ester	57.10
8	27.054	54624	2.05	25316	1.92	Octane, 3,4,5,6-tetramethyl-	57.00
9	28.436	96413	3.62	25334	1.92	Sulfurous acid, decyl 2-propyl ester	57.05
10	29.880	106772	4.00	32770	2.48	Decane, 1,1'-oxybis-	57.05
11	31.341	118995	4.46	34952	2.65	Dodecane, 1-fluoro-	57.10
12	32.540	114376	4.29	15450	1.17	2-Methyl-5-t-butyl-1,3-oxathiane	73.00
		2666803	100.00	1318846	100.00		

Chromatogram of Control Sample Collected Over a 48-Hour Period

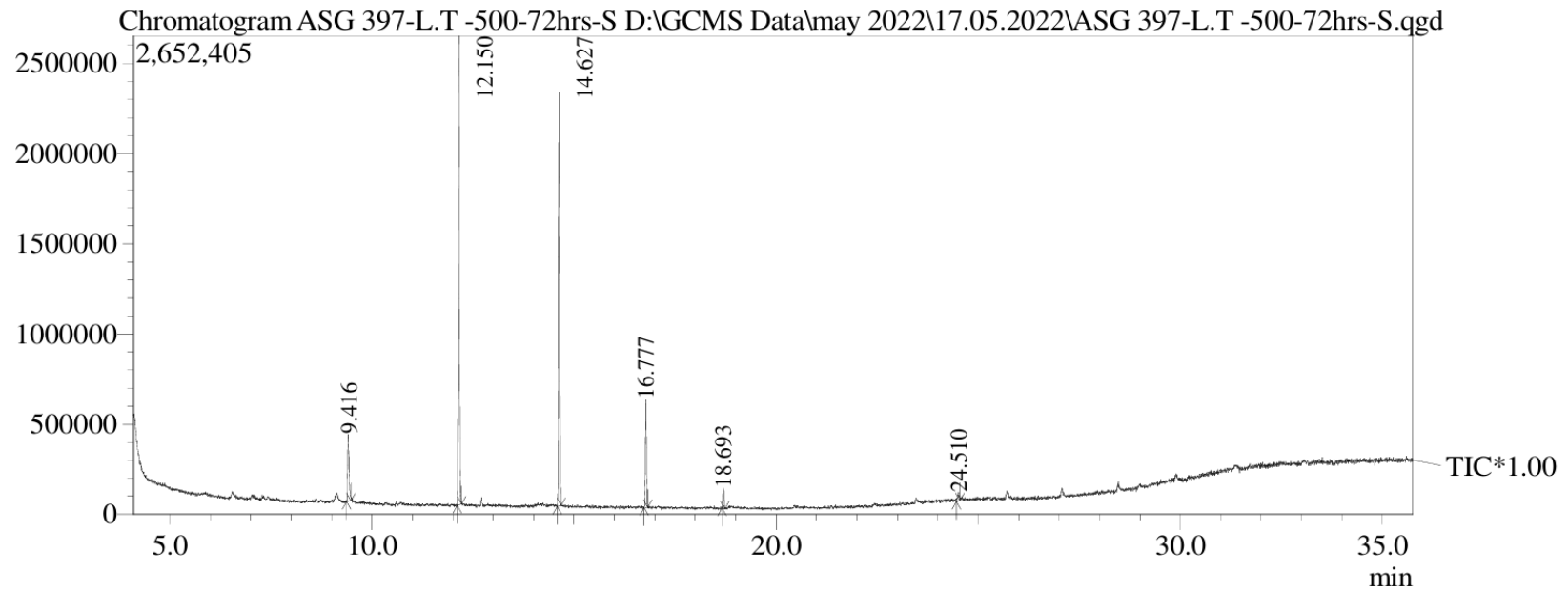


Peak Report of Control Sample Collected Over a 48-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.415	59390	2.95	30765	3.11	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.147	443411	22.06	243167	24.55	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.624	537427	26.73	336133	33.93	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
4	16.772	135677	6.75	86060	8.69	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
5	24.485	113790	5.66	44326	4.47	Undecane, 5,7-dimethyl-	57.05
6	25.230	155481	7.73	50861	5.13	Diisooctyl phthalate	148.95
7	25.687	110847	5.51	43126	4.35	Decane, 2,3,5,8-tetramethyl-	57.05
8	27.035	140261	6.98	53351	5.39	Eicosane, 2-methyl-	57.10
9	28.432	117646	5.85	50936	5.14	2-Bromo dodecane	57.10
10	29.869	196272	9.76	51972	5.25	Sulfurous acid, 2-propyl undecyl ester	57.05
		2010202	100.00	990697	100.00		

Chromatogram of Volatile Compounds Emitted by 500 Beetles After 72 Hours of Aggregation

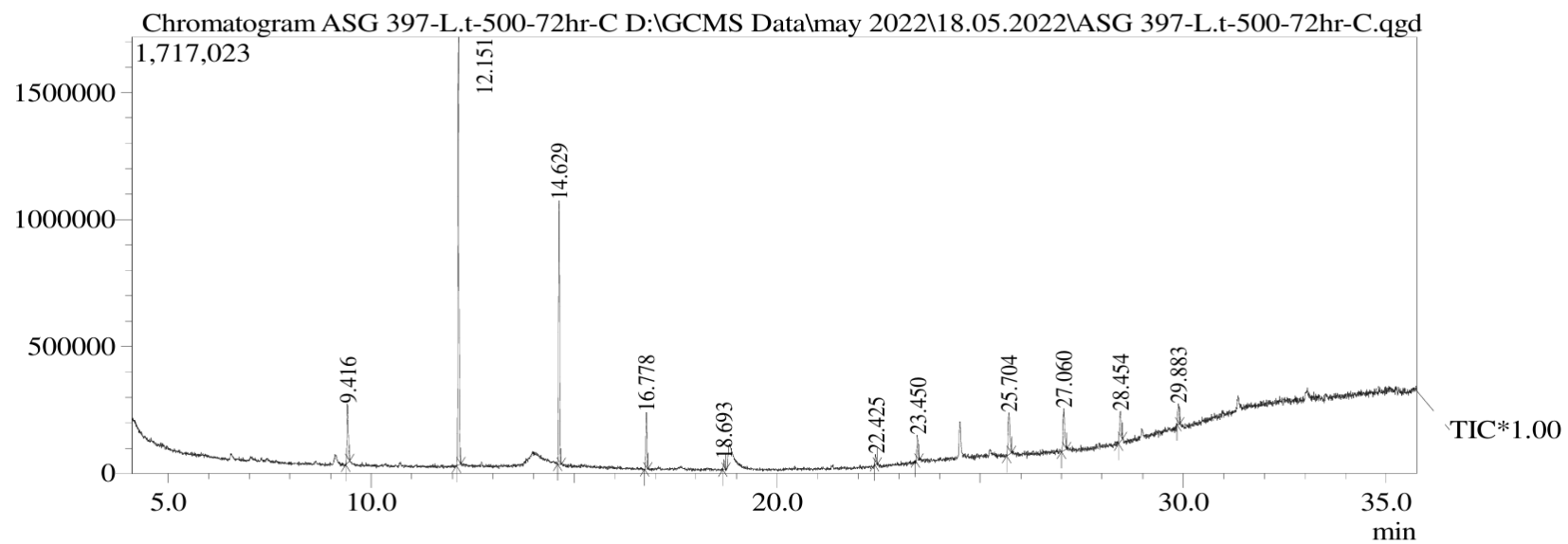


Peak Report of Volatile Compounds Emitted by 500 Beetles After 72 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.416	905037	8.19	370497	6.15	Cyclohexasiloxane, dodecamethyl-	73.05
2	12.150	4709678	42.61	2599488	43.15	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
3	14.627	4084151	36.95	2295920	38.11	Cyclooctasiloxane, hexadecamethyl-	73.05
4	16.777	1030017	9.32	597961	9.93	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	73.05
5	18.693	173332	1.57	111524	1.85	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
6	24.510	152021	1.38	49269	0.82	Sulfurous acid, 2-propyl tridecyl ester	57.05
		11054236	100.00	6024659	100.00		

Chromatogram of Control Sample Collected Over a 72-Hour Period

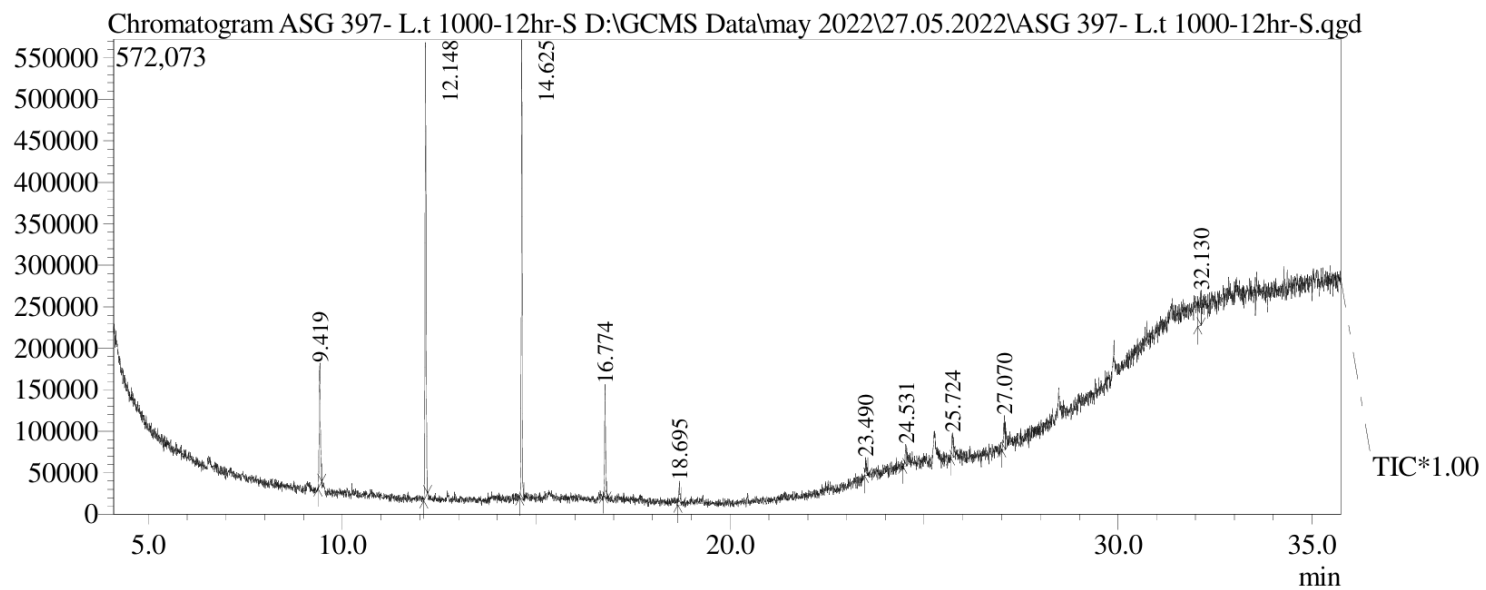


Peak Report of Control Sample Collected Over a 72-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.416	538567	7.08	233204	6.00	Cyclohexasiloxane, dodecamethyl-	73.05
2	12.151	3047563	40.04	1683681	43.33	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.629	1773075	23.30	1036958	26.69	Cyclooctasiloxane, hexadecamethyl-	73.05
4	16.778	391179	5.14	223157	5.74	Cyclohexasiloxane, dodecamethyl-	73.05
5	18.693	64050	0.84	40957	1.05	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
6	22.425	71659	0.94	39603	1.02	2,3,5,8-Tetramethyldecane	57.05
7	23.450	216145	2.84	100408	2.58	2-methyloctacosane	57.05
8	25.704	473519	6.22	164861	4.24	Heptadecane, 2,6,10,15-tetramethyl-	57.05
9	27.060	470341	6.18	164608	4.24	Eicosane, 10-methyl-	57.10
10	28.454	350615	4.61	118662	3.05	Heptadecane, 9-octyl-	57.10
11	29.883	214121	2.81	79500	2.05	Eicosane	57.10
		7610834	100.00	3885599	100.00		

Chromatogram of Volatile Compounds Emitted by 1000 Beetles After 12 Hours of Aggregation

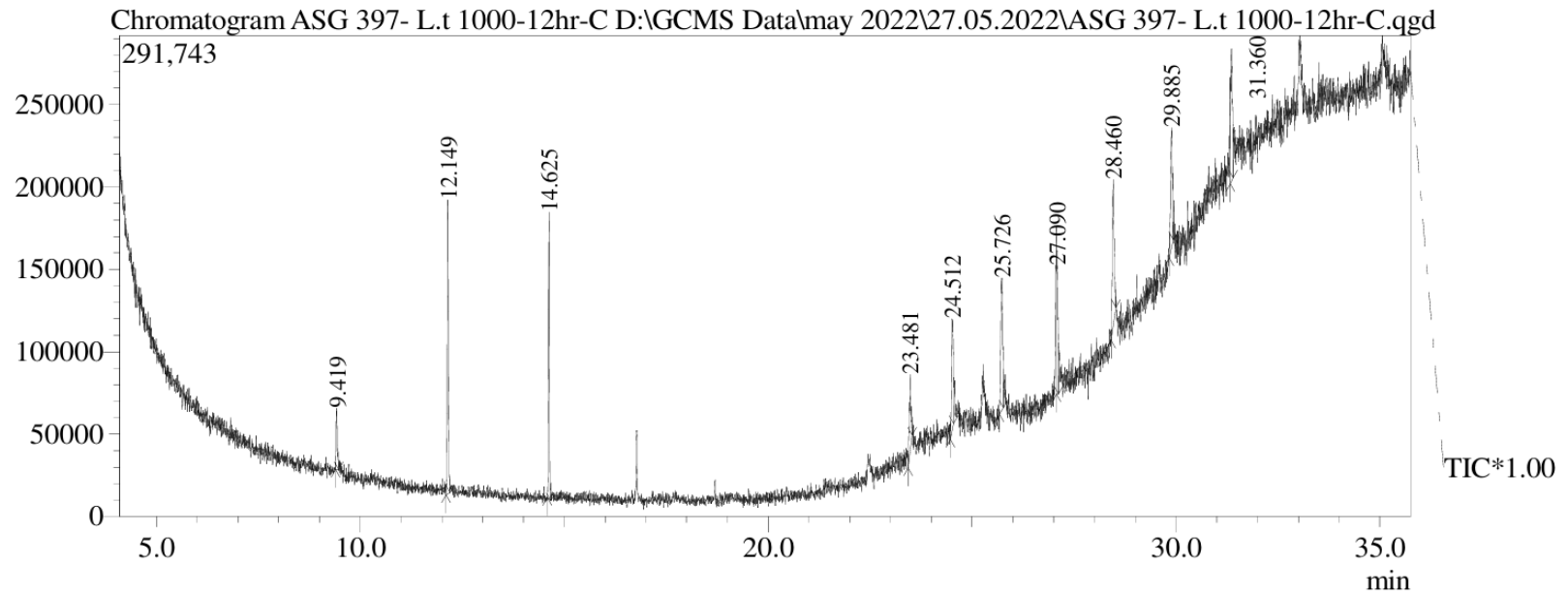


Peak Report of Volatile Compounds Emitted by 1000 Beetles After 12 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.419	343448	11.57	147488	9.37	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.148	1018110	34.30	547737	34.79	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.625	917432	30.91	553740	35.17	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
4	16.774	220618	7.43	135927	8.63	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	73.00
5	18.695	62988	2.12	29116	1.85	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
6	23.490	44096	1.49	21692	1.38	Di-n-decylsulfone	57.05
7	24.531	59571	2.01	25866	1.64	2-Nonen-1-ol, (E)-	57.05
8	25.724	76026	2.56	29862	1.90	Eicosane, 2-methyl-	57.05
9	27.070	96968	3.27	41410	2.63	Stearic acid, 3-(octadecyloxy)propyl ester	71.00
10	32.130	129164	4.35	41510	2.64	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	206.90
		2968421	100.00	1574348	100.00		

Chromatogram of Control Sample Collected Over a 12-Hour Period

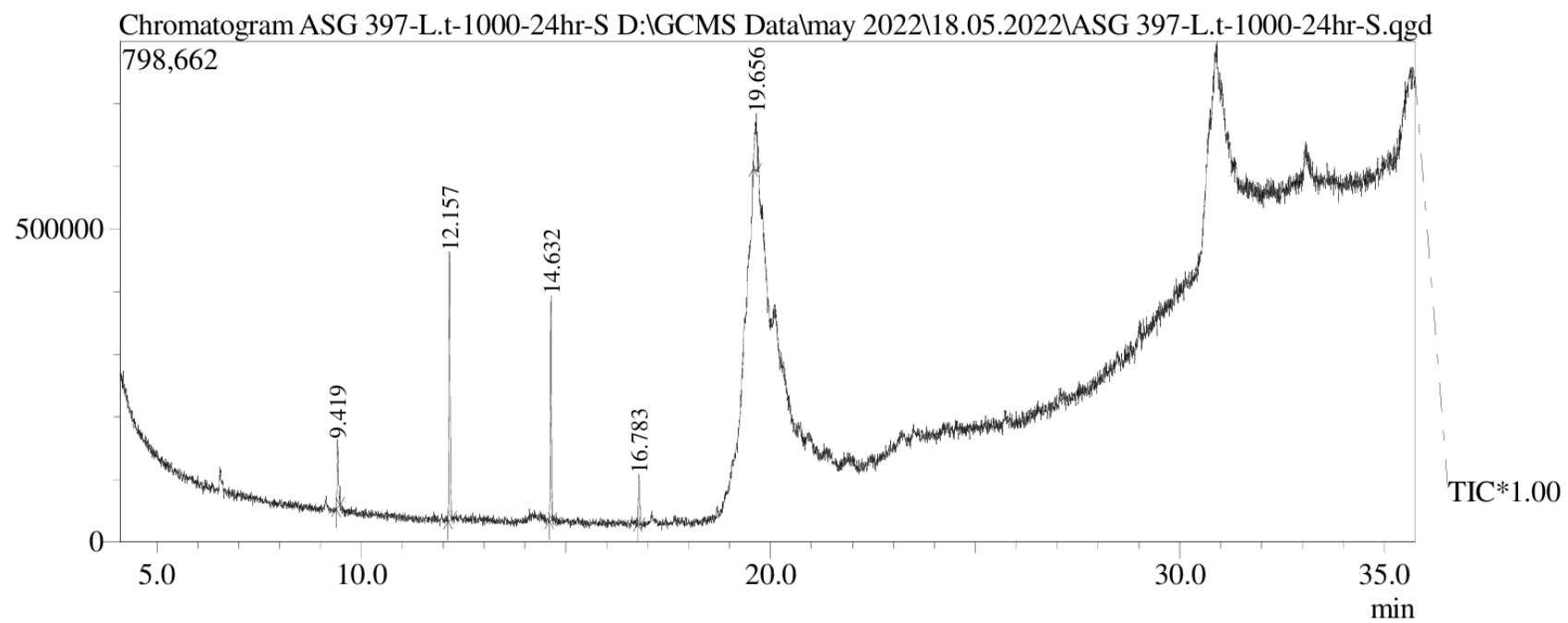


Peak Report of Control Sample Collected Over a 12-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.419	75496	3.51	37032	4.10	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.149	323678	15.03	178130	19.70	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.625	286339	13.30	174027	19.25	2-(2',4',6',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
4	23.481	117044	5.44	46501	5.14	Sulfurous acid, 2-propyl tetradecyl ester	57.10
5	24.512	200255	9.30	68043	7.53	2-methyloctacosane	57.00
6	25.726	257548	11.96	80326	8.88	2-Bromotetradecane	57.00
7	27.090	170614	7.92	77944	8.62	Nonanal	57.05
8	28.460	282878	13.14	88321	9.77	Tritetracontane	57.05
9	29.885	226901	10.54	73135	8.09	10-Methylnonadecane	57.05
10	31.360	212271	9.86	80621	8.92	Octadecane, 1-bromo-	57.05
		2153024	100.00	904080	100.00		

Chromatogram of Volatile Compounds Emitted by 1000 Beetles After 24 Hours of Aggregation

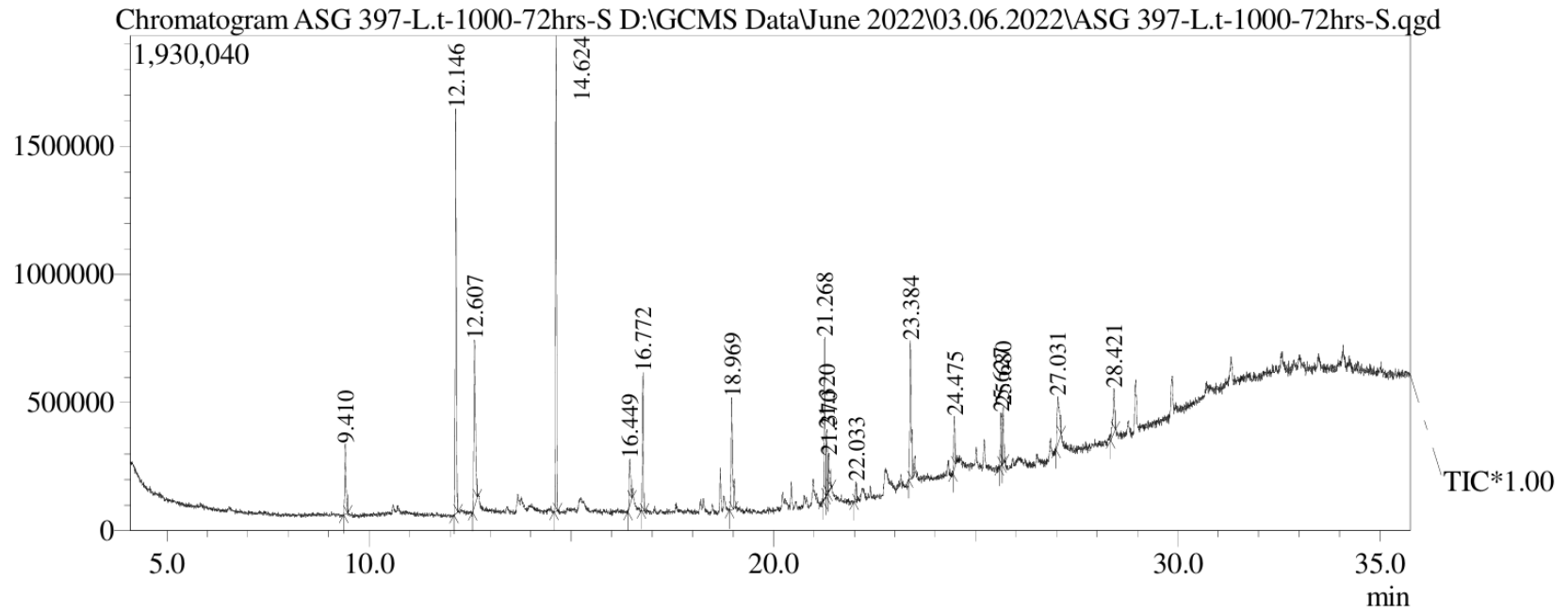


Peak Report of Volatile Compounds Emitted by 1000 Beetles After 24 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.419	257416	12.32	109332	10.22	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.157	779189	37.29	428801	40.09	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.632	631407	30.21	359491	33.61	Cyclooctasiloxane, hexadecamethyl-	73.00
4	16.783	144413	6.91	80027	7.48	Cyclohexasiloxane, dodecamethyl-	73.00
5	19.656	277382	13.27	92046	8.60	2-Thiophenepropanamine, N,N-dimethyl-	58.05
		2089807	100.00	1069697	100.00		

Chromatogram of Control Sample Collected Over a 24-Hour Period

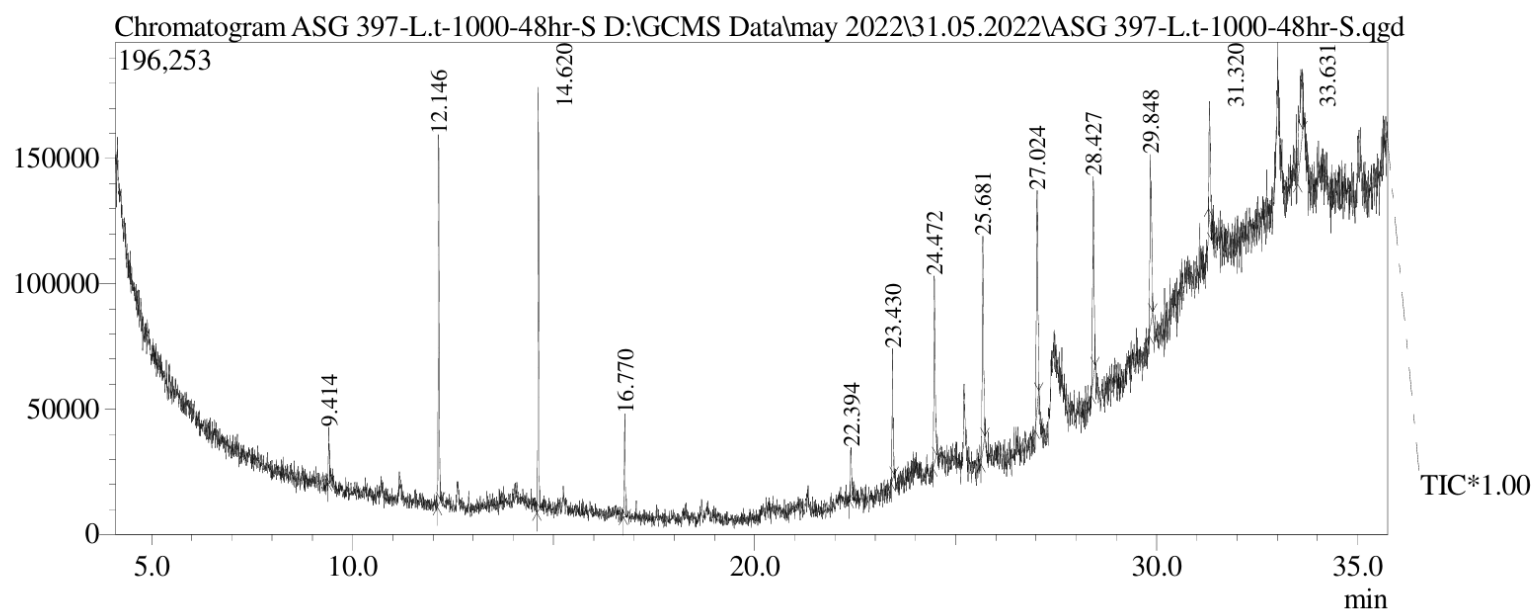


Peak Report of Control Sample Collected Over a 24-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.423	99936	4.65	53226	4.96	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.156	781005	36.32	453442	42.29	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.634	531930	24.74	326003	30.40	Cyclooctasiloxane, hexadecamethyl-	73.00
4	16.784	110208	5.13	67680	6.31	Cyclohexasiloxane, dodecamethyl-	72.95
5	24.526	250284	11.64	60523	5.64	Eicosane	57.00
6	25.745	202525	9.42	52961	4.94	10-Methylnonadecane	57.05
7	27.080	174285	8.11	58401	5.45	7,7-Diethylheptadecane	57.05
		2150173	100.00	1072236	100.00		

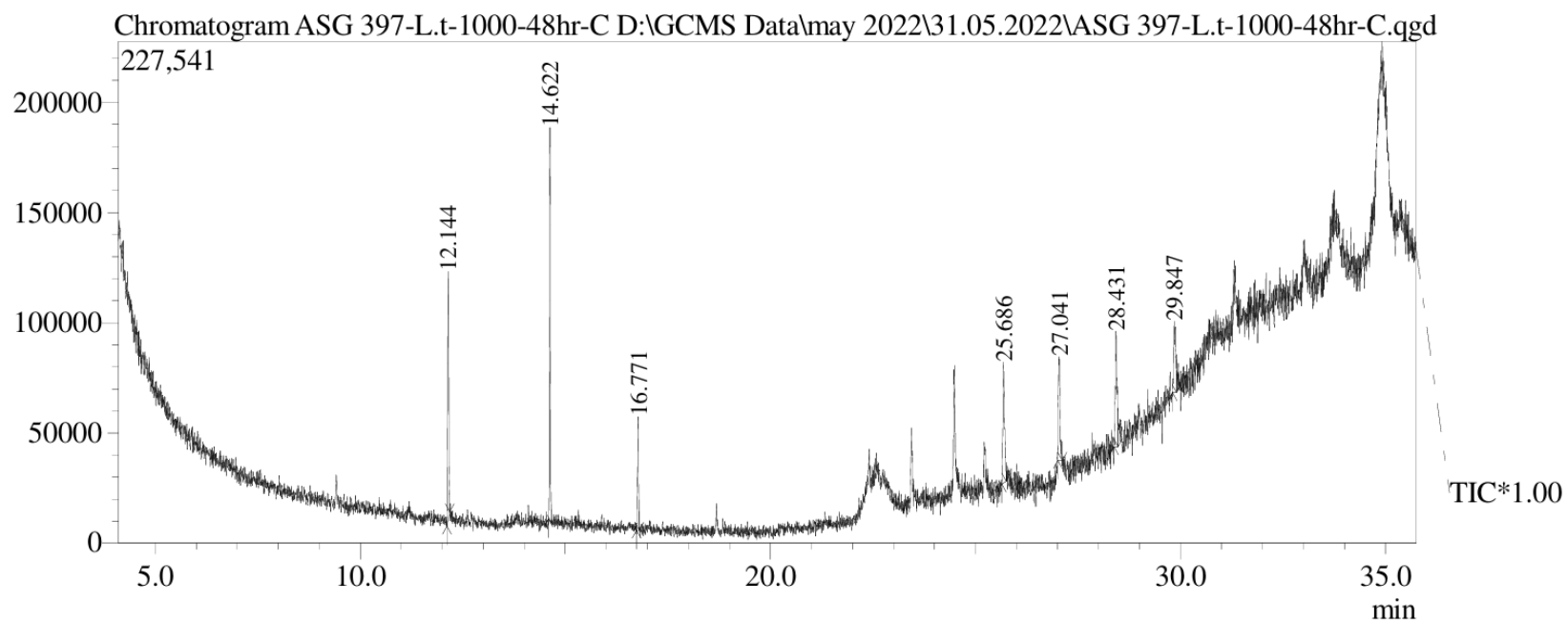
Chromatogram of Volatile Compounds Emitted by 500 Beetles After 48 Hours of Aggregation



Peak Report of Volatile Compounds Emitted by 500 Beetles After 48 Hours of Aggregation

Peak Report TIC							
Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.414	35035	1.72	22275	2.41	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether	73.05
2	12.146	249331	12.24	147216	15.95	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.620	279710	13.73	168785	18.29	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.05
4	16.770	63196	3.10	41194	4.46	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether	73.05
5	22.394	47552	2.33	20947	2.27	Oxalic acid, allyl decyl ester	57.05
6	23.430	87005	4.27	52412	5.68	Decane, 2,3,5,8-tetramethyl-	57.05
7	24.472	178124	8.74	74937	8.12	Nonadecane, 2-methyl-	57.10
8	25.681	212487	10.43	85131	9.22	Nonadecane, 2-methyl-	57.10
9	27.024	215445	10.57	88389	9.58	Eicosane, 2-methyl-	57.10
10	28.427	204719	10.05	80381	8.71	Methoxyacetic acid, 3-tetradecyl ester	57.05
11	29.848	183692	9.01	68741	7.45	2-Bromotetradecane	57.05
12	31.320	117335	5.76	47341	5.13	Undecane, 2,7-dimethyl-	57.05
13	33.631	164073	8.05	25273	2.74	2,3-O-Benzal-d-mannosan	71.05
		2037704	100.00	923022	100.00		

Chromatogram of Control Sample Collected Over a 48-Hour Period

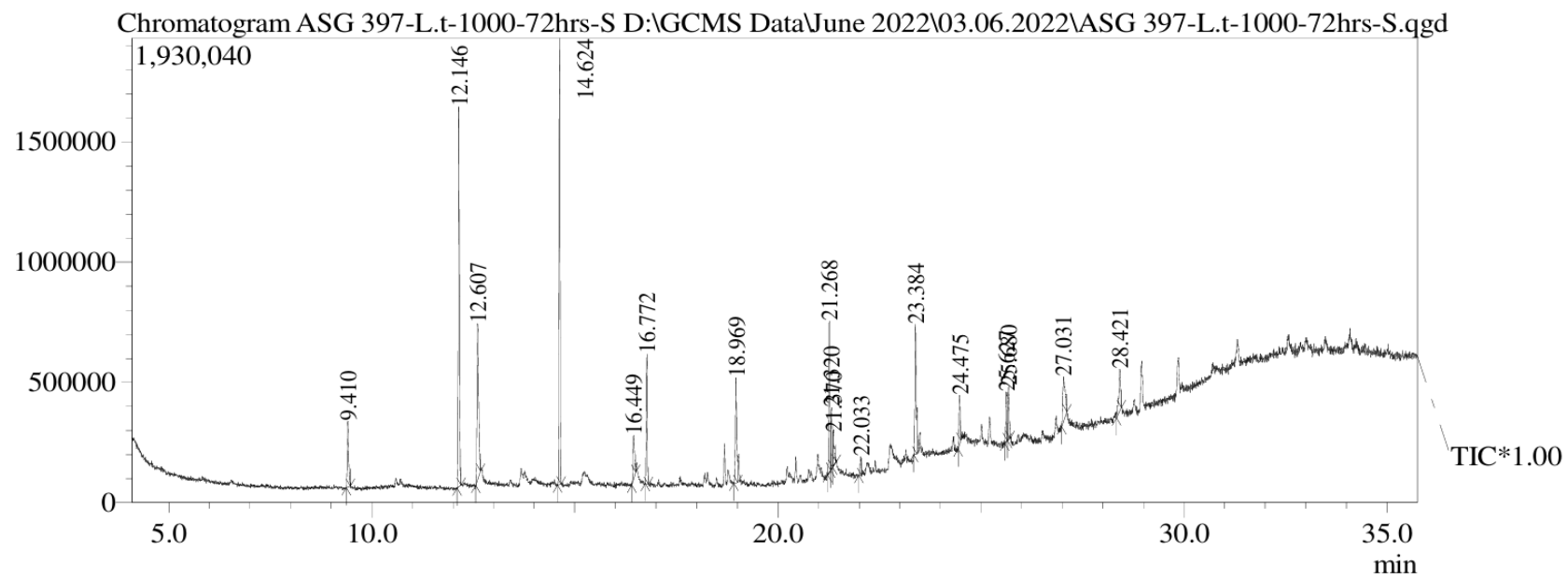


Peak Report of Control Sample Collected Over a 48-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	12.144	197655	18.27	112398	21.25	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
2	14.622	283887	26.24	178511	33.75	2-(2',4',6',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	73.00
3	16.771	81929	7.57	51477	9.73	1-Propene-1-thiol	73.05
4	25.686	144838	13.39	54804	10.36	2-Bromo dodecane	57.10
5	27.041	125752	11.62	47029	8.89	Sulfurous acid, 2-propyl undecyl ester	57.10
6	28.431	147650	13.65	52449	9.92	Decane, 2,3,5,8-tetramethyl-	57.05
7	29.847	100308	9.27	32291	6.10	Tridecane, 4-methyl-	57.10
		1082019	100.00	528959	100.00		

Chromatogram of Volatile Compounds Emitted by 500 Beetles After 72 Hours of Aggregation

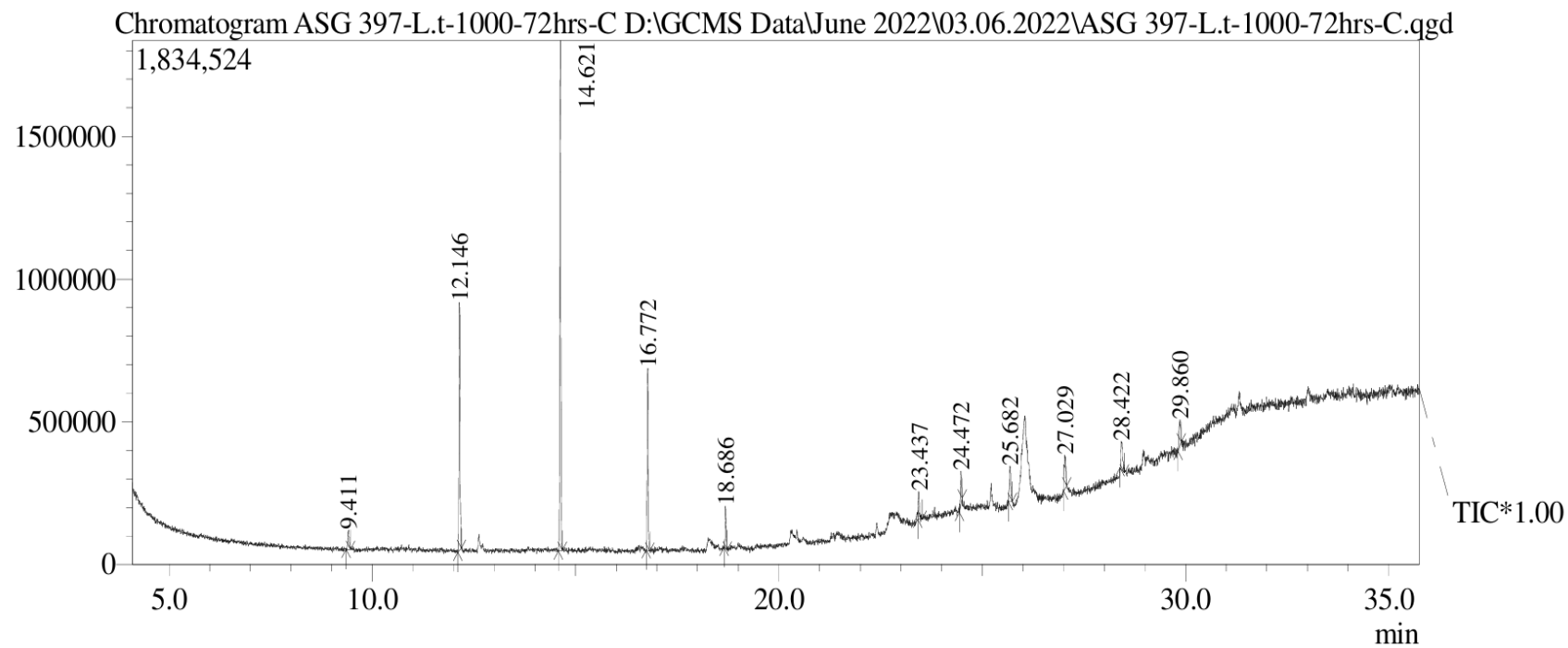


Peak Report of Volatile Compounds Emitted by 500 Beetles After 72 Hours of Aggregation

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.410	656960	3.84	274608	3.37	Cyclohexasiloxane, dodecamethyl-	72.95
2	12.146	2834015	16.58	1578237	19.36	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	12.607	1739881	10.18	649969	7.97	Phenol, 2,4-bis(1,1-dimethylethyl)-	191.00
4	14.624	3210884	18.78	1854822	22.75	Cyclooctasiloxane, hexadecamethyl-	73.00
5	16.449	455747	2.67	182505	2.24	E-15-Heptadecenal	55.05
6	16.772	933857	5.46	540254	6.63	Cyclohexasiloxane, dodecamethyl-	73.00
7	18.969	976908	5.71	437432	5.37	1-Heneicosanol	57.05
8	21.268	1321105	7.73	627943	7.70	Trifluoroacetic acid, pentadecyl ester	55.00
9	21.320	609823	3.57	255197	3.13	Eicosane	57.00
10	21.370	352096	2.06	137453	1.69	Melochinin	152.95
11	22.033	135416	0.79	78479	0.96	Heptasiloxane, hexadecamethyl-	73.00
12	23.384	1132800	6.63	539656	6.62	n-Nonadecanol-1	55.00
13	24.475	417949	2.44	204956	2.51	Tritetracontane	57.05
14	25.627	532191	3.11	206721	2.54	n-Heptadecanol-1	55.05
15	25.680	498556	2.92	223882	2.75	Heneicosane	57.05
16	27.031	731610	4.28	186712	2.29	Heptadecane, 9-octyl-	57.05
17	28.421	555467	3.25	172870	2.12	2-methyloctacosane	57.05
		17095265	100.00	8151696	100.00		

Chromatogram of Control Sample Collected Over a 72-Hour Period



Peak Report of Control Sample Collected Over a 72-Hour Period

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.411	152283	1.99	66656	1.60	Cyclohexasiloxane, dodecamethyl-	73.00
2	12.146	1539919	20.14	868688	20.91	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.00
3	14.621	3025472	39.58	1785993	42.98	Cyclooctasiloxane, hexadecamethyl-	73.00
4	16.772	1091438	14.28	638000	15.36	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	73.00
5	18.686	245717	3.21	147338	3.55	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	73.00
6	23.437	213329	2.79	97200	2.34	Nonadecane, 2-methyl-	57.00
7	24.472	248509	3.25	121413	2.92	Octadecane	57.00
8	25.682	312798	4.09	123240	2.97	Eicosane, 10-methyl-	57.05
9	27.029	300413	3.93	119201	2.87	Tetratetracontane	57.00
10	28.422	284440	3.72	97060	2.34	Eicosane, 7-hexyl-	57.05
11	29.860	230392	3.01	90140	2.17	Tridecanol, 2-ethyl-2-methyl-	57.05
		7644710	100.00	4154929	100.00		