Central University of Technology, Free State

THE USE OF ESSENTIAL OILS TO CONTROL PATHOGEN-CARRYING COCKROACHES FOUND IN MANGAUNG FOOD MARKETS

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DECLARATION OF INDEPENDENT WORK

I, Gosego Keotshepile Mapula Sedikelo, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MASTER OF HEALTH SCIENCE: ENVIRONMENTAL HEALTH is my own work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

Signature of student | Date

14 September 2021

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Life is The River

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SUMMARY/INTRODUCTION

Integrated pest management is a broad approach to pest control that includes biological control, habitat manipulation, and changes in cultural practices. It focuses on long-term pest prevention or elimination through a combination of strategies such as biological control, habitat manipulation, and cultural change (Kloosterman and Mager, 2014). It also emphasizes the importance of food safety research and outreach programs from producer to consumer on how to reduce or remove pests coming in contact with food. (2019, Dara). Integrated pest management (IPM) is widely regarded as the future approach for pest control that incorporates many elements for pest management (Stenberg, 2017). With the recent developments in food industries, changing consumer trends and awareness of food safety, there is a growing need to revisit the integrated pest management model to fit modern times (Dara, 2019). These elements include prevention of pests before problems are encountered, accurate identification of pests for proper and effective treatment, regular monitoring of possible pest infestation, and lastly, acting by choosing the best method to eliminate pests, which can be biological, chemical or a combination of the two (Stenberg, 2017).

Food market owners must consider three essential factors to control pests in food industries: pests must not be capable of penetrating the buildings, pests should be unable to access food, and pests must not be capable of hiding or nesting within the building (Kloosterman and Mager, 2014). Ideally, pests must not have any opportunity to enter the buildings. However, they always find their way into households via different pathways because of poor hygiene and improper disposal of food waste (Kloosterman and Mager, 2014). The presence of pests suggests trouble for food industries and business owners because of the spoilage of foodstuffs caused by microbiological contaminants and pathogens they could carry (Kloosterman and Mager, 2014). As a result, food processing plants have seen production losses and increased production costs due to contamination of foodstuffs, which has led to the increase in retail prices resulting in a negative financial impact on consumers (Trebicki and Finlay, 2019).

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Consumers globally trust that the food they purchase and prepare daily is safely produced, properly packaged and distributed to various food markets (Kloosterman and Mager, 2014). From the point of manufacture to the point of consumption, the package must be designed to protect the product throughout the value chain (Mullen and Pedersen, 2000). Despite the efforts, foodborne outbreaks continue to increase, despite existing food safety procedures, highlighting the gaps in ensuring food safety (Nyarugwe *et al.*, 2016). As a result, the food safety culture has grown in popularity. Food safety culture is defined by the Global Food Safety Initiative (GFSI) as shared values, beliefs, and conventions that influence an organization's thinking and behavior toward food safety, including pest management programs in the food industry that must be implemented to prevent pest infestation. However, each food company has its own food safety culture, and there are few published papers on this evaluation.

Food must be produced and stored under strict good manufacturing practices (GMPs) (Heeps, 2016). GMPs are the fundamental operating procedures and environmental conditions that must be met in order to produce safe food. (De Oliveira *et al., 2016*). They're put in place to ensure that ingredients, products, and packaging materials are effective, and that food is prepared in a safe environment. (De Oliveira *et al.,* 2016). Pests penetrate through minor cracks and faults in the package seal because they are opportunistic (Mullen and Pedersen, 2000). As a result, packaging must be constructed to keep pests out by using folds and adhesive seals that are impenetrable to bugs (Mullen and Pedersen, 2000). Seals with a high level of structural integrity also operate as a barrier to escaping food odours, reducing pest attraction (Morrison et al., 2021). Packaging provides control and protection for food products against external and internal contaminants such as germs, gases, and odours, which attract vectors such as cockroaches (Youssef and EL-Sayed, 2018). Shrink wrapping enhances the potential to create such an environment (Bell, 2014). This strategy is similar to the notion of hermetic storage, which is used to keep moisture and insects out of crops during storage (Navarro and Navarro, 2018).

By investing in good manufacturing practices, food processing plants reap benefits such as cost reduction and quality improvement (Gouda and Saranga, 2020). This will

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not only ensure that those food products manufactured are not adulterated with foreign materials but can also prevent pest problems (Heeps, 2016). Pest control is vital in food safety because vectors such as cockroaches pose a microbiological hazard to consumers (Pretty and Bharucha, 2015), leading to infection in humans by pathogens that cause some fatal diseases such as listeriosis (Pretty and Bharucha, 2015). "Listeriosis is a rare foodborne illness caused by *Listeria monocytogenes"* (Madjunkov *et al.,* 2017). It is spread through consumption of contaminated ready-to-eat foods, items with a longer shelf life, meat products, and soft cheeses *(*Madjunkov *et al., 2017).* Some pathogens carried and transmitted by cockroaches have developed resistance to certain antibiotics, making it difficult to treat them (Pretty and Bharucha 2015). Infections caused by bacteria resistant to antibiotics can result in the failure of standard treatments, the need for prolonged treatments, and even death in humans. (Adesoji *et al.,* 2016). Therefore, it is essential to have effective pest control in food processing plants to minimize and control pest infestation, automatically decreasing the chances of food contamination (Adesoji *et al.,* 2016).

Control materials should be labelled and applied in a way that poses the least amount of danger to human health, non-target creatures, and the environment (Pretty and Bharucha, 2015). Dermatological, gastrointestinal, neurological, carcinogenic, pulmonary, reproductive, and endocrine health effects have all been linked to chemical pesticides environment (Pretty and Bharucha, 2015). Pesticide poisoning, whether unintentional or intentional, can result in hospitalization and death environment (Pretty and Bharucha, 2015). Pesticide use and execution of legislation governing it are often inadequate in developing countries such as South Africa (Mngadi, 2016), resulting in a demand for alternatives from plant sources such as essential oils (Ros, 2016). Essential oils are aromatic, volatile liquids collected from plant material by steam distillation and named after the plant from which they were taken. (Ríos, 2016) & (Chaudhari *et al*., 2021). Essential oils are defined as blends of scented substances or fragrant and odourless substances (Ríos, 2016). They are chemically pure compounds that are volatile under standard environments.

Certain essential oils have long been alleged to repel insects in households and food processing facilities (Isman, 2000; Chaudhari *et al*., 2021). For example, Java citronella (*Cymbopogon winterianus*) essential oil. Citronella oil is made by steam distillation of citronella grass. It is commonly used in the household setting as a repellent against mosquitoes. Furthermore, *Thymus vulgaris* has been tested against mosquitoes as a possible repellent (Singh and Sheikh, 2021).

Pesticides originating from organic sources are called "green" pesticides because they are less harmful to animal health and human, as well as ecosystem habitats and the (Nollet and Rathore, 2017). In this study, essential oils Java citronella and red thyme were used and demonstrate to share features of potential cockroach repellent properties that may be used in pest control. This research further discusses the applications and limitations, including the recent advances in nanotechnology, which can be used to increase the protection time of these oils when applied in an open environment and generally present the status of plant essential oil-based pesticides in integrated pest management programmes.

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CHAPTER ONE

Literature review

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1.1 Cockroaches: behavioural ecology and pathogen transmission

Cockroaches are among the most ancient and primordial insects, going back to the Permian age (approximately 275 million years ago) and having termite heritage (Tang et al., 2019). Only a few species have advanced in close to human habitations; the remainder are wild (Tang et al., 2019). Due of their excellent adaptability, prolific reproduction, and widespread pesticide tolerance, they are becoming increasingly difficult to control (Smidova and Lei, 2017). Cockroaches can harbor a wide range of microorganisms, including bacteria, viruses, fungus, and parasites, which have evolved a symbiotic connection with their hosts over time (Zhang and Zhang, 2018). The Blatella germanica (German cockroach), Periplanetta americana (American cockroach), and Blatta orientalis (Oriental cockroach), for example, are considered significant food pests (Marriot *et al.,* 2018). They are one of the potential vectors for spreading disease and health problems in humans by contaminating foods or food storage. Because of their habitats and life in dirty places, they can carry human pathogens in their exoskeleton and body. They carry and transmit bacteria of some fatal diseases, including plague, leprosy, typhoid fever, listeriosis, dysentery, campylobacteriosis, and salmonellosis (Oyeyemi, 2016).

German cockroaches are a group of the most common pests found in urban areas globally and the number one cockroach pest species worldwide (Tang *et al*., 2019). They adapt well to human environments, preferring similar humidity and temperature levels as humans (Tang *et al*., 2019). Moreover, due to urbanization and industrialization, food establishments/warehouses are being developed in areas where these pests thrive, and individuals bring along all necessities they require for survival, such as harbouring places, drainage systems, stagnant water, food, waste and warm places (Downs *et al*., 2016). In addition, anthropogenic factors such as delayed repair of broken drainage systems, improper disposal of waste and leaving uncovered food around for long periods create an environment suitable for cockroaches to reproduce and multiply (Downs *et al*., 2016; Shabbir *et al*., 2020).

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Very few insects are detested like cockroaches, and they are known for their ferocious desire to reproduce (Hamilton *et al.*, 2019). Cockroaches, like the German cockroach, have peripheral vision and use their antennae to detect food and water and also threatening odours (Hamilton *et al.*, 2019). When cockroaches congregate, they emit a pheromone that alerts other roaches that they've discovered a suitable nesting site. As part of their normal routine, they might also expel fluids. Female cockroaches may attract hordes of male roaches with only a whiff of their scent. Pheromones are chemical molecules produced by insects that act as sexual attractants. These chemicals are used to attract insects as well as predict population increase (2017, Stökl and Steiger)

Their tail plays a considerable role in their defence mechanism, and the sensitive hairs on their body signal to them when a human is about to make a move, and they can take off at speeds of 0.3 m/s (Hamilton *et al.*, 2019). Cockroaches are tropical insects that dwell in close proximity to people. In temperate zones, most species live in areas of buildings with appropriate warmth, moisture, and food (Nelapa, 2018). Cockroaches live in clusters and are most active at night, hiding in cracks and crevices in walls, doorframes, and furniture, as well as in bathrooms, cupboards, steam tunnels, animal houses, basements, televisions, radios, and other electrical equipment, drains, and sewer systems (Nelapa, 2018). Cockroaches are particularly sensitive to light due to their nocturnal nature, and will typically retreat for cover when a light is switched on (Nelapa, 2018).

Multiple variables support insect infestations in food markets, allowing pests access and growth (Smit et al., 2011). These elements include the building's physical construction and the immediate environment surrounding the building, both of which can be pest infestation initiators (Smit et al., 2011). In developing countries, low socioeconomic urban areas are characterized by a lack of environmental cleanliness. Poor surface and home wastewater drainage, non-selective garbage disposal, and inadequate refuse collection are examples of these issues, which serve as breeding grounds, easy access to food, and shelter for pests (Mngadi, 2016). Accessible waste, which supplies food for pests, is another environmental factor that favors insect infestations (Mngadi, 2016).

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Climate also plays a role because studies have shown a rapid increase of pests in tropical climates where environments are ideal for rapid population increase, contrary to cooler climates which do not support the development and several physiological processes (Bell, 2014; Ma *et al.*, 2020). Stagnant water inside or around buildings can serve as breeding grounds for cockroaches and is particularly hazardous because microbes can multiply rapidly under these conditions (Shabbir *et al.*, 2020).

Additionally, excess or uncontained water, such as leaking pipes or drains, steam or water vapour, condensation and rainwater, can also serve as breeding grounds for these pests (Shabbir *et al.*, 2020). It is also vital to note that floors, ceilings and walls need to be designed not to give insects and other invertebrates the chance to infest households (Wang *et al.*, 2008; Lelieveld and Holah, 2014). Worldwide the infestation trend of cockroaches has increased due to urbanization, where humans are bringing the three elements needed for their survival: food, water and shelter (Shahraki *et al.*, 2010). Cockroaches are also ready to reproduce two months after birth (Shahraki *et al.*, 2010). Studies show a substantial connection between infestation, poor sanitation and mismanagement of waste (Shahraki *et al.*, 2010). Integrated pest management (IPM) prevents and reduces the infestation of cockroaches and can result in long-term reductions in cockroaches, ensuring in a healthier environment for humans (Portnoy *et al.*, 2013). Food is a key factor in transmitting infectious pathogens from cockroaches to humans (Donkor, 2020). Because of their unclean behavior and presence in areas where food is stored or handled, cockroaches appear to be appropriate mechanical transmitters for a broad range of foodborne pathogenic bacteria (Donkor, 2020). Cockroaches transmit medically important bacteria that cause infectious illnesses such as dysentery (Morenikeji et al., 2016).

1.2 Antibiotic resistance

Antimicrobial agents were discovered to treat infections, which revolutionized modern medicine and increased the therapeutic standard (Munita and Arias, 2016). Antibiotic therapy is a challenge in many nations due to rising resistance to routinely used antibiotics (Munita and Arias, 2016).

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Antibiotics have become one of the most important medical interventions in the development of advanced medical techniques, such as the treatment of lethal bacterial infections like Listeria monocytogenes, which causes listeriosis. 2016 (Munita and Arias). Antibiotic resistance among common bacterial pathogens, on the other hand, is now endangering this therapeutic execution, as well as the effective outcomes of critically ill patients (Munita and Arias, 2016). The World Health Organization (WHO) has listed antibiotic resistance as one of the top public health challenges of the twenty-first century (Munita and Arias, 2016). Antibiotic-resistant germs that are difficult to treat are on the rise, posing a global health threat (Blair et al., 2015). Antibiotic resistance is encoded by a number of genes that can be passed between bacteria (Blair et al., 2015).

Different resistance mechanisms are frequently described, and new genes and transmission vectors are regularly identified (Blair *et al*., 2015). Multidrug-resistant microorganisms have emerged in the hospital setting and have been identified in community settings, suggestive of that reservoirs of antibiotic-resistant bacteria are existing outside the hospital (Munita and Arias, 2016). Antibiotic resistance occurs when bacteria develop resistance against one or more antibiotics (Adesoji *et al.,* 2016). Antibiotic resistance can be through random or spontaneous mutation; this results in the accumulation of non-lethal mutated genes (Shibai *et al.,* 2017). The genes can be plasmid or chromosomal genes, and this is where the resistant gene develops. The resistant gene is then transferred to the next generation through vertical or horizontal gene transfer (Sultan *et al.,* 2018). In vertical gene transfer, the bacteria replicates and is divided into two, and in horizontal gene transfer the bacteria has three types, transduction, conjugation and transformation (Sultan *et al.,* 2018). These processes involve all genetic events, including plasmids, bacterial phases, and transposons (Sultan *et al.,* 2018).

The first mechanism in antibiotic resistance is drug inactivation or drug modification, and this is where the drug is inactivated or modified into a new structure so that it is unable to work in the way it was designed to work (Eichenberger and Thaden, 2019). The bacterial resistant genes make antibiotic counteracting substances which can be a protein made from resistant genes (Sultan *et al.,* 2018). An example of this is B-lactamases produced by the bacterial strain that inactivates the penicillin gene

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(Eichenberger and Thaden, 2019). The second mechanism is the alteration of the target or binding side (Sultan *et al.,* 2018). In this mechanism, the target side of the bacteria is altered so that the antibiotic remains ineffective to the bacteria (Sultan *et al.,* 2018). This type of mechanism has been seen in methicillin-resistant *Staphylococcus aureus*, where a penicillin-binding is altered, and penicillin is unable to bind to it, resulting in antibiotic resistance (Wijesundara *et al.*, 2019). The third mechanism is the use of active efflux pumps (Pacheco *et al.*, 2017).

In this mechanism, the toxins, foreign material and drugs from bacterial cells are eliminated from the bacterial cell (Pacheco *et al.,* 2017). The genes that encode efflux pumps are naturally selected, and they result in overexpression of efflux pump proteins, whereby the bacteria pump out the antibiotics from the cellular interior to the external environment and later result in antibiotic resistance (Pacheco *et al.,* 2017).

Bacteria are often not susceptible or resistant to the same antibiotics (Eichenberger and Thaden, 2019). Within related bacterial families, resistance levels might differ dramatically (Eichenberger and Thaden, 2019). The minimum inhibitory concentration (MIC), which is the lowest drug concentration that will stop bacteria from multiplying, is used to determine susceptibility and resistance. (De Francesco et al., 2019). Susceptibility is a range of average MICs for a given antibiotic among bacteria of the same species (De Francesco *et al.*, 2019). If a species' average MIC falls inside the resistant range, the species is said to have intrinsic resistance to that drug (Raygaert, 2018). The fundamental example of bacterial adaptability is the bacterial reaction to antibiotics (Munita and Arias, 2016). Bacteria have a high level of genetic flexibility, allowing them to respond to a wide range of environmental stressors, including the presence of antibiotic compounds, which could risk their survival. The early use of amoxicillin in animal feed to enhance development and improve feeding in the early 1950s is an example of this (Lees et al., 2021). Microorganisms found in animal feed are thought to contain a significant amount of nutrients, as well as hinder absorption from the intestine and release toxins that harm the animal (Madhab, 2014; Lees et al., 2021). Antibiotics decrease these dangerous germs by boosting their growth (Lees et al., 2021).

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However, this, on the other hand, promotes the emergence of antibiotic-resistant strains (Lees *et al*., 2021). Resistant bacteria have been reported in effluents, processing water and products from a number of parts of the world (Adesoji *et al.,* 2016). Pests are diverse and can thrive in many environments, and some antibiotics are poorly absorbed in the digestive tract of animals and are therefore released to the environment as faeces.

With the continuing urban expansion into agriculturally zoned outplaces and urban/industrial areas, there is a public health concern associated with them carrying pathogens from such environments in their gut and exoskeleton that are resistant to some antibiotics. In addition, due to the travelling nature of pests and their attraction to areas where there is food and water, cross-contamination can occur, resulting in the introduction of antibiotic-resistant bacteria to food during production (Adesoji *et al.,* 2016).

1.3 Pesticides

Research has shown that pests/insects of medical importance such as cockroaches, have the ability to carry and transmit antibiotic resistance bacteria in food production facilities and in households, presenting the need to have scientific measures in place to control them (Pedigo *et al*., 2021). The South African Pest Control Association was founded in 1964 to represent the pest control industry in South Africa, and it is governed by the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act of 1947. (Act No. 36 of 1947). Pesticides have become an integral part of modern life in agriculture, homes, health care, and food markets because they are regularly used to control insect infestations of numerous pests and disease carriers, such as mosquitoes, ticks, rats, and mice (Kim ,2017) Toxic insecticides, mousetraps, and electric flycatchers (EFCs) are examples of pest management resources and materials (Kumar et al., 2018). Organophosphate insecticides are the most widely used pesticides in the world, with a wide range of applications (Kumar et al. 2018). These compounds may turn out to be bioavailable for microbial degradation (Kumar *et al*., 2018).

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Concerns have been raised concerning environmental dangers associated with pesticide exposure through multiple channels such as residues in food and drinking water due to non-species-specific modes of action for administering these pesticides (Kim, 2017). While the risks of such hazards can range from short-term effects such as skin and eye irritation, dizziness, headaches, and nausea to long-term effects such as cancer, asthma, and diabetes, they are difficult to predict due to the interplay of various factors such as period and level of exposure, pesticide type (toxicity and persistence), and the environmental characteristics of the affected areas (Kim, 2017).

Inappropriate pesticide use is a concern since pesticide exposures, particularly those containing chlorpyrifos (CPS), an organophosphate sold under many brand names, can put people, especially children, at risk of adverse health effects (Mngadi, 2016). Synthetic pesticides consist of residual sprays, insecticides, aerosols baits and traps and repellents. Residual sprays are colourless and odourless sprays applied within households and food establishments using plunger-type sprayers or hand compression air sprayers (Dengela *et al.*, 2018). Pin stream nozzles on the sprayers allow the pesticide to be sprayed into cracks and other hard-to-reach regions (Dengela et al., 2018). Insecticide resistance has been a persistent impediment to cockroach management since the 1950s, despite the fact that pesticides are necessary for reducing cockroach populations and improving health outcomes (Gao et al., 2020). Due to resistance development and environmental concerns, chlorinated hydrocarbon insecticides have been substituted with biodegradable organophosphorus and carbamate insecticides, synthetic pyrethroids, and insect growth regulators. (Gao et al., 2020).

Insect growth regulators are very poisonous substances that prevent insect larvae and pupae from developing into adults (Petrescu and Ilia, 2017). Non-target creatures are not harmed by them. Due to their high cost and scarcity, their use is restricted. 2017 (Petrescu and Ilia). Aerosols, like pesticides, frequently contain pyrethrins, pyrethroids, or other irritants to drive cockroaches out of their hiding places and reduce the time it takes to kill them (Scheff et al., 2019). Although the aerosol treatment can quickly reduce cockroach numbers, a residual spray treatment may be necessary for longer-term control (Scheff et al., 2019).

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Baits are commonly used in cockroach control and are still utilized in some contexts, such as offices and laboratories, especially if some of the insecticides used are resistant (Pol et al., 2017). Many commercially available products work by luring cockroaches to a specific place, where they can be captured or destroyed, and the trapping element could be a mechanical trap or a sticky material. (Pol et al., 2017)

The use of repellents to control cockroaches is gaining popularity (Mengoni and Alzogaray, 2018). They could be very useful for hiding areas in shipping containers, cases, and boxes storing drinks, food, and other products, especially in the food industry (Mengoni and Alzogaray, 2018). Keeping cockroaches out of these spaces prevents insects from spreading from one spot to the next (Aharonov et al., 2018). Repellents can also be used in vending machines that sell food and beverages and in kitchen cupboards (Aharonov et al., 2018).

Despite efforts to employ synthetic pesticides to reduce pest infestations, their negative effects on humans and increasing cockroach resistance to widely used pesticides have created concerns. (Nerio *et al.*, 2010). Concerns regarding the environment and human health have been raised by the use of synthetic pesticides to control insects and arthropods. (Nerio *et al.*, 2010). An alternative is the use natural products that are both effective and environmentally friendly (Nerio *et al.*, 2010).

1.4 Essential oils

Essential oils (EOs) are complex compositions of volatile organic molecules produced by plants as secondary metabolites (Cappellari et al., 2020). Hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds such as alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers make up these substances (Cappellari et al., 2020). At ordinary room temperature, they are organic compounds with a high vapour pressure that quickly turn into vapours or gases (Sharififard et al., 2016). Essential oils from various plants have been intensively examined as a possible natural resource to evaluate its repelling qualities (Sharififard et al., 2016).

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Essential oil repellence has been proven, and the significance of the synergistic effects among their components means they can also work well when mixed (Sharififard *et al.*, 2016).

Monoterpenes, a type of terpene that consists of two isoprene units and has the chemical formula C10H16, have been connected to the repellent activity. (Nerio *et al.*, 2010) & (Cappellari *et al.*, 2020). Terpenes are a broad and diversified group of organic chemicals made by a wide range of plants, especially conifers (cone-bearing plants with vascular tissue) and some insects. (Nerio *et al.*, 2010) & (Cappellari *et al.*, 2020). They often have a strong odour and may protect the plants that generate them by repelling herbivores and attracting herbivore predators and parasites. (Booth *et al.,* 2016). Furthermore, adding other natural items to the mix, such as vanillin, may extend the duration of protection, amplifying the repellent action of some essential oils. (Nerio *et al.,* 2010).

Vanillin is an organic chemical with the molecular formula C8H8O3 (Wang et al., 2016). It's a phenolic aldehyde that is used for tanning, preserving, and as a germicide, fungicide, and pesticide for plants and vegetables, but its main purpose is in the creation of polymeric materials (Surburg and Panten, 2016). Despite the fact that synthetic chemicals are still more commonly employed as repellents due to cost, essential oils have the potential to produce effective and safer repellents for humans and the environment (Nerio et al., 2010) & (Cappellari et al., 2020).

1.5 Rationale

Cockroaches are one of the most common pests in both commercial and residential settings (Gwenzi et al., 2021). They've demonstrated great ability of adjusting to human life. Cockroaches are not only tenacious pests, but they can also spread human disease infections, posing a public health risk (Gwenzi et al., 2021).

Due to their close association with humans and their environments, they are considered potential carriers and transmitters of pathogens resulting in some foodborne diseases (Naher et al., 2018). In developing countries, including South Africa, information is limited regarding cockroaches as vectors/carriers of pathogenic

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bacteria in food markets and currently, there is a battle to control the spread of foodborne pathogens and the possible rise in foodborne outbreaks (Cholewiński *et al.*, 2017). This is a major concern for local and global food markets, invigorating the need for new and safe methods to minimize the development and spread of foodborne pathogens (Cholewiński *et al.*, 2017). Pathogens transmitted by pests are considered a serious public health problem regardless of developments in science, presenting a threat to humans and the environment we live in (Cholewiński et al., 2017). And cockroaches have built resistance against synthetic pesticides used, making it difficult to control their spread. As a result, the current study will focus on using natural products such as essential oils to control pathogen-carrying cockroaches found in food markets, which is a safer method of controlling pest infestation as it poses minimal threat to humans and non-target organisms compared to synthetic pesticides.

Cockroaches, specifically *Blatella germanica* and *Periplanetta americana,* have been found to carry disease-causing organisms, typically those causing gastroenteritis that can be resistant to commonly used antibiotics. They carry pathogens on their legs, bodies and in their gut and deposit on food through mechanical and biological transmission as they forage (Sarwar, 2015). These pathogens include *Salmonella* spp. *Salmonella typhi* causing typhoid, *Entamoeba histolytica* causing amoebiasis, *Shigella dysenteriae* causing dysentery, *Listeria monocytogenes* causing listeriosis and potentially also the *Poliomyelitis virus* responsible for polio (Sarwar, 2015).

Often leaking pipes and random non-designated areas used as dumping sites create breeding grounds for pests like cockroaches. Unlike the American cockroach and the Oriental cockroach, which thrive mostly in sewage and drainage systems, the German cockroach was selected for this study as it is the most found cockroach living amongst humans and in food establishments. German Cockroaches have a preference to live in warm, humid environments near food and moisture sources. They are most commonly found in home and commercial kitchen facilities, as well as food processing factories; these are the most likely locations for an infestation.

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1.6 Study aims

1. Profiling of microorganisms carried by cockroaches isolated from a meat processing plant in Thaba Nchu and identifying antibiotic-resistant bacteria isolated from cockroaches.

2. Testing the efficacy of essential oils, namely Java citronella (*Cymbopogon winterianus*) and red thyme (*Thymus zygis*), against the German cockroach (*Blattela germanica*) using contact toxicity, fumigant toxicity and repellence.

1.7 Study objectives

1. Isolate and characterize microbes associated with cockroaches using standard microbiological techniques such as a RapID Kit.

2. Assess antibiotic-resistant profiles of bacteria carried by cockroaches using the Kirby-Bauer disc diffusion method.

3. Evaluate the chemical composition of Java citronella (*Cymbopogon winterianus*) and red thyme (*Thymus zygis*).

4. Evaluate the contact toxicity, fumigant toxicity and repellence of natural plant essential oils against the German cockroach.

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CHAPTER TWO

PROFILING OF MICROORGANISMS ISOLATED FROM COCKROACHES

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2.1 Abstract

Background: Integrated pest management emphases on long-term prevention of pests or their control through combined strategies such as habitat manipulation, biological control and change of cultural practices. However, pests always find their way into households and food processing plants via various pathways, and their presence suggests challenges because of possible spoilage of foodstuffs caused by microbiological contaminants and pathogens they will carry. Therefore, this chapter focuses on profiling microorganisms carried by cockroaches identified from a meat processing plant in Thaba Nchu and identifying antibiotic-resistant bacteria isolated from cockroaches.

Materials and methods: Effluent sample of was collected using sterile 50ml plastic test tubes with lids and cockroaches were collected using designed and custom-made cockroach traps, and they were placed at different areas within the facility between June and July. The trapped cockroaches were then blended, and microorganisms quantified using serial dilution on Plate count agar (PCA) and Nutrient agar (NA) identified using a RapID Identification kit. Afterwards, the Kirby Bauer disc diffusion assay was used for antibiotic sensitivity tests using Mueller-Hinton agar and selected antibiotics, namely ampicillin (10 mcg), ciprofloxacin (5 mcg), chloramphenicol (30 mcg), gentamicin (10 mcg), streptomycin (10 mcg), penicillin and tetracycline (30 mcg).

Results: The cockroaches collected were identified as German cockroaches. Results showed a high bacterial count from the cockroaches and the effluent from the meat plant. Antibiotic resistance was measured as <14 mm resistant, 15-17 mm intermediate and >18 mm susceptible. Two common bacteria were isolated from the meat processing plant's effluent and the cockroaches, namely *Yersinia enterocolitica* and *Yersinia kristensennii. Y enterocolitica* showed resistance against ampicillin, penicillin and streptomycin.

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Conclusion: This study confirmed the presence of bacteria on German cockroaches and further showed that the isolated microorganisms are resistant to commonly used antibiotics such as ampicillin, streptomycin and penicillin. The identified bacteria may possibly pose a public health problem in food processing facilities.

Keywords: antibiotic resistance, foodborne pathogens, cockroaches

2.2 Introduction

Cockroaches have adapted to live in a variety of environments and are consequently found all over the world (Donkor, 2019). Only thirty (30) cockroach species are connected with human habitations out of four thousand (4000), including Blattella germanica, the German cockroach (Donkor, 2019). The German cockroach is a widespread indoor pest that poses a major health risk to humans. It is also a substantial public health hazard. It grows in damp, dark, and dirty interior conditions, where it is exposed to a variety of microbes and can easily contaminate food (Pan and Zhang, 2020). The cockroach is an ideal biological vector because it can acquire and mechanically transmit a wide range of human infections, the majority of which are bacteria (Pan and Zhang, 2020). *Periplanetta americana*, the American cockroach, has one of the largest genomes of any studied insect, making it a resilient survivor (Pan and Zhang, 2020). Cockroaches can quickly breed to enormous numbers making them difficult to control (Sayyad *et al.*, 2016). Cockroaches have chemoreceptors, one hundred and fifty-four (154) olfactory receptors for smell, twice as many as other crawling insects and sixty (60) gustatory receptors for taste (Wada-Katsumata *et al.*, 2018).

They are known as omnivorous because they feed on various materials, including each other, aiding in the mechanical transmission of various pathogenic microorganisms to humans (Zahraei-Ramazani *et al.*, 2018). This diverse diet makes it easier for them to find food in any environment, even if it is poisonous to other insects (Hu *et al.*, 2020). Because cockroaches have a cytochrome P450 which codes for detoxifying enzymes that makes it possible for cockroaches to withstand toxic plants and chemicals, these enzymes keep them safe (Hu *et al.*, 2020).

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They are a major nuisance found in flats, households, food-handling establishments, hospitals, and healthcare facilities all over the world (Moges et al., 2016). The cockroach can survive without its head for up to a week. This is due to the fact that it does not have a high-pressure blood network that may cause it to bleed out; instead, because to its tremendous regenerating abilities, it seals off its neck. (Jayaram et., 2018). The German cockroach, which is an indoor species, takes advantage of dense human populations and poor living circumstances (Moges et al., 2016). Their nocturnal and filthy habits make them ideal carriers of different pathogenic bacteria that they transmit to food, dining utensils, kitchen surfaces, and other areas around the house. (Moges *et al.*, 2016).

Cockroaches' function in human infections has been a source of contention (Donkor, 2019). Recent study has made a significant contribution to our understanding of this topic (Donkor, 2019). Few review studies, on the other hand, consolidate the research data on a single platform to provide a global perspective of cockroaches' function in disease transmission (Donkor, 2019). Antibiotic-resistant microorganisms have been found to be transmitted by cockroaches. Antibiotic resistance is a condition in which bacteria are unaffected by antibiotics and continue to thrive (Nhabe, 2017). Efflux, cell membrane alteration, mutations where antibiotics bind, and enzymatic inactivation are all mechanisms of resistance (Spengler et al., 2017).

Antibiotics normally enter cells and induce cell death or growth suppression, however in the efflux process, bacteria produce a membrane protein that removes the antibiotic from the cell, allowing the bacterial cell to live (Spengler et al., 2017). In the modification of the bacterial cell membrane, the bacteria modify the membrane in such a way so that antibiotics cannot enter the cell (Schaenzer and Wright, 2020). Mutation, where antibiotics bind, is seen mainly with antibiotics that inhibit protein synthesis (Foster, 2017). The antibiotics usually bind at the 50s and 30s cell unit of the ribosome. The 50s and 30s are two sub-units of the bacterial ribosome, and they represent the different sedimentation rates of the ribosomes during centrifugation (Foster, 2017). Antibiotics fail to attach to the ribosome if the antibiotic binding sites at certain cell units are mutated. The process of protein synthesis and cell development continues in this instance (Foster, 2017).

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Antibiotics are enzymatically inactivated in enzymatic inactivation, which implies that an existing cellular enzyme is changed to react with the antibiotic so that it no longer affects the pathogen (Foster, 2017). Bacteria are killed or their growth is impeded in the presence of antibiotics in normal circumstances, however the bacteria are unaffected when resistance genes are present (Nhabe, 2017).

As a result, treating bacterial infections caused by multidrug-resistant (MDR) bacterial strains has become a challenge, necessitating frequently expensive combination therapy (Setlhare, 2017). Infections produced by multidrug-resistant (MDR) organisms have a higher death rate than infections caused by sensitive bacteria, and they cost a lot of money (Giraldi et al., 2019). In this chapter, common species of cockroaches found in a meat processing facility were identified, microorganisms carried and transmitted by cockroaches isolated, and antibiotic-resistant bacteria were determined using the disc diffusion method.

2.3 Materials and methods

2.3.1 Cockroach traps

Custom-made traps (Figure 2.1), using two-litre plastic bottles, were used to trap the cockroaches. The bottle was cut in half, and the top part of the bottle was inserted back in the bottom part inside out with the lid of the bottle removed. It was designed to make it easier for cockroaches to enter but difficult to escape. These traps (Figure 2.1) were assembled because the traps and baits available in the market use synthetic chemicals to attract cockroaches, which was avoided to not interfere with the results.

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Figure 2.1: Custom-made trap design and assembly.

The bottles were washed thoroughly with soap and warm water, and then each bottle was disinfected with 96% ethanol (Le Dare and Gicquel, 2019). Peanut butter was smeared at the opening of the bottles as bait for the cockroaches. Because cockroaches rely on their sense of smell to find food, the strong smell of peanut butter served as an ideal bait (Ibrahim *et al.*, 2017). The two parts were then sealed together using a glue gun and glue sticks. The traps were then placed inside a cooler box that was also washed and disinfected with 96 % ethanol, and the traps were placed in a walk-in fridge overnight before being taken for sampling (Le Dare and Gicquel, 2019). The sixteen traps were collected after five days, this is for the reason that though cockroaches can survive a month without food, they can live one week without water, which is why they are usually found in humid or high-moisture areas around the household, such as basements and bathrooms (Pol *et al.*, 2018).

2.3.2 Sampling Facility

The samples were collected between June and July 2019 at a meat factory under Mangaung Metropolitan Municipality jurisdiction in Thaba Nchu. The traps (Figure 2.1) were placed in storage facilities for spices, processed meat products, cold rooms, and waste disposal areas inside the facility's premises. Figures 2.2 to 2.6 show that despite extensive efforts to minimize or prevent pests such as cockroaches from gaining access to the facility, they still find a way of accessing the area, mainly in search of food and water. The traps were placed at areas with easy access to water and food, including different areas at meat processing sites, storage areas, effluent

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discharge areas and the dumping site. These sites were picked based on the smell or odour they produce that can attract cockroaches and the availability of elements needed for cockroaches to thrive. Cockroaches connect through chemical pheromones they secrete as they travel, the aggregation of these pheromones increases the number of cockroaches in that particular area, which later results in an infestation (Holdcraft *et al*., 2016).

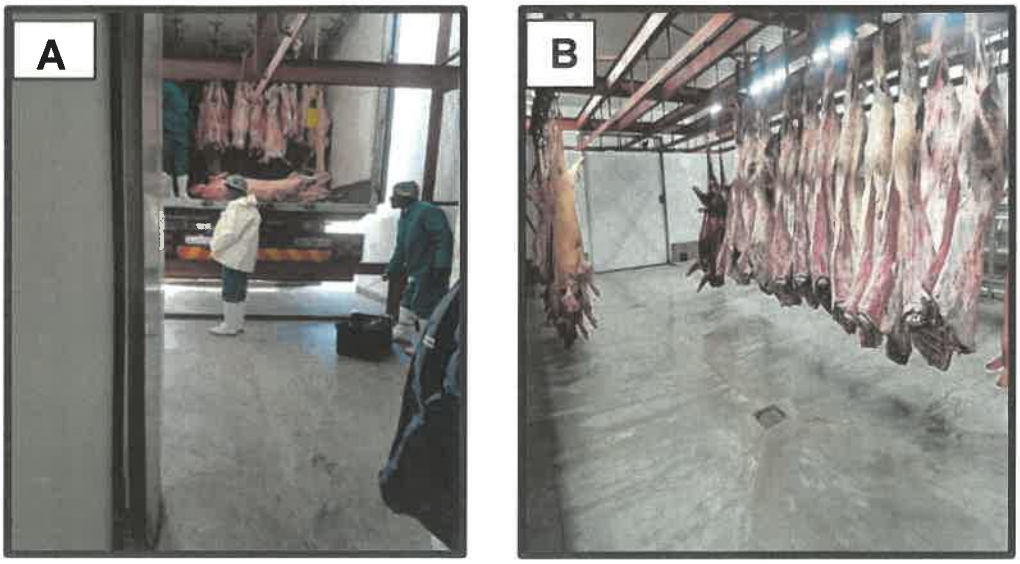

Figure 2.2: Showing (A) carcass delivery and (B) the storage of carcass in cold rooms inside the meat factory where some of the traps where placed.

Figure 2.3: Boot wash facilities. Every division of the meat factory has a boot wash basin where employees wash and disinfect their boots to avoid cross-contamination.

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Figure 2.4: Storage room for meat products. Following meat processing, all meat products are stored in the cold room.

Figure 2.5: Colour-coded uniforms issued to employees indicating their designated work sites. Employees are restricted to their work sites to prevent cross-contamination.

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Figure 2.6: Effluent discharge point at the production site where the fat is mechanically separated from the water to prevent it from blocking the main municipal sewage line running from the plant to the wastewater treatment plant.

Cockroaches are known to travel through drainage systems, and they can remain underwater for 10 minutes (Naqqash *et al.*, 2014). This means they can carry different pathogenic microorganisms on their exoskeleton from one place to another. Therefore, to investigate this possibility, effluent discharge from the meat processing plant (Figure 2.6) was collected for analysis and compared to bacteria isolated from the blended cockroaches. The sample was collected using sterile 50 ml test tubes and kept in a cooler box. All the samples were kept at a temperature of 4 ^{0}C to inhibit the development of endogenous microbes, and they were transported to Central University of Technology laboratory for analysis.

2.3.3 Microbial quantification

In preparation of microbial quantification, live cockroaches where blended to create a liquid substance for serial dilutions. Serial dilution was performed using 4.5 ml distilled sterilized water and 0.5 ml of the sample. It was then diluted from 10^{-1} and 10^{-5} into three different groups of test tubes.

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The serial dilution method was used to obtain single colonies from the plates. The objective of the serial dilution method was to attain plates with CFUs in the range of 30–300. A pipette was used to pour 0.1 ml of each tube on plate count agar (PCA), and nutrient agar (NA), respectively, that had already been sterilized. The plates were incubated at 37 °C for between 18 and 24 hrs. Colonies were counted with a colony counter, and colonies with dissimilar morphologies were observed on the plates and transferred. Afterwards, Staph plus and Strep test inoculation fluids were used for the identification of bacterial species.

2.3.4 RapID-ONE system identification

The RapID-ONE system is a qualitative micro-method for identifying important pathogenic enterobacteriaceae and other oxidase-negative, Gram-negative bacteria isolated using conventional and chromogenic substrates (Rafiullah *et al.*, 2018). The RapID-ONE system's tests are built on the detection of microbial degradation of certain substrates using different indicator systems. A combination of traditional testing and single-substrate chromogenic assays are used in the experiments (Rafiullah et al., 2018). Peptides that react with proteolytic enzymes in a coloured reaction are known as chromogenic substrates. They are synthesized and engineered to have a selectivity for the enzyme that is similar to that of a natural substrate (Rafiullah *et al.*, 2018).

The contents of the inoculation solution were transferred into the upper right-hand corner of the panel using a disposable sterile plastic pipette. The peel-back tab was then pressed back into place to secure it. The panel back was slanted at a 45° angle away from the reaction cavities after the test suspension was added. The panel was tilted forward toward the reaction cavities while remaining level and horizontal until the inoculum flowed along the baffles into the reaction cavities. The panels were then incubated at 37°C for 4 hours (Figure 2.7).

Two drops of RapID-ONE Reagent were applied to cavities 15 (PRO) through 17 after the RapID-ONE panel was peeled off (PYR). Scores were recorded from cavities 1 (URE) through 18 (ADON) from left to right using the interpretation guide presented.

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Following that, two drops of RapID Spot Indole Reagent was added to cavity 18 (ADON/IND). Results were recorded after 10 seconds. The reference microcode obtained on the report form in ERIC was used for the identification (Thermo Scientific SA).

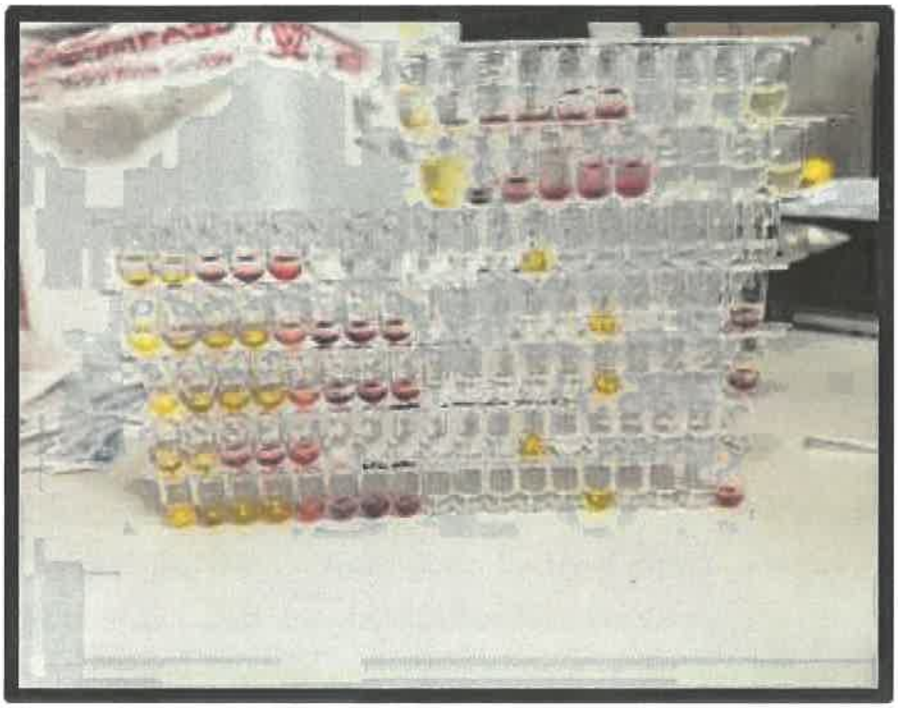

Figure 2.7: RapID-ONE system inoculation ports.

2.3.5 Antibiotic-resistance test

Antibiotic resistance was assessed using the Kirby-Bauer disc diffusion method, which is amongst the most flexible susceptibility testing methods (Mousa and Shama, 2020). This test is used to assess whether microorganisms are resistant or sensitive to various antibiotics, which can then be used to treat bacterial infections in patients. The presence or absence of an inhibitory zone around the disc indicates if the bacteria are drug-sensitive.

On the surface of the Muller Hinton agar medium, a turf of bacteria was created using the spread plate method. The spread plate method is a technique for plating bacteria-containing liquid samples.

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Figure 2.8: Disc diffusion assay - no growth zone of inhibition is observed around the ampicillin (AMP) disc.

For Kirby-Bauer disc diffusion testing, the bacteria are evenly spread out on the plate, and antibiotic discs (Figure 2.9) are placed on the plate. Areas of clear media around the discs put forward that the antibiotic limits bacterial growth after allowing the bacteria to grow overnight. With growing distance from the source, the concentration of antibiotic that disperses into the media drops. As a result, the greater the clear bacteria-free zone that grows around the disk containing that antibiotic (Figure 2.9), the more susceptible the bacteria are to that antibiotic (Mousa and Shama, 2020). The zone sizes (areas of no growth surrounding the disc) are measured in millimetres using a ruler after an overnight incubation.

The following antibiotic discs were used: ampicillin (10 mcg), gentamicin (10 mcg), ciprofloxacin (5 mcg), chloramphenicol (30 mcg), streptomycin (10 mcg), penicillin and tetracycline (30 mcg). Results were classified as susceptible, intermediate and resistant, while multidrug-resistant bacteria were carefully chosen based on their resistance to over three classes of antibiotics.

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2.4 Results and Discussion

The German cockroach, *Blattella germanica* (Figure 2.9), is a diverse synanthropic pest; it thrives near and benefits from association with humans and artificial habitats that people create around themselves (Wada-Katsumata *et al.*, 2018). Forty-four cockroaches were collected, which included twenty-seven nymphs and seventeen adult cockroaches. They were blended separately using a sterile blender disinfected with 70% alcohol because adult cockroaches can travel longer distances, possibly acquiring more microorganisms when compared to the nymphs, which are not fully developed, and their failure to travel further. The blended solutions were stored in separate sterile tubes. Figures 2.10 and 2.11 show pictures of cockroach types collected at the meat processing plant.

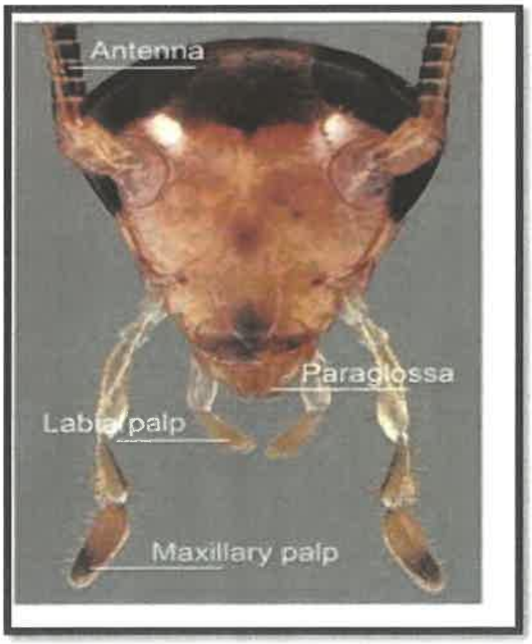

Figure 2.9: Image revised from Wada-Katsumata et al., 2018 showing the head of a German cockroach, *Blattella germanica*, with its four sensory appendages (antennae, labial palps, maxillary palps, paraglossae).

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Figure 2.10: Showing the German cockroach nymphs.

Figure 2.11: Showing adult German cockroaches.
The images were taken using a Nikon RZ100 light microscope.

Figures 2.12 and 2.13 show the total aerobic count using plate count agar (PCA) and nutrient agar (NA), respectively. On the plate count agar in Figure 2.12, 7.86 log cfu/ml total aerobic bacteria were observed from the third cockroach sample and 5.10 log cfu/ml from effluents at the processing plant. The cockroach and the effluent on the first and second samples showed colony forming units (cfu) levels that were too high to count. In Figure 2.12, using nutrient agar, the minimum cfu level of 4.69 log cfu/ml on the fourth sample from effluents was observed, and samples one, two and three for cockroaches had cfu levels that were too high to count. No colonies were observed in samples that were used for control. The samples were taken from the traps that only contained cockroaches.

In this study, the samples isolated from cockroaches exceeded the limit (10^5 cfu/cm^2 or 5.0 $\log_{10}$ cfu/cm^2) of total plate count for meat processing plants set by the World Health Organisation (WHO). The present study results are shown in Figures 2.12 and 2.13. Three biological replicates were done and the results revealed the presence of microbes on cockroaches found in the sampling area.

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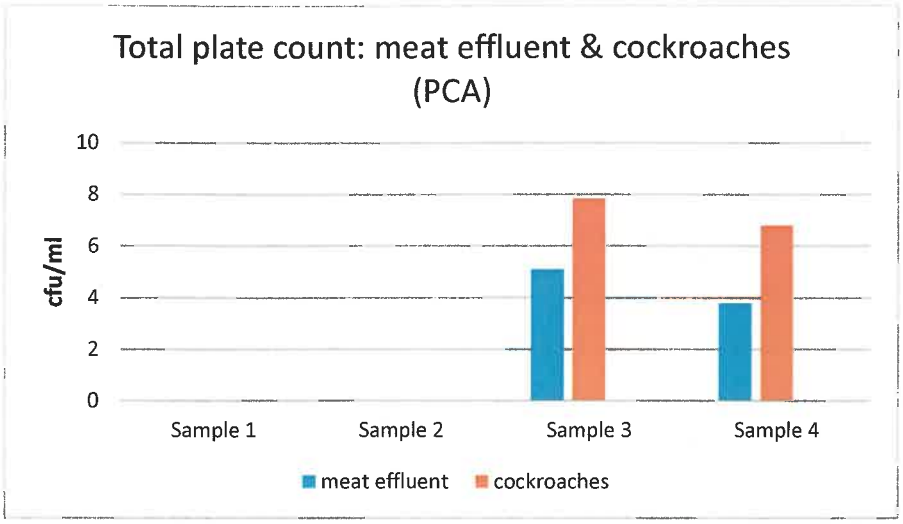

Figure 2.12 Total bacterial count on plate count agar (PCA).

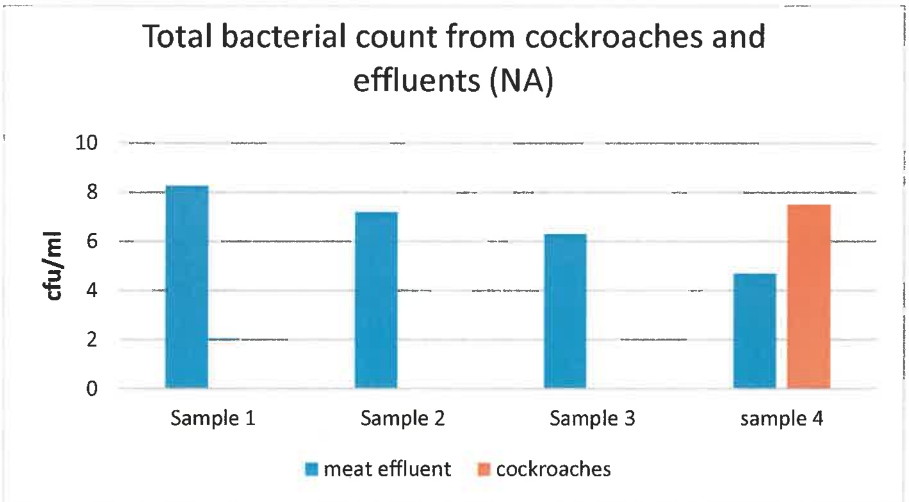

Figure 2.13: Total bacterial count on nutrient agar (NA).

High bacterial counts observed from cockroach samples also suggest that the environment has a direct link between the cockroaches and the effluents, and there is a greater possibility of cockroaches encountering the objects used in the production side of the factory or coming into direct contact with the meat and meat products and thus contaminating them with these pathogens (Salehzadeh *et al.*, 2007). These findings also confirmed that the German cockroaches are reservoirs and possible mechanical transmitters of pathogenic microorganisms.

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Bacteria were isolated from cockroaches and effluents and identified by using a Remel RapID-One system. Two common bacteria were isolated from the meat processing plant's effluent and the cockroaches: Yersinia enterocolitica and Yersinia kristensennii. Y enterocolitica showed resistance against ampicillin, penicillin and streptomycin. Y. enterocolitica and Y. kristensennii belong to the Yersiniaceae. They are mobile at 22-29 °C, but at normal human body temperature they are non-motile. Most Yersinia species are isolated during cold seasons or in cold environments (Khademi and Sahebkar, 2019). Meat processing plants generally function at low temperatures, making an ideal environment for the bacteria to thrive (Bonardi et al., 2018; Gnanasekaran et al., 2018). The focal reservoirs of Yersinia strains are domesticated pigs, which carry the pathogens in lymph nodes and the intestinal tract asymptomatically resulting in the spread to the carcass during slaughter processes, and to cockroaches encountering the contaminated water in the drainage systems (Bonardi et al., 2018; Gnanasekaran et al., 2018).

Table 2.1 represents the bacterial species that were identified and their antibiotic-resistance patterns. The resistance was measured in <14 mm resistant, 15-17 mm intermediate and >18 mm susceptible.

Table 2.1: Zone diameter of antibiotic resistance standards in millimetres (*mm)*

Antibiotic	**AMP**	**CIP**	**C**	**CN**	**S**	**TE**	**P**
Meat processing plant effluents							
Y. kristensenii	0 mm	23 mm	20 mm	20 mm	18 mm	15 mm	0 mm
Y enterocolitica	0 mm	26 mm	0 mm	18 mm	6 mm	13 mm	0 mm
Cockroaches							
Shigella sp.	10 mm	21 mm	26 mm	25 mm	20 mm	13 mm	0 mm
Y. kristensenii	0 mm	23 mm	20 mm	20 mm	18 mm	15 mm	0 mm
Y enterocolitica	0 mm	26 mm	0 mm	18 mm	6 mm	13 mm	0 mm
S. odorifera	0 mm	24 mm	18 mm	19 mm	18 mm	15 mm	0 mm

CODE: **AMP** ampicillin, **CIP** ciprofloxacin, **C** chloramphenicol, **CN** gentamicin, **S** streptomycin, **TE** tetracycline, **P** penicillin

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B-lactam antibiotic resistance was found in both bacteria. B-lactam antibiotics work by inhibiting cell wall production by targeting penicillin-binding protein. Penicillin-binding proteins are in charge of constructing the cell wall. The cell wall is damaged and dies as a result of this damage. When bacteria with a resistant gene die and free DNA is released into the environment, if similar bacteria to the dead bacteria are present, they will re-uptake the gene that makes them resistant to B-lactam antibiotics. Once inside the bacteria, the resistant gene can be transferred from naked DNA to the host bacteria's chromosome by a process known as homologous transformation, which involves the exchange of segments between two DNA molecules based on sequence similarity. Over time the bacteria gain sufficient of these genes to be resistant to B-lactam antibiotics.

These altered proteins can now synthesize the cell wall and develop resistance to B-lactam antibiosis. Cockroaches in the facility are possibly responsible for spreading the bacteria that contain these resistant genes to the surrounding meat and meat products. This could later pose a health threat to consumers, which could possibly lead to foodborne outbreaks that are difficult to treat using first-line antibiotics. This can be due to a process that began in the early 1950s where penicillins were used in animal feed of domesticated animals, including pigs, cattle and sheep, to prevent infection from harmful bacteria. Such animals were used to produce meat products at the particular meat factory where the samples were taken. Therefore, gentamicin and chloramphenicol would be the choices to treat *Yersinia* infections due to their high sensitivity.

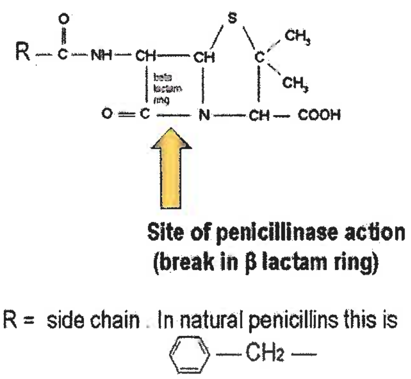

Figure 2.14: The structure of penicillin. (Harrison and Bratcher, 2008. Cephalosporins: A Review. *Pediatrics in Review*, p.264)

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Shigella spp. was also isolated from cockroaches. *Shigella* is a Gram-negative, facultative anaerobic bacteria and is closely related genetically to *E. coli* (Mani *et al.*, 2016). It causes dysentery during infection, a form of gastroenteritis that leads to bloody diarrhoea (Redrobe and Steinmetz, 2017). Signs of infection may include fever, stomach pain and an incomplete sense of defecation. Dehydration may result as a complication (Redrobe and Steinmetz, 2017). It is usually found in faeces, water contaminated, food or by an infected person and also on surfaces touched by infected people (Redrobe and Steinmetz, 2017). Since the cockroach is known to travel anywhere for food, it can come in contact with these bacteria, carrying them on its exoskeleton and depositing the bacteria on stored food products as it passes through them.

The bacteria are not necessarily found in meat processing plants unless cross-contamination has resulted from a sick employee. It also showed resistance against penicillin and ampicillin and showed high sensitivity to gentamicin, ciprofloxacin, streptomycin and chloramphenicol, which can be the drugs of choice in the case of infection. The last bacteria isolated from cockroaches was *Serratia odorifera,* a Gram-negative facultative anaerobe, an important nosocomial pathogen that can cause a wide range of infections, most particularly of the urinary tract and bloodstream (Poopola *et al.*, 2019). *Serratia* usually is not harmful to healthy people, but it is an opportunistic pathogen.

The connection between the cockroaches carrying a nosocomial infection-causing bacteria at a meat factory can be due to the close proximity of a clinic to the meat factory and cockroaches possibly coming in contact with the waste or travelling through the drainage system and making their way to the meat factory, the possibility of this hypothesis will be investigated in future studies. The bacteria also showed resistance to ampicillin and penicillin and showed sensitivity to ciprofloxacin, chloramphenicol, gentamicin and streptomycin, which can be the drug of choice where infection occurs. Antibiotic-resistant bacteria infections are on the rise around the world, posing a serious public health problem and posing a challenge to healthcare systems (Redrobe and Steinmetz, 2017).

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Antibiotic resistance can develop in bacteria in humans as a result of overuse and misuse, making infections that would normally respond to antibiotic treatment difficult, if not impossible, to treat. Treatment failures result in an increase in disease cases and deaths, an increasing challenge in developing new antibiotics, and, as a result, a severe economic impact, especially those living in poverty. A foodborne outbreak from this meat processing plant resulting from one of the resistant bacteria will negatively impact the communities that use food produced at the plant and the plant itself. This can lead to the plant's closure and discarding all the meat and meat products that could have been contaminated with the bacteria. This will also result in significant monetary loss as all the smaller butcheries will also close or cease to function, pending investigations that will take place. An example of this includes the listeriosis outbreak in South Africa that resulted in recalling processed meat products from stores and the death of some of the infected people. Moreover, for small communities such as Thaba Nchu, where access to healthcare is already a challenge, an outbreak will cause increased pressure on an already struggling health system, leading to more people becoming sick due to lack of prompt management and antibiotics that can cure the sick effectively.

2.5 Conclusion

The results from this study show that cockroaches are not only a nuisance in our communities but are reservoirs and potential disseminators of pathogenic microorganisms and potential health hazards in food processing plants. It is also evident that cockroaches carry drug-resistant bacteria, such as *Shigella*, *Yersinia* species and *Serratia*, which are of medical importance, were found from cockroaches that thrive at the meat processing plant. German cockroaches can act as mobile reservoirs of such bacteria and possibly lead to a foodborne outbreak coming from the meat factory. Favourable environmental conditions, like the one present at the meat factory while having cockroaches, potentially can transmit microorganisms such as *Yersinia enterocolitica* and *Yersinia kristensennii. Y enterocolitica* causes harmful diseases resistant to some conventional antibiotics, including ampicillin, penicillin and streptomycin.

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The results also show that antibiotic resistance should be of public concern at the community level, especially in developing countries like South Africa, where the most affordable antibiotics like ampicillin have become resistant to some foodborne pathogens. The spread and development of antibiotic resistance in bacteria are universal threats to both animals and humans. Nevertheless, it can be controlled and this must be undertaken in the most effective way possible by preventing these pathogens from directly or indirectly encountering humans or the food we eat.

The introduction of bacteria to foodstuffs in food processing plants or meat factories by pests such as cockroaches can cause food spoilage, resulting in production loss and increased retail prices of meat products to counteract the monetary loss in the food due to spoilage. Therefore, food establishments need to understand the importance of proper food storage and good hygiene practices, including proper and safe waste disposal and regular maintenance of water pipes. Prompt and effective management of these factors can minimize pest infestation and concurrently decrease the spread of these pathogenic bacteria.

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CHAPTER THREE

Chemical characterisation of *Thymus vulgaris* and *Cymbopogon winterianus* essential oils

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3.1 Abstract

Background: Plant derived essential oils present a broad spectrum of activity against pests as possible insecticides, repellents, or deterrents. These oils have also been used to protect stored goods for a long time. The octopaminergic nervous system in insects is disrupted by several chemical elements of these oils. Most essential oil compounds are relatively non-toxic to mammals, including humans and domesticated animals, and meet the criteria for reduced-risk pesticides; this target site is not shared with mammals.

Materials and methods: The chemical composition of the two studied essential oils was analysed using gas chromatography-mass spectrometry (GC-MS).

Results: A total of 53 compounds were identified in red thyme; p-Thymol and p-Cymene were the main constituents of the oil. Furthermore, Java citronella presented with 24 compounds, with 3,5,5-Trimethylhexanol, Citronellal, α-Terpineol being the major constituents of the oil. Red thyme had a much lower percentage of α-Terpineol of 0.290, which plays a role in how effectively the oil can act as an insect repellent. The difference in percentages shows that Java citronella is more potent as a possible repellent over red thyme.

Conclusion: The study further provides evidence that plant essential oils can be explored as insect repellents for future pest management programmes as they comprise compounds that are known to be effective in preventing pest infestations.

Keywords: Java citronella, red thyme, repellent

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3.2 Introduction

Essential oils (EOs) are concentrated hydrophobic liquids that are easily evaporated at room temperatures. These types of liquids are known as volatile chemical compounds (Salar-Garcia *et al.*, 2017). They are also known as ethereal, a solution with diethyl ether $(C_2H_5)_2O$ as a solvent, and they get their name from the plant from which they were extracted (Butnariu and Sarac, 2018). The word essential oil is derivative in that it contains the essence of the plant's characteristic fragrance from which it is derived (Hanif *et al.*, 2018; Batnariu and Sarac, 2019). Essential oils are secondary plant metabolites that give plants their fragrant properties (Sharififard et al., 2016). Plants produce them as communication and defense molecules through auxiliary metabolic pathways (Sharififard et al., 2016). Secondary metabolites' main role is to protect plants from predators and pathogens by repelling microorganisms and herbivores (Zaynab et al., 2018). EOs are responsible for the flavor and perfume of aromatic plants, in addition to their communication and defense functions (Pavela and Benelli, 2016). Steam distillation is the most popular method for extracting essential oils, but additional processes include expression, solvent extraction, and absolute oil extraction (Hanif et al., 2018) & (Batnariu and Sarac, 2019).

Extracting essential oils at temperatures near 100 °C, followed by condensation to generate an immiscible liquid from which the essential oil can be separated in a clarifier, is known as steam distillation (Bozovic et al., 2017). Solvent extraction is the process of partially removing a constituent from a solution or combination by dissolving it in a more soluble immiscible solvent (Ghahramanloo et al., 2017). The plant is put into a bath of solvents during solvent extraction, which melts the plant material, forming the essential oils. Alcohol is then used to help separate the essential oils. In the perfume industry, this process of extracting essential oils is frequently utilized (Ghahramanloo et al., 2017). Citrus oils do not hold up well when heated, hence the expression process is employed to extract essential oils from the peels of citrus fruits (Ghahramanloo et al., 2017). In this method, a machine grinds the seeds or fruits to extract the oil, or pierces the fruit's rind and peel while rotating to extract the oil (Stratakos and Koidis, 2016).

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Finally, absolute oil extraction is a technique utilized in aromatherapy and perfumes. Absolutes are concentrated, highly scented, oily mixes produced from plants, similar to essential oils. Raw plant material is immersed in a solvent under vacuum in this process. Evaporation is then used to remove the solvent (Ignatius, 2019). While essential oils are less hazardous than conventional chemicals, inappropriate usage can create problems such as allergic responses and skin irritation, and children are especially vulnerable to the toxic consequences of wrong use (Bingham et al., 2019).

An essential oil consists of different compounds that make up the whole oil and to analyse this, gas chromatography-mass spectrometry (GC-MS) is used (Al-Rubaye *et al.*, 2017). The primary purpose of any chromatographic process is to separate compounds to be studied independently from the others due to their complex composition. The process combines the features of gas chromatography (GC) and mass spectrometry (MS) to identify different substances in a test sample (Wesołowska *et al.*, 2019). GC is another type of chromatographic method used in analytical chemistry for separating and analysing compounds that can be vaporized without decomposition (Al-Rubaye *et al.*, 2017).

Because GC relies on gases to process, the molecules to be separated must be gaseous (Al-Rubaye *et al.*, 2017). The majority of chemicals in plants, such as sugars and pigments, are not volatile. Their boiling point is so high that they normally degrade before becoming gases due to high temperatures. Essential oils, on the other hand, are made up of volatile molecules and so easily transition to the gas phase. As a result, this sort of chromatography is the best option for studying essential oils. MS is an analytical technique for determining the mass ratio of ions (Al-Rubaye *et al.,* 2017).
The findings are given in the form of a mass spectrum for both pure samples and complex mixtures (Al-Rubaye *et al.*, 2017). In essential oils, GC-MS is used to determine the chemicals responsible for the distinctive floral or fragrance scent or taste of some expensive oils (Al-Rubaye *et al.*, 2017).

Other methods for analyzing essential oil ingredients include gas chromatography flame-ionization detection (GC-FID), which is the typical approach for quantification, and gas chromatography mass spectrometry (GC-MS), which is the most frequent analytical method for qualitative analysis.

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For lipid analysis, GC-FID is commonly utilized. High-performance liquid chromatography (HPLC), a method in analytical chemistry used to separate, identify, and quantify each constituent in a mixture, is an alternative to GC analysis. The fundamental distinction between the two procedures is that HPLC employs a liquid mobile phase while GC employs a gas carrier (Asteggiano *et al.*, 2021).

GC-MS used to determine the composition of red thyme (Thymus zygis) and Java citronella (Cymbopogon winterianus) essential oils responsible for insecticide and pesticide properties on insects (Rodríguez-González et al., 2019). GC-MS can be used to specify elements of an essential oil with their relative proportions because this type of testing allows us to discover many volatile constituents (Rodríguez-González *et al.*, 2019). This can then be used to verify a claimed botanical origin (Rodríguez-González *et al.*, 2019). Cymbopogon winterianus is a clump-forming, evergreen grass with several upright culms that can reach 2.5 metres in height (Rodríguez-González *et al.*, 2019). *Thymus zygis* is a blossoming plant in the family Lamiaceae. Examples include sage and mint (Dauqan and Abdullah, 2017). Its leaves are thin and about 8 mm in length, and its mainly used as a herb (Dauqan and Abdullah, 2017). The aim in this chapter is to separate volatile organic compounds found in *Thymus zygis and Cymbopogon* winterianus and distinguish their individual characteristics.

3.3 Materials and methods

3.3.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Two essential oils were used, *Cymbopogon winterianus* (Java citronella) and *Thymus zygis* (red thyme). The plants were bought at Thitapoho farm, and the essential oils were extracted through the steam distillation method. The essential oils were then taken to the University of South Africa for GC-MS analysis. The two oils were selected based on their high affinity as potential repellents over other essential oils.

A GC-MS analysis was performed to characterize the essential oils that will be used in the current study. The oils were tested on an Agilent 7890B GC system that was immediately connected to a 5977A mass spectrometer.

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A 0.2L sample was diluted with n-hexane (2 percent v/v) and injected with a split ratio (100:1) using an autosampler at 24.79 pressure and a 220 °C inlet temperature. The Agilent 19091S 433 UI: HP5-MS UI (30 m x 250 m x 0.25 m) column was used in the GC system. The oven temperature was gradually increased from 60 to 280 degrees Celsius. Helium was used as the carrier gas at a steady flow rate of 30 m/s. Electron impact at 70 eV was used to acquire spectra that ranged from 40.5 to 415 m/z. Without utilizing correction factors, the peak areas of the selected GC elements were represented as percentages of the total of all total ion count (TIC) peak areas determined by mass spectrometry detection (MSD, transfer line 240 °C; source 230 °C; MS Quad 150 °C). The compounds were identified by comparing peak retention durations and mass spectra with data in the Adams' database [n-Pentadecane was used as a reference] and utilizing the NIST11 and Agilent Flavor2 mass spectra libraries. The purity of the oil can be determined by using this approach and comparing the results to the standards.

3.4 Results and discussion

The results on (Table 3.1) show different compounds of the two tested essential oils. Three compounds were found to be the significant constituents, accounting for 53.77% of the Java citronella essential oil, namely; 3,5,5- Trimethylhexanol ($C_9H_{20}O$) at 14.61%, Citronellal ($C_{10}H_{18}O$) at 15.94% and a-Terpineol ($C_{10}H_{18}O$) at 23.22%. Furthermore, for red thyme, two main compounds accounted for 68,45% of the oil: p-Cymene ($C_{10}H_{14}$) at 22.154% and p-Thymol ($C_{10}H_{14}O$) at 46.393%.

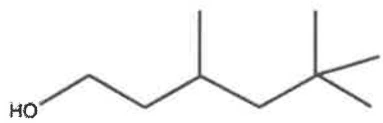

Figure 3.1: Chemical structure of 3,5,5-Trimethyl-1-hexanol. (McGinty *et al.,* 2010. *Food and Chemical Toxicology).*

3,5,5-Trimethyl-1-hexanol is an ingredient used in cosmetics for fragrances, shampoos, soaps and household cleaners, and detergents. It is a colourless oily liquid

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with an oily-herbaceous texture (McGinty *et al*., 2010). This compound is responsible for giving the oil its unique fragrance (Dring *et al*., 2017).

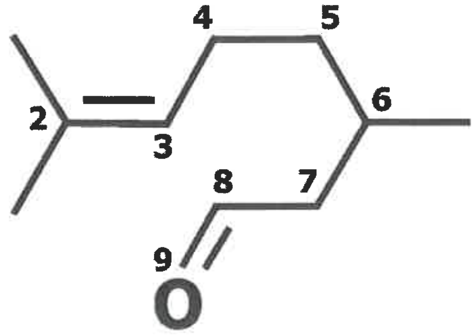

Figure: 3.2: Chemical structure of Citronellal (Domingos *et al.,* 2016. *Physical Chemistry*).

The essential oil is of good quality due to its higher Citronellal concentration. Citronellal is a *monoterpenoid* aldehyde (Kaur and Kaur, 2021). Aldehydes are usually irritating to the skin, and in the citronella oil, they are responsible for side effects related to skin inflammation when the pure oil comes in contact with the skin. Monoterpene aldehydes are Citronellal, and citral has a lemon-like smell that is very identifiable (Eden *et al*., 2018; Abril-Sanchez *et al*., 2019). Citronellal is a primary isolate in distilled oils from the plants *Cymbopogon*, lemon-scented gum, and lemon-scented tea tree and has insect repellent properties against mosquitoes (Eden *et al*., 2020).

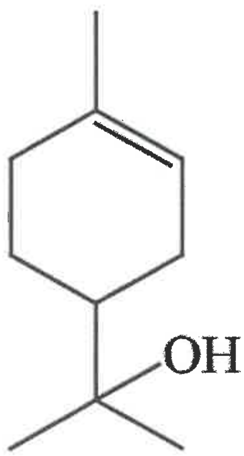

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Figure 3.3: Chemical structure of *a*-Terpineol. (Khaleel *et al.*, 2018. *Open Chemistry*).

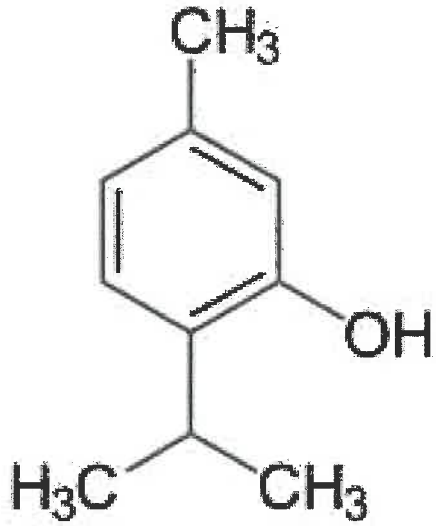

Figure 3.4: Chemical structure of *p*-Thymol. (Front Matter. Nomenclature of Organic Chemistry: *IUPAC Recommendations and Preferred Names 2013 (Blue Book).* Cambridge: The Royal Society of Chemistry, 2014).

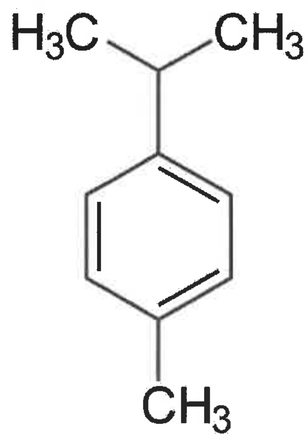

Figure 3.5: Chemical structure of *p-Cymene*. (Nomenclature of Organic Chemistry: IUPAC Recommendations and Preferred Names 2013 (Blue Book). Cambridge: The Royal Society of Chemistry, 2014, pp.139, 597).

α-Terpineol is a terpene alcohol that is found in natural oils. Moreover, with other biological properties they present with (Figure 3.4), *terpenoids* have presented

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promising insecticidal activities against anthropods. A study was done where essential oils from plants belonging to a number of species that present a high percentage of a-Terpineol were tested to assess their repellent and even insecticidal properties against *Aedes aegypti,* and it was found that a-Terpineol was a repellent at a minimum effective dosage (MED) of 0.039 ± 0.008 mg/cm^2 (Khaleel *et al*., 2018). Table 3.2 compares the same types of essential oils commonly used with *Cymbobogon winterianus Jowitt,* Ceylon type *Cymopogon nardus rendle* and CUT EO-19-10-1, the essential oil presented in this table.

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Table 3.1: Essential oil composition of Java citronella and Thymus zygis (red thyme)

Java citronella				Red thyme		
Retention time (min)	Compound name	% Area		Retention time (min)	Compound name	% Area
5.94	α-Pinene	10.31		7,76	α-Thujene	0,813
6.35	Camphene	0.20		8,00	α-Pinene	1,149
6.91	3,5,5-Trimethylhexanal	7.73		8,62	Camphene	0,395
7.13	β-Pinene	2.54		10,23	β-Pinene	0,417
8.29	1,4-Cineole	0.22		11,81	β-Myrcene	2,958
8.6	p-Cymene	0.34		12,86	α-Terpinene	0,736
8.75	D-Limonene	0.72		14,44	p-Cymene	22,154
8.83	1,8-Cineole (Eucalyptol)	0.38		15,98	γ-Terpinene	8,429
9.33	3,5,5-Trimethylhexanol	14.61		16,14	*trans*-β-Ocimene	0,145
9.8	γ-Terpinene	0.13		16,95	α-Terpinolene	0,327
10.91	Terpinolene	1.89		18,62	S-(+)-Linalool	6,192
13.45	Citronellal	15.94		20,14	Iso-Propyl–2-furan acetaldehyde	0,048
13.59	Isopulegol	1.77		20,89	2-Ethyl-5-methylfuran	0,144
13.96	Isoborneol	0.90		21,46	Terpinen-4-ol	0,243
14.45	Terpinen-4-ol	0.75		24,82	Thymol methyl ether	0,569
15.03	α-Terpineol	23.22		25,92	Carvacrol methyl ether	0,232
15.28	γ-Terpineol	4.03		26,37	α-Terpineol	0,290
16.6	Citronellol	1.92		29,82	p-Thymol	46,393
17.12	β-Citral (neral)	0.11		30,02	Carvacrol	2,657
18.37	α-Citral (geranial)	0.10		30,93	Thymol acetate	0,107
21.7	α-Terpinyl acetate	0.44		32,54	Caryophyllene	1,781
21.89	Citronellyl acetate	0.40		33,24	Alloaromadendrene	0,194
24.01	Longifolene	0.48		33,86	Humulene	0,084
24.61	(E)-β-Caryophyllene	0.37		34,58	*p-Tert*-butyl catechol	1,037
				35,57	Ledene	0,174
				36,39	γ-Cadinene	0,095
				36,85	δ-Cadinene	0,260
Total		89.50		Total		98.03

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Table 3.2: International market comparison of citronella oils with Java citronella.

ISO 3848:2016 Standard	Market specifications (Chinese/Sri-Lankan oil)			
Compound name	Java type (commonly used) *Cymbobogon winterianus Jowitt*		Ceylon type *Cymopogon nardus rendle*	CUT EO-19-10-1
α-Pinene				10.31
3,5,5-Trimethylhexanal				7.73
β-Pinene				2.54
D-Limonene	2.0-5.0	1-10 (1-4)	9-11	0.72
3,5,5-Trimethylhexanol	14.61			14.61
Terpinolene				1.89
Linalool	0.5-1.5			
Citronellal	31.0-40.0	25-50 (32-45)	5-15	15.94
Isopulegol	0.5-1.7	1-10		1.77
α-Terpineol				23.22
Citronellol	8.5-14.0	10-25	6-8	1.92
β-Citral (neral)				0.11
Geraniol	20.0-25.0	10-25 (11-13)	18-20	
α-Citral (geranial)	0.3-1.0	Total Citral: 0.1-1		0.10
Citronellyl acetate	2.0-4.0	1-10	0.40	0.40
Eugenol	0.5-1.0	1-10		
Methyl isoeugenol			7-11	
Germacrene-D	1.5-3.0			
β-Elemene	0.7-2.5			
δ-Cadinene	1.5-2.5			
(E)-Geranyl acetate	2.5-5.5	1-10 (3-8)		
Elemol	1.3-4.8			

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Table 3.3: Comparison with the international market specifications of Red thyme.

Compound name	ISO standard: 19817:2017(E)	Market specifications: Spanish standard	CUT-21-1-1
α-Thujene	0.5-1.5		0,813
α-Pinene	0.5-2.5	1.0-3.0	1,149
β-Myrcene	1-2.8		2,958
α-Terpinene	0.9-2.6	15-28	0,736
p-Cymene	14.0-28.0	5-10	22,154
γ-Terpinene	4.0-13.0	4-6,5	8,429
S-(+)-Linalool	0.5-6.5	0,2-2,5	6,192
Terpinen-4-ol	0.1-2.5		0,243
Carvacrol methyl ether	0.1-1.5	36-55	0,232
p-Thymol	35.0-55.0	1-4	46,393
Carvacrol	0.5-5.5		2,657
Caryophyllene	0.5-4.0		1,781

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The results show that CUT-19-10-1 has the highest percentage of a-Terpineol, the compound responsible for insecticide and repellent activity of an essential oil. The comparative results showed considerable variations in the quantitative composition of the essential oils particularly with the compound a-Terpineol, this may be due to different cultivars during the year, number of factors such as genotype/chemotype, geographical differences, season and pedogenetic factors (Alencar *et al.*,2017). This shows that the oil can be used to assess insecticidal properties using contact toxicity, fumigant toxicity and repellence. Compared with red thyme, Java citronella presents a higher percentage of α-Terpineol of 23.22%, while red thyme is at 0,290%. This could prove that Java citronella can be a better insect repellent than red thyme.

3.5 Conclusion

The application of GC-MS plays a vital role in analytical chemistry, particularly when it comes to natural-based products such as essential oils. With the increasing need of the world to "go green", the application of essential oils, when properly grown, extracted and bottled, plays an important role in scientific research as some of the oils present profound healing properties that are antimicrobial, antifungal and antibacterial.

In this chapter, Java citronella illustrates that some of the complex interactions that essential oils collectively have, can contribute to the repellent activity against insects. Many essential oil compounds have different modes of action such as insecticidal, feeding deterrence, repellence, growth inhibition and even attractant activities. When used in combination, they either reduce the concentration needed to achieve the desired effect, or the synergistic phenomenon can increase their effectiveness. Based on the results found in the study, two essential oils (Java citronella and red thyme) had compounds that may have insecticidal properties. However, this will have to be assessed further to get evidence.

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CHAPTER FOUR

Contact toxicity, fumigant toxicity and repellence assessment of *Thymus vulgaris and Cymbopogon winterianus* against *Blattela germanica*

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4.1 Abstract

Background: Natural materials such as plant essential oils provide an exceptional alternative to synthetic pesticides to control pests and decrease the negative impact the environment and human health. The move towards using green products and the continuing need to develop new methods to control and prevent pest infestation is increasing. Pests such as cockroaches have demonstrated a growing resistance against the common synthetic pesticides. However, synthetic pesticides have been shown to have detrimental effects on humans and the environment. Thus, the need to develop safer ways for pest management.

Materials and methods: This study evaluated the effect of Java citronella and red thyme essential oils against the German cockroach, *Blatella germanica*, using three bioassay methods to determine the contact toxicity, fumigant toxicity and repellent activity. The essential oils were diluted in hexane. The dilutions were 5%, 10%, 15%, 30%, and 100% essential oil for all Java citronella and red thyme assays. Aliquots of 2.5 ml were used for every assay to treat the filter papers.

Results. Generally, the results showed a higher insecticidal effect in test groups treated with Java citronella oil than in groups treated with red thyme essential oil.

Conclusion: Java citronella and red thyme have been shown to have an insecticidal effect against the German cockroach, with Java citronella showing more affinity over red thyme as a potential cockroach repellent. This further proves that essential oils can be explored as a safer alternative pesticide.

Keywords: repellence, insecticidal, fumigant toxicity, contact toxicity

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4.2 Introduction

Cockroach infestations are prevalent in warm areas, especially in residences without sufficient ventilation, hospitals, restaurants, and businesses with a high ambient temperature and humidity (Sharififard et al., 2016). Conventional insecticides are the most used way for treating cockroach infestations, despite widespread concerns about their potentially harmful side effects (Sharififard *et al.*, 2016). In addition, due to the risk of poisoning, insecticides are prohibited in food preparation facilities, restaurants, storage buildings, and apartments. As the use of chemical insecticides is restricted, there is a greater desire for safer options to combat cockroach infestations (Sharififard et al., 2016). Even though essential oils may not be the perfect replacement for all synthetic pesticides due to their high cost, they have prospects for urban pest control; they have shown to be safer and pose less threat to human health and the environment (Isman, 2020).

In research, several essential oils were found to be equivalent to diethyltoluamide (DEET), a prominent active ingredient in insect repellents that is now advertised as the most efficient mosquito repellent (Sparks *et al.*, 2018). DEET works by stopping insects from activating the l-octen present in human breath and sweat by blocking their olfactory receptor, which they use to find food (Sparks *et al.*, 2018). The use of DEET, on the other hand, has prompted concerns about environmental and human health dangers, particularly in children (Sparks *et al.*, 2018). Essential oils have thus been investigated as alternatives to standard synthetic insecticides due to their repelling properties and low toxicity to the environment, non-target creatures, and people (Pavela and Benelli, 2016). Overuse of synthetic insecticides containing organophosphates to control cockroaches has resulted in major issues that have affected non-target organisms, contaminated land and aquatic habitats, and resulted

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in insecticide resistance (Soares *et al.*, 2019; Kah et al., 2018) & (Yeguerman et al., 2020).

Insect repellents provide major barriers of personal protection against arthropod-borne infectious illnesses by topical application (Robert and Debboun, 2020). Although essential oils can be used as a mosquito repellent when applied to the skin, they can only be effective at the vapour stage (Burfield and Reekie, 2005) & (Pujiarti, 2017).

The German cockroach (Blattella germanica L.) is a widespread synanthropic pest (Yeom et al., 2018). B. germanica is a hazard to human health because it produces asthma-inducing allergens, acts as a mechanical vector for transporting harmful bacteria, and contributes to unsanitary indoor settings (Dietrich et al., 2014; González-Diaz et al., 2019). While pesticides are critical for reducing cockroach populations and improving health outcomes, insecticide resistance has proven to be a persistent cockroach control challenge (Fardisi et al., 2019).

The development of environmentally friendly approaches has been deliberated upon worldwide to replace conventional pesticides, and evidence suggests essential oils could be used for pest control (Campolo *et al.*, 2017). The active compounds in essential oils are biodegradable and have lower negative effects on mammals and the environment (Bedini *et al.*, 2018). Thus, it has been suggested that they could be used on a small scale to treat cockroach infestations in human residences and workplaces (Moretti *et al.*, 2017). The major components of the oils reportedly affect the insect's nutritional physiology and behaviour (Moretti *et al.*, 2017). Furthermore, other studies have reported that essential oils demonstrate lethal and sublethal effects against German cockroaches (Yeom et al., 2018).

However, factors such as great volatility and rapid oxidation affect their biological activity and resolution, reducing their prospect of being used in large-scale applications

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(Campos *et al*., 2019). Thus, the aim of this chapter is to test the efficacy of essential oils as potential alternative pesticides against cockroaches.

4.3 Materials and methods

4.3.1 Cockroaches

A total of 800 German cockroaches were used for this study. The German cockroach was chosen for this study because it is an urban pest and it is commonly found in human dwellings and food establishments, and it is responsible for transmitting disease-causing microorganisms, a source of nuisance and social stigma where the infestation is present. The cockroaches were sourced from an existing culture at Clinvet Research Facility, where they were bred under conditions that mimic their natural habitat under room temperature (~26 °C) with access to dog food pellets and water *ad libitum*.

These cockroaches have been cultured for several generations with the frequent infusion of 'wild' ones to maintain genetic heterozygosity and limit laboratory adaptations and inbreeding depression. From the foregoing, it is therefore assumed that the behavioural and physiological responses assessed in this study are a true reflection of free-living wild cockroaches that are not kept under controlled conditions on a standard diet.

Transportation of cockroaches was done in perforated polyethylene containers (Figure 4.1) to the University of the Free State, where they were kept under controlled conditions (~26 °C; 65% RH) prior to the onset of experiments.

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Figure 4.1: Showing live cockroaches in polyethylene containers used for transportation.

4.3.2 Bioassays: Contact and fumigant toxicity and repellence

Two essential oils, *Cymbopogon winterianus* (Java citronella) and *Thymus zygis* (red thyme) were used; the oils were sourced from Thitapoho farm, where they were extracted through the steam distillation method.

Contact Toxicity: The bioassay method developed by WHO (World Health Organization, 1975) was used to determine resistance or susceptibility of the cockroaches to the selected essential oils with some modifications. Briefly, treatment solutions for the bioassay were prepared by dissolving essential oils in hexane as the solvent (v/v) at concentrations of 5%, 10%, 15%, 30% and 100%.

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Thereafter, aliquots of 2.5 ml for each treatment solution were used to immerse/imbed filter papers before immediately placing them at the base of desiccation jars (27 cm diameter: 28 cm height).

In each jar, five male or female cockroaches were then placed before closing with a lid smeared with petroleum jelly (Vaseline®) to ensure an airtight seal. In all cases, the control group consisted of jars with only the organic solvent hexane on the filter paper. Each jar was regarded as a replication for each treatment, and a total of five replications were done to yield a sample size of 25 cockroaches used for control.

Fumigant Toxicity: Essential oil treatment mixtures were prepared in hexane as the solvent (v/v) at concentrations of 5%, 10%, 15%, 30% and 100%. Thereafter, aliquots of 2.5 ml for each were used to immerse filter papers and immediately place them at the top of desiccation jars at 10 ml solution. Groups of five cockroaches were transferred using a mechanical aspirator into desiccation jars (27 cm diameter: 28 cm height). Care was taken to ensure that filter papers were not soggy (too wet) and dripping such that any cockroach mortality would have been because of fumigant toxicity and not coming in direct contact with the oil.

In each jar, five male or female cockroaches were then placed before closing with a lid smeared with petroleum jelly (Vaseline®) to ensure an airtight seal. In all cases, the control group consisted of jars with only the organic solvent hexane on the filter paper. Each jar was regarded as a replication for each treatment, and a total of five replications were done to yield a sample size of 25 cockroaches used for control. The mortality for both assays was calculated between the moment of exposure to the first 24 hours. Mortality was assessed by the cockroaches laying on their backs and the ability of cockroaches to move when poked with a brush on their stomachs.

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Repellency: The methods by Appel, Gehret, and Tanley (2001), Manzoor, Munir, Amdreen, and Naz (2012) and Sharififard, Safdari, Siahpoush, and Kassiri (2016) were used for this study with some modifications. A total of 360 cockroaches were used in this method.

A filter paper was cut into two equal pieces, and one half was treated with Java citronella and red thyme, respectively, using a pipette, and the other half was treated with hexane. Different concentrations of (5%, 10%, 15%, 30% and 100% v/v) oil solutions were used at five replicates per concentration. Each treated filter paper was left for two minutes to dry before the start of the repellence test. It was followed by placing the filter paper at the bottom of the rectangular airtight, transparent box divided in the middle and sealed with parafilm. The filter paper treated with hexane was placed on the right side of the assay box. The five adult German cockroaches were released at each replicate into the centre of each assay box. The distribution of the test subjects was observed at 0, 5, 10, and 15 minutes.

4.3.3 Data Analyses

The effects of essential oil type and concentration on contact toxicity and repellence were compared using Two-Way ANOVA in Statistica 9.0 (Statsoft, Tulsa, OK, USA). In this instance, the type of essential oil and concentration were regarded as the categorical predictors whilst either survival (contact toxicity assays) or the number of cockroaches away from the odour source (repellence assays) were dependent variables. Key assumptions of ANOVAs for homogeneity of variance and normality of data distributions were met following tests using the Hartley-Bartlett test and Shapiro-Wilk test, respectively. Tukey's HSD *post-hoc* tests were used to identify statistically homogenous groups at $p = 0.05$.

4.4 Results and discussion

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Contact toxicity: Concentrations of 15%, 30% and 100% of Java citronella essential oil caused 80%, 92% and 100% mortality, respectively, after the first 24 hours. The same concentrations of red thyme essential oil resulted in mortality rates of 44%, 52%, and 72% after 24 hours, which differed compared to Java citronella.

Furthermore, a difference was noted at the lowest concentration between the two oils, where Java citronella caused mortality rates of 52% and 64% at 5% and 10% concentrations and red thyme had mortality rates of 20% and 15% at 5% and 10% concentrations. The results demonstrate that Java citronella has higher activity against the German cockroach than red thyme as a possible insecticide. A similar study was done where insecticide properties of essential oils and some of their constituents were tested on the Turkestan cockroach. In this study, cockroaches were shown to avoid the essential oil, further proving that essential oils present insecticide activity against arthropods (Sudip *et al.,* 2017).

Figure 4.2: Showing dead cockroaches following contact toxicity treatment.

Fumigant toxicity: Fumigation with Java citronella caused mortality rates of 60%, 72% and 96% and 15%, 30% and 100% compared to red thyme at the same concentrations, but with mortality rates of 40%, 36% and 84% using red thyme

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essential oil, mortality rates were 44%, 52% and 72%, after 24 hours. Furthermore, a difference was noted at the lowest concentration between the two oils, further proving that Java citronella is more effective as a possible fumigant insecticide against the German cockroach over red thyme. No mortality was observed for the control (hexane) in all replications of both contact toxicity and fumigant toxicity.

Figure 4.3: Showing dead cockroaches following contact toxicity treatment.

Table 4.1: Summarized output of a Two-way ANOVAs testing the effects of essential oil type and concentration on the mortality of German cockroaches following direct contact exposure and fumigation using Java citronella and red thyme. Significant treatments are denoted in bold font. The results show that Java citronella has more affinity over Red thyme on contact exposure and fumigant exposure, and can be explored as a safer alternative pesticide, that is less harmful to humans and non-target organisms.

	Source/Effect	**SS**	**DF**	**MS**	**F**	**P**
Contact exposure	Oil type	11760	1	11760	65.3	**<0.001**
	Concentration	47333.3	5	9466.6	52.6	**<0.001**
	Oil type Concentration	2960	5	592	3.3	**0.012**
	Error	8640	48	180		

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Fumigation exposure	Oil type	2940	1	2940	13.6	**0.001**
	Concentration	48753.3	5	9750.7	45	**<0.001**
	Oil type Concentration	1340	5	268	1.23	0.306
	Error	10400	48	216.7		

Repellence: The repellent effect of Java citronella and red thyme against the German cockroach is presented in Figure 4.4. The study revealed an increasing repellent effect on the German cockroaches when Java citronella was used at a lower concentration of 5%. In comparison, the repellence effect started showing against red thyme at concentrations of 15% of the oil solution. An increased repellence was observed in all concentrations with increased exposure time, with Java citronella still showing the most repellent effect against the German cockroach.

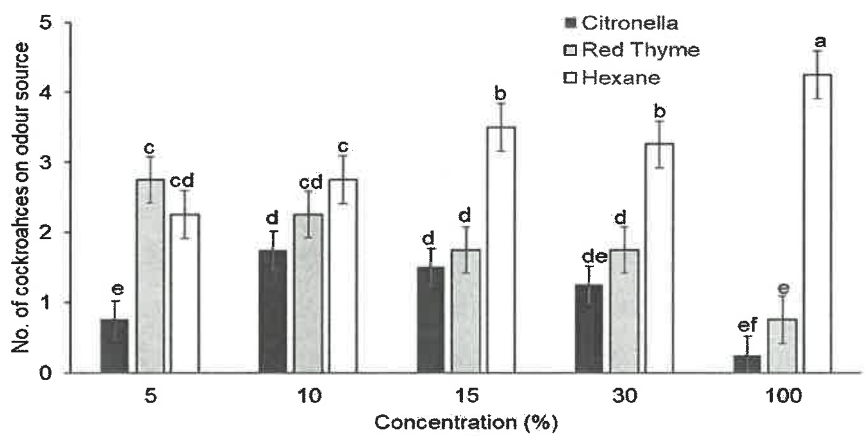

Figure 4.4: Repellent effect of Java citronella and red thyme against the German cockroach.

4.4.1 Mechanism of action of Java citronella against the German cockroach

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Java citronella *(Cymbopogon winterianus)* shows to be the more effective oil that presents with insecticidal activity; this is because of the active compound in the oil, *a*-Terpineol. Therefore, it is suggested that α-Terpineol may be a potential agent for developing natural insecticides or fumigants and repellents to control insects. Cockroaches have strong chemoreceptors, consisting of 144 olfactory receptors for smell and 544 gustatory receptors for taste.

As a result, they consume anything they encounter, including non-food items such as cardboard and plants that can poison them and other animals. Therefore, essential oils, due to their pungent aroma, serve as the best alternative to get rid of these pests by attracting cockroaches to them, posing toxicological effects, and potentially killing them, but that is not the case. This is because cockroaches have an enzyme called cytochrome p450s which helps them withstand poisonous chemicals. These genes help the cockroach by detoxifying the poison and keeping it safe.

However, most essential oils, like Java citronella, work as neurotoxins to the cockroach by disrupting the neurotransmitter, and other compounds like tannin are also found in plants, which are inhibitors of enzyme activities that aid digestion in insects (Ajayi *et al.,* 2018). The chemoreceptors in the cockroach are responsible for the attraction of the cockroach to the oil; the toxins work by penetrating the insect's body through its respiratory tract as soon as the oil makes contact with the cockroach's body (Feroz, 2020). Although cockroaches can detoxify most chemicals, they cannot do the same with Java citronella because the mode of action is different.

4.5 Conclusion

Because the German cockroach can be found in all areas of a building, including garment lockers, mattresses, furniture, and cupboards, spraying chemical insecticides in these areas might be difficult. Synthetic pesticides are also prohibited in the food industry due to their long-term effects, which can be detrimental to consumers.

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Because essential oils are less hazardous to humans and leave no toxic residues, they are a safe choice for indoor cockroach control. Considering all of the active chemicals in the oil that are responsible for its efficacy, Java citronella oil demonstrated a significant effect against *Blatella germanica* at the lowest concentration of 5%, and so can be recommended as a potential repellent.

Investigation on a larger scale and under different field conditions is needed to assess its efficacy and the practical application, and develop favourable formulations of essential oils against cockroaches. However, polymeric nanoparticles' design is an innovative idea for new bioinsecticides technologies. These technologies include incorporating EOs in a nanoencapsulation to prevent the rapid degradation and evaporation of EO constituents, increasing their toxicity and shelf life and improve the handling.

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CHAPTER FIVE

General discussion, conclusions and recommendations

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5.1 Conclusion and recommendations

5.1.1 Introduction

Cockroaches have proven to be a nuisance and have caused social stigma within communities for many years. They have also affected food manufacturing industries negatively because of economic losses suffered due to spoiled or contaminated foodstuffs because of pest infestation by these arthropods (Materechera, 2016). They also have been shown to cause or aggravate some illnesses, such as asthma, where the presence of cockroaches in a household can worsen an asthma sufferer's symptoms (Mendy *et al*., 2020).

This is because they leave droppings, shed parts or saliva, which can trigger an allergic reaction (De Waal, 2019). Cockroaches also crawl from sewers and garbage cans into kitchens, and they spread harmful bacteria causing human diseases such as dysentery, cholera, leprosy, plague, typhoid fever, and viral disease such as poliomyelitis (Greenberg, 2019). These could lead to greater problems if infestations occur at big meat processing plants (Greenberg, 2019).

The results in this study also show that *Y. kristensenii, Y. enterocolitica, S. odorifera* and *Shigella spp* are resistance to Ampicillin and Penicillin, which are commonly used antibiotics. Yersinia belongs to the Enterobacteriaceae family and is a widely spread gastrointestinal pathogen spread by the faecal–oral route after ingestion of contaminated food or contact with infected or colonized animals. Antimicrobial therapy is required to treat immunocompromised individuals, but due to the growing resistance against this antibiotics, health workers experience treatment failures resulting in unwanted fatalities. Infections caused by *Shigella* and *S.odorifera* are also treated with B-lactam antibiotics, these antibiotics are those that are mostly available in small community clinics and local hospitals in South Africa (Chigome *et al*., 2020).

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Failure to respond to these antibiotic therapies leads to complications such sepsis, which can later result in death (Uppu *et al.*,2015). This is why it is important to introduce measures, such as pesticides, in place to prevent pest infestations as pests can carry these pathogens. But the pesticides currently used have shown to have harmful effects on humans and non-target organisms, this is why it's vital to have safer alternatives such essential oils included future pest management strategies.

However, there has been few studies to establish the effectiveness of essential oils like citronella as insect repellents (Asadollahi et al., 2019). When the oil is exposed and left in typical environmental conditions, it quickly evaporates, resulting in a loss of efficacy (Zhang et al., 2020). However, studies have shown that mixing the essential oil of *Cymbopogon winterianus* with a large molecule like vanillin increases protection time that can be considerably prolonged by reducing the release rate of the volatile oil (Monteschio *et al.*, 2019). Vanillin is an organic compound and has shown to be useful as a herbicide and pesticide (Monteschio *et al.*, 2019).

5.2. General discussion

The meat processing plant at hand receives carcasses from the abattoir, and they produce meat and meat products such as sausages, cold meat, biltong, beef, chicken and pork. These foodstuffs are then transported to smaller butcheries around the Free State and Lesotho.

According to Mangaung environmental health inspectors, the facility complies with all the standards, and they strive to maintain cleanliness and ensure that the food they produce is safe and of good quality. Despite their efforts, cockroaches still manage to find their way into the facility. The possible cause of this could be the geographical location of the facility. It is at the outskirts of Thaba Nchu, and it brings along all the elements cockroaches need for survival, namely: food, water, and shelter.

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If an outbreak occurs in such an environment, the spread of the disease can reach other places quicker, and it will be difficult to contain due to their customers' locations. The facility has a pest control programme in place, but fumigating the area, especially inside the facility where production takes place, becomes difficult because conventional pesticides have compounds that have been proven to be harmful to humans when ingested. This is the reason why natural substances, such as essential oils, should be explored as possible pesticides, as they pose much lower risks compared to synthetic pesticides currently in use.

5.3 Conclusion and recommendations

Cockroaches are known cause nuisance within communities and food processing industries. Infested facilities experience production loss as these pests contaminate food causing food spoilage. Not only that but economic loss as news on infestation will spread within the community, resulting in consumers being hesitant to purchase any food produced from the identified facility. This is why it's important for business owners to understand best hygiene practices and pest management. And also the importance of education and training of their employees on personal hygiene and general hygiene especially when handling food. Food establishment owners also need to understand different pesticides used in their facilities, their applications and the residual effects it will have on food and consumers.

To prevent infestations in food processing plants, workers need clean up production sides and spilled food. Throw away any food that is left out on the counters that is not going to be used, Wipe all surface of all food preparation areas after end of every shift. Clean under appliances and counter tables and make sure to rinse out containers and buckets used before putting them away for the next day.

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Ensure that the indoor garbage disposal site, if there is any, is empty or closed with tight fitting lids. Store food in hermetic containers or plastic wraps and elevate them off the floor. cockroaches communicate through chemical pheromones they secrete as they travel. Paper and cardboard are excellent absorbers of these pheromones. Substitute cardboard boxes with plastic containers if possible.

The interior and exterior of the building should be inspected periodically and maintenance should seal any gaps or crevices they find because cockroaches can squeeze through the tiniest openings to get into the building. Get rid of any standing water in and around the facility and check for leaks and repair them promptly. It is also recommended that food processing facilities have a clearly structured waste collection plan to prevent waste standing out too long which can attract unwanted pests. Should the measures in place not be sufficient in controlling cockroach infestation, other methods of controlling them can be explored. Essential oils are currently being studied as one of the future pest management strategies, but due to their volatile nature, their application pose a challenge, particularly in open environments.

Nanotechnology has recently emerged as a viable option for extending the protection duration of essential oils by allowing for slower oil release rates. Encapsulated citronella oil nanoemulsions generated by high-pressure homogenization of 2.5 percent surfactant and 100 percent glycerol created persistent droplets that increased oil retention and slowed release, according to one study (Akbas, 2018). Microencapsulation with gelatin-Arabic gum was used in another investigation to extend the action of a natural pesticide. The citronella repellency of treated fabric held at room temperature (22 °C) was sustained for up to 30 days.

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The application of nanotechnology to improve the performance of essential oils has the potential to transform the pesticide industry and make essential oils a more feasible option for long-lasting repellents. Essential oils could be used as part of an integrated pest management program to keep cockroaches away from access points into a facility like the meat processing plant in this study. This will assist the facility's hygiene management in preventing cockroaches from entering the facility by applying the essential oil, which has been developed as a repellent, to recognized access sites of these arthropods, keeping them outside and away from any plant-produced products.

However, essential oil formulations should not be a stand-alone treatment for application against the pest infestations of German cockroaches; other means need to be incorporated in these management programmes, such as an effective pest management programme where regular inspections are done to identify problems before they get out of hand. The chemical formulae of essential oils present a limiting factor that will need to be explored when it comes to their application in open environments, and further studies need to take place, particularly in nanoencapsulation as a method to delay the volatilization of active ingredients and to protect them from environmental factors that cause their degradation.

Since the vapour stage is brief, polymer mixtures that include nanoencapsulation can be used to prolong the vapour period of effective repellence. This is important, particularly when dealing with bigger arthropods like cockroaches requiring prolonged exposure to reach maximum dose effect. Further studies in nanotechnology will be done to explore methods that can be used to prolong the exposure time of the essential oil without the oil losing its effectiveness.

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