EXPLORING THE CHEMICAL & BIOLOGICAL PROPERTIES OF AROMATIC PLANTS AS NATURAL GRAIN PROTECTANTS AGAINST STORED GRAIN BEETLES

A dissertation submitted by

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Under the Guidance of

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CERTIFICATE

I hereby certify that this dissertation entitled "**Exploring the chemical & biological properties of aromatic plants as natural grain protectants against stored grain beetles**" submitted by **Ms. Arunima Shine V (Reg No. VM23FPT006)** to **St. Teresa's College (Autonomous), Ernakulam, Mahatma Gandhi University, Kottayam, Kerala**, in partial fulfilment of the requirement for the award of the degree of **Master of Vocation (M. Voc) in Food Processing Technology** is a bonafide report of original research work done by her under my supervision and guidance at the **Department of Food Protectants and Infestation Control (FPIC), CSIR-CFTRI, Mysuru, Karnataka** during **December 2024-April 2025**. It is also certified that this dissertation has not been submitted for any degree to any other University.

The report hereby approved as a bonafide and creditable dissertation work carried out and presented by her in a manner to warrant its acceptance in partial fulfilment of the required credits for the degree of **Master of Vocational (M. Voc) in Food Processing Technology.** However, the undesigned do not necessarily endorse to take responsibility for any statement or opinion expressed or conclusion drawn therein, but only approve the dissertation report for the purpose for which it is submitted.

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DECLARATION

I **ARUNIMA SHINE V** (Register No: VM23FPT006), hereby declare that this dissertation project entitled "**Exploring the chemical & biological properties of aromatic plants as natural grain protectants against stored grain beetles**" submitted to **St: Teresa's College, Ernakulam, Kerala** in partial fulfilment of the requirements for the award of the degree of **Master of Vocation in Food Processing Technology** is an authentic record of the original research work carried out by me during the period from **December 2024 to April 2025** under the supervision and guidance of **DR. RAJASHEKAR .Y**, Principal Scientist, Department of Food Protectants and Infestation Control, CSIR-Central Food Technological Research Institute, Mysore.

I also declare that the project has not been submitted to any other Universities or Institutions for award of any degree.

ARUNIMA SHINE V

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LIST OF ABBREVIATIONS

%	-	percentage
EO	-	Essential Oils
ml	-	milli liter
ml/l	-	milli liter per liter
L	-	Linnaeus
LD	-	Lethal Dose
LC	-	Lethal Concentration
+	-	Plus
–	-	Minus
Pvt Ldt	-	Private Limited
GC-MS	-	Gas Chromatography-Mass Spectrometry
HPLC	-	High Performance Liquid Chromatography
°C	-	degree Celsius
±	-	plus, or minus
g	-	gram
kg	-	kilo gram
=	-	equal to
/	-	divided by
×	-	multiplied by

No.	-	number
cm	-	centimeter
µl	-	micro liter
µl/L	-	micro liter per liter
h	-	hours
UV	-	Ultra Violet nano meter
nm	-	Standard Error
SE	-	Lower limit Upper Limit retention factor

ABSTRACT

Stored grain insect pests pose a serious threat to global food security, reducing the quantity and quality of products that are stored. Even though they are very effective, traditional chemical pesticides often lead to issues like residual toxicity, environmental pollution, and resistance. Essential oils from plants have emerged as a sustainable environment-friendly alternative in recent times. *Sitophilus oryzae* (rice weevil) and *Tribolium castaneum* (red flour beetle), two of the most important stored grain insects of pest status, are the focus of this research's analysis of the chemical composition and insecticidal activity of essential oils of some aromatic plants: Eucalyptus globulus, *Pimenta dioica* (allspice), and *Cinnamomum verum* (cinnamon).

The major chemical constituents of the essential oils were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The fumigant and contact toxicity of the oils were investigated under laboratory conditions. Cytotoxic activity was exhibited significantly in the findings, and rates of mortality differed based on exposure time, concentration, and application method. Significantly, eucalyptol, α-pinene, eugenol, and cinnamaldehyde were revealed to be the major bioactive constituents responsible for toxicity.

The findings point to these essential oils' promise as natural pesticides for integrated pest management (IPM) of stored grain systems. They are ideal candidates for environmentally friendly pest control methods due to their dual roles as bioactive compounds and insect repellents. To further enhance their utility in practice, additional research on formulation, synergism, and field application is recommended.

1. INTRODUCTION

With the growing human population and the increasing demand for food crops, the need for insecticides to control insect pests is increasing at an accelerated rate. India produced 281.37 million metric tons of food grains in 2018–19, according to NAAS (2019). Post-harvest management and food security are an integral aspect of the Indian economy. Since they provide the majority of Indians with their main source of energy, cereals and pulses are essential components of the human diet.

Cereal grains have been the principal component of the human diet. Wheat and rice account for more than half of global grain production. The major staples of the globe are rice, wheat, and maize, with sorghum and millet being less common. Consuming whole grains has been linked in studies to a lower incidence of major diet-related conditions such as coronary artery disease, inflammatory bowel disease, abnormal laxation, and several types of cancer. A great source of carbohydrates, wheat also contains minerals like P, Mg, Fe, Cu, and Zn, as well as essential elements like protein, thiamine, riboflavin, niacin, and vitamin E (Siddiqui and Sarwar, 2002; Sarwar and Sattar, 2007). These nutrients are essential for supporting a number of body processes and preserving general health. Wheat is an important part of a balanced diet since its fibre content also supports digestive health and aids in blood sugar regulation.

Due to poor post-harvest management, over one-third of the world's yearly agricultural output is wasted. 20 to 50 percent of post-harvest losses are caused by inadequate grain storage management. Poorly designed storage structures and insufficient post-harvest management techniques are mostly to blame for these post-harvest losses. For a steady supply all year long, post-harvest must be stored securely using the right preservation techniques. Nevertheless, post-

harvest storage loss is a serious problem and a major obstacle to humanity's ability to meet the demands of an expanding population. Grain must be stored correctly after harvesting to maintain its nutritional value and quality and increase its shelf life. However, inadequate maintenance and storage capacity frequently result in grain spoiling by producing harmful compounds, reducing nutrients, using up surplus resources, and ultimately depreciating them in the marketplace. The damages that mainly happen to grains are qualitative and quantitative losses. While qualitative losses include the loss of flavour, nutritional value, and vulnerability to elevated mycotoxin levels, quantitative losses are the decrease in grain weight and moisture content brought on by the faulty respiration process.

About 10% of all food grains are lost after harvest as a result of improper storage, rodents, insects, microorganisms, etc. Two important biotic agents that harm stored food grains are insect pests and storage fungus. In India, annual storage losses have been estimated at 14 million tonnes worth Rs. 7,000 crores, of which insects alone are responsible for around 1,300 crores. The major causes for the storage losses of grains are attacks by insects, rodents, birds, and dampness.

In developed countries grains are kept in well-maintained silos with aeration and drying reduces storage losses. In contrast, storage losses are higher in less developed nations, especially when it comes to cover and plinth storage with poorly maintained storage facilities. Inadequate post-harvest management procedures and badly planned storage facilities are typically the cause of high food grain losses (Anon, 1989).

Silos are storage buildings that work well for keeping food grains fresh for extended periods of time. These buildings are typically composed of concrete or steel and are thought to be an alternative to the traditional bag storage system. One of the main obstacles to the adoption of these structures by small and medium-sized farmers is the high initial cost of manufacture.

Fumigation is the most common chemical control method for protecting stored food grains from insect pests. Phosphine (PH_3) and methyl bromide (CH_3Br) fumigants have been used for more than decades to control stored product insect pests (Islam *et al*., 2009). CH_3Br has been banned in many countries due to the depletion of the ozone layer (MBTOC, 2010; Schneider et al., 2003). Both phosphine and methyl bromide fumigant usages are highly restricted in India (CIBRC, 2022). Some of the stored product insect pest species developed resistance against phosphine, which leads to control failures in different regions of the world (Montzka et al., 2011).Due to the adverse effects of these chemicals on the environment and human health, researchers have been finding natural alternatives like botanical insecticides derived from essential oils or plant extracts (Zenoozia et al., 2022). Effectiveness against target pests without endangering people or the environment is the primary goal of biopesticides. The majority of the chemical insecticides are neurotoxic, with action on targets in the central nervous system like the membrane ion channels (DDT, pyrethroids), the enzyme acetylcholinesterase (organophosphate, carbamate), and the neurotransmitter receptors (avermectins, neonicotinoids) (Rajashekar et al., 2012)

Researchers have started discovering natural alternatives, such as botanical pesticides made from essential oils or plant extracts, as a result of these chemicals' detrimental impacts on the environment and human health (Zenoozia et al., 2022). Effectiveness against target pests without endangering people or the environment is the primary goal of biopesticides. Inorganic solvent-produced edible plant extracts are safe options for application as contact insecticides, fumigants, or repellents (Nikolaou et al., 2021). Insecticidal action against important stored goods insect pests has been observed for a number of plant species.

Plant essential oils are made up of a mixture of different compounds, such as esters, monoterpenes, aldehydes, ketones and sesquiterpenes. These chemical compositions are involved

in plants' defense systems against microorganisms and insect pests of stored products, and they are essential in eliminating stored insect pests. The antibacterial activity of essential oils demonstrated that their hydrophobic components might alter the cell membranes of microbes, hence altering their cell structure and membrane permeability and ultimately resulting in cell death (Zhang et al., 2016.) The antibacterial properties of essential oils are derived from the interactions between their chemical constituents (Chouhan et al., 2017; Marchese et al., 2017).

Eucalyptus globulus is classified as an aromatic medicinal plant and belongs to the myrtle family. The essential oils of many eucalyptus species are widely used in the pharmaceutical and cosmetic industries due to their antibacterial and antioxidant properties. Numerous investigations have demonstrated the potent insecticidal effects of eucalyptus oil. The essential oil's preservation qualities enable it to prolong the shelf life of products in addition to being used as a flavouring enhancer. Several studies demonstrate its strong antibacterial properties and its ability to stop the growth of a variety of microorganisms. Oxygenated sesquiterpenes and both oxygenated and unoxygenated monoterpenes make up the majority of the essential oil. 1,8-cineole (eucalyptol), aromadendrene, globulol, D-limonene, and pinene are the main constituents of E. globulus essential oils; their concentration varies according to the plant's age, agronomic conditions, and plant parts (Topiar et al., 2015; Armando et al., 1997).

Pimenta dioica, commonly known as allspice, is reported to have several health-promoting effects. It is a type of aromatic plant that belongs to the family Myrtaceae and is native to the West Indies. There have also been reports of antibacterial and antioxidant qualities in the essential oil of the aromatic herb *Pimenta dioica* (Dima et al., 2014). The essential oil of P. dioica leaves from Jamaica contained eugenol, α-pinene, caryophyllene, limonene, and 1,8-

cineole [3], whereas the essential oil of P. dioica leaves from Sri Lanka contained eugenol (85.33%), β-caryophyllene (4.36%), cineole (4.19%), linalool (0.83%), and α-humulene (0.76%).

Cinnamomum verum is well-known for its medicinal and pharmacological properties. antimicrobial, wound-healing, antidiabetic, anti-HIV, anti-anxiety, and anti-Parkinson's diseases. Trans-cinnamaldehyde, cinnamyl acetate, eugenol, L-borneol, caryophyllene oxide, b-caryophyllene, L-borneol acetate, Enerolidol, alpha-cubebene, alpha-terpineol, terpinolene, and alpha-thujene are some of the essential oils found in cinnamon.

Storage grain pests are categorized into two kinds: primary and secondary storage pests. Primary storage pests can damage sound grains, and it includes *Sitophilus oryzae* (rice weevil), *Rhyzopertha dominica* (lesser grain borer), *Trogoderma granarium* (khapra beetle), and Callosobruchus chinensis (pulse beetle).Secondary storage pests damage broken or already damaged grains and include *Tribolium castaneum* (red flour beetle), *Oryzeaphilus surinamensis* (saw-toothed grain beetle), and *Cryptolestes pusillus* (flat grain beetle).

Sitophilus oryzae is the most damaging pest of stored grain and an internal feeder. mostly infesting maize, wheat, barley, rice, and sorghum. Damage is caused by both adults and grubs. Before harvesting and storing rice, sorghum, wheat, barley, and maize grains, developing larvae reside and feed inside the grain, creating irregular holes that are 1.5 mm in diameter. Weevils do more damage than they consume, resulting in large financial losses for grain storage facilities and farmers.

External feeders called Tribolium castenum infest dry fruits, legumes, wheat flour, and prepared cereal products like cornflakes. Damage is caused by both larvae and adults, with the most

severe damage occurring during the hot and muggy monsoon season. Food always contains larvae. As they pass through flour and other granular foods, adults create tunnels, which further contaminates and degrades the food.

When it comes to fumigant action, essential oils and their constituents may be superior to conventional fumigants due to their rapid degradation, local availability, and low toxicity to mammals. Compounds found in plant extracts exhibit ovicidal, repellant, antifeedant, sterilizing, and poisonous actions on insects (Nawrot and Harmatha, 1994; Isman, 2006).

Using plant products as grain protectants is one of the cost-effective and environmentally friendly ways to prevent insects from attacking the food grains that are being kept. The application of some native plant products as grain protectants has been reported with good results (Jotwani and Sircar, 1965; Saramma and Verma, 1971; Chander and Ahmed, 1983; Jacob and Sheila, 1990; Abdallah et al., 2001; Hassan, 2001; and Bhargava and Meena, 2002).

In this study, it was aimed to extract phytochemicals from the leaves of Eucalyptus, Allspice, and Cinnamon. Further, it was planned to evaluate the insecticidal activities of the extracted phytochemicals against stored product insect pests. Also to identify the moisture content, fat, and protein losses brought on by insect pests in stored grains.

2. REVIEW OF LITERATURE

The Food and Agriculture Organization of the United Nations (FAO) reports that 17% of the food produced worldwide is currently lost during storage (10% due to insects and 7% due to illnesses, mites, and rodents). The estimation shows that destruction by pests is thought to affect between 7% and 50% of all crops each year (Pimentel and Rattan 2009; Sallam 2013; Calliney et al. 2014; Oliveira et al. 2014). Insect pests attack a variety of crops throughout the year, resulting in losses of over $1 billion USD annually worldwide (Boyer et al., 2012). The Food and Agriculture Organization of the United Nations (FAO) reports that 17% of the food produced worldwide is currently lost during storage (10% due to insects and 7% due to illnesses, mites, and rodents). Pests like moths and storage beetles are threatening the world's food security.

Essential oils have a long history in medical and dietary uses and are “generally recognized as safe” even though they demonstrated toxic effects against stored product insects as well as agricultural pests. They may act as fumigants, contact insecticides, antifeedants, or repellents. Essential oils can be produced from different plant parts such as flowers, herbs, buds, leaves, fruit, twigs, bark, seeds, wood, rhizomes, and roots.

Essential oil-producing plants, often known as aromatic plants, are found all over the world and are divided into a small number of families: The families belonging to aromatic plants are Lauraceae, Rutaceae, Myrtaceae, Piperaceae, Poaceae, Cupressaceae, Asteraceae, and Lamiaceae (Svoboda and Greenway 2003; Bruneton 1999). EOs are made up of a mixture of 20 to 70 chemical compounds, some of which make up over 80% of the contents as an appendix. For example, limonene, the primary compound in Sweet Orange EO, makes up 88–97% of the entire

oil. In general, the primary constituents define the EOs' biological activity. EOs are soluble in organic solvents, hydrophobic, and typically lipophilic. They also have a density that is frequently lower than that of water.

According to Zebec et al. (2016), monoterpenes and sesquiterpenes, which are produced in the cytoplasm and plastids, make up the majority of essential oils. Research on the potential use of plant extracts as substitutes for synthetic insecticides has gained traction due to the documentation of the harmful effects of synthetic pesticides on the environment and human health as well as the stricter environmental regulations of pesticides (Isman, 2000). There are around 2000 plant species known to have some insecticidal properties (Klocke, 1989). These main components typically dictate the biological characteristics of the essential oils. Two groupings of different biosynthetic origins are among the constituents (Pichersky et al., 2006). The primary group is made up of terpenes and terpenoids, while the other group consists of aliphatic and aromatic components. All of these substances have a low molecular weight (Bakkali et al., 2008). There are four main commercial applications for plant essential oils: as pesticides, flavor enhancers in various food products, odorants in fragrances, and pharmaceuticals (Pushpanathan et al., 2006). Research is being done on the properties of essential oils, such as their ability to operate as insect growth regulators, fumigants, repellents, and antifeedants (Weaver and Subramanyam, 2000). These investigations demonstrated that essential oils and their components might be a viable substitute for the fumigants currently in use (Tunc et al., 2000). Of the more than 17,000 plant species identified globally, only 10% are categorized as aromatic plants due to the presence of essential oils. There are many fascinating applications for natural substances known as essential oils. Plants are used to extract essential

oils using both conventional and innovative methods. A variety of encapsulation techniques have been developed and published in the literature to encapsulate biomolecules, active chemicals, nanocrystals, oils, and essential oils for a variety of applications, including in vitro diagnostics, therapy, cosmetics, textiles, food, etc.

Essential oil extracted from Elsholtzia densa was tested against insect pests by Liang et al. in 2021. For *Tribolium castaneum* and Lasioderma serricorne, they assessed the toxicity of fumigant and contact insecticides. A total of 45 components, or 98.74 percent of the total essential oil, were identified using GC-MS. Acetophenone, 1,8-cineole, r-cymen-7-ol, 1-O-cerotoylglycerol, limonene, b-caryophyllene, r-cymene, trans-phytol, a-terpineol, linalool, and palmitic acid were all isolated from the essential oil in the meantime. The repelling properties of the essential oil and its chemical components differ. The significance of looking into these materials' potential for insecticidal activity and for enhancing human health is demonstrated in part by this work.

Around the world, eucalyptus essential oils are used extensively. The US Food and Drug Administration deems them safe and non-toxic, and the Council of Europe has even authorized their use as a food flavoring agent (Batish et al., 2008). Numerous studies have examined the antioxidant capacity of essential oils derived from different species of Eucalyptus, including *E. polyanthemos, E. perriniana*, and E. camaldulensis (Barra et al., 2010; Lee and Shibamoto, 2001; Singh et al., 2012). Antibacterial, larvicidal, fumigant, antioxidant, and anthelmintic qualities are attributed to the abundance of essential oils, flavonoids, or tannins found in eucalyptus leaves. 1,8-cineole (eucalyptol), aromadendrene, globulol, D-limonene, and pinene are the main

constituents of *E. globulus* essential oils; their concentration varies according to the plant's age, agronomic conditions, and plant parts (Topiar et al., 2015; Armando et al., 1997). Only one study has examined the essential oils from E. globulus leaves' ability to inhibit S. mutans (Goldbeck et al., 2014), and very few have documented the antioxidant activity of these oils (Mishra et al., 2010, Noumi et al., 2011). To manage stored product insects, plant volatile aldehydes are employed as natural pesticides. Garlic essential oils contain methyl allyl disulfide and diallyl trisulfide, which are utilized to keep pests out of stored goods. Di-n-propyl disulfide is a volatile component that was taken from di-n-propyl disulfide, a volatile component, was isolated, and used as a fumigant against adults of *Sitophilus oryzae* and adults and larvae of *Tribolium castaneum*. In both insects, di-n-propyl disulfide moderately inhibited food consumption while dramatically reducing growth rate and dietary use.

Investigated the repellent effects of P. dioica leaf essential oils by contact and fumigation on adult C. maculatus. The results showed that all concentrations produced noticeably greater repellent effects than the control. With an increase in concentration, contact and fumigation repellency gradually surged. Strong contact and fumigation repellent effects were demonstrated by the maximum concentration, which produced 98.0 and 92.0 rates, respectively. More than 50% repellant activity was produced in both experiments at the lowest concentration. According to contact and fumigation toxicity results, the beetle died 100% of the time after two and twelve hours of exposure. The fact that more than 80% of adult deaths for both toxicity tests occurred just 30 minutes after treatment was also very striking. The essential oil is quite effective as a contact and a fumigation toxicant, as evidenced by the relatively low LC50 values of 0.3 (v/v%) for contact toxicity after 12 hours and 0.3 v/v% for fumigation toxicity after 2 hours of exposure,

according to the probit analysis. Accordingly, these results support P. dioica's extremely successful role in controlling C. maculatus in storage. Therefore, it can be stated that the essential oil of P. dioica elicited a higher toxic effect within a very short period of time. The essential oil of *Pimenta dioica* contains eugenol, pinene, caryophyllene, cineole, linalool, and methyl eugenol, all of which have significant insecticidal properties. A relatively comparable investigation into phytochemical elements has shown that P. dioica essential oil contains eugenol, α-pinene, caryophyllene, 1,8 cineole, linalool, and humulene (Dharmadasa et al., 2015).

Using the usual procedures previously outlined in the Bhavya et al. article, the fumigant impact of C. verum's leaf and flower essential oils was assessed. The repellent properties of C. verum's essential leaves and flowers were assessed using the methods outlined by Kłyś et al. The techniques of Patiño-Bayona et al. were used to conduct contact toxicity profiles of the various essential oils. Aedes aegypti, Armigeres subalbatus, and Culex tritaeniorhynchus mosquito cultures were gathered and kept in typical atmospheric conditions. Each mosquito's larval stages were kept in glass jars, and the larvicidal investigations were conducted on mosquitoes in their third instar of development. Each culture was divided into about 50 larvae, which were then placed in separate glass chambers with varying amounts of essential oils (0–100 µg/mL) and left for 24 hours. The LC50 value was calculated by counting the average fatality in each concentration. The experiment was carried out in triplicate three times each. The antibacterial activity of the essential oils from the flowers and leaves was assessed against several bacterial and fungal species. As previously mentioned, the antibacterial activity was measured using the lowest inhibitory concentration and the agar disc-diffusion method. To guarantee accuracy, the experiment was carried out in triplicate and five times. The study's microbiological strains,

which included Salmonella enteritidis, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli, were pertinent to human disorders.

The toxicological impact of synthetic materials prompted fresh research on the use of essential oils as natural preservatives and antioxidants in the pharmaceutical, dietary supplement, and food processing industries (Wei and Shibamoto, 2007). Over time, the use of different synthetic pesticides and fumigants at grain storage facilities has resulted in a number of issues, such as chemical insecticide residues in food (Longobardi et al., 2008; Bilgin et al., 2009; Phillips and Throne, 2010) and insecticide and fumigant resistance (Lorini et al., 2007; Sousa et al., 2009). The most widely used fumigants that quickly destroy insect pest life stages in storage facilities are methyl methylbromide, formate, and sulfuryl fluoride (Isman 2006). The widespread use of synthetic pesticides during storage may have been exacerbated by factors such as market availability, convenience of handling, high residual activity, and broad-spectrum insect activity (Rajashekar et al., 2012). According to Adesina and Ofuya (2015) and Khani and Heydarian (2014), frequent and repeated use of these chemical compounds has been shown to have a number of negative effects on human health and survival, as well as the development of insect resistance and ecological imbalance. Since the 1980s, plants or plant parts that are readily available in the area have been utilized as botanical pesticides to preserve stored grains for three to four months (Talukder, 2009). Numerous plant powders, extracts, and essential oils have been shown to have insecticidal properties against cowpea beetles and other storage insects, including oviposition deterrents, toxicants, repellents, and anti-feedents (Isaman 2006). Due to their low toxicity to mammals, quick breakdown, and local availability, plant essential oils have demonstrated numerous advantages over traditional pesticides (Pugazhvendan et al. 2012).

Insect infestation alters the protein and amino acid content, the amount of carbohydrates and fats that are available, and the organoleptic properties of food that has been kept. Moreover, the presence of insect populations in preserved foods often leads to microbial contamination. Fungi, like aflatoxins, can produce mycotoxins that harm food safety and quality.

3. OBJECTIVES

- Evaluate the insecticidal activity of eucalyptus, allspice, and cinnamon essential oils against *Sitophilus oryzae* and *Tribolium castaneum*.
- Compare the fumigant toxicity of these essential oils against S. oryzae and *Tribolium castaneum*in food grain conditions.
- Assess the efficacy of combined essential oils against *S. oryzae* in non-food conditions.
- Analyze the impact of treatments on moisture, protein, and fat content in food grains.

4. MATERIALS AND METHODS

4.1 Food grains

The wheat and wheat flour were procured from the local market, Mysuru, Karnataka. The grains were stored at -20°C for disinfestation until the use for insect culture and experiments. The wheat grain (*Triticum aestivum*) and wheat flour were dried in sunlight and conditioned before using for insect culturing.

4.2 Culturing of insects

Sitophilus oryzae

The stock culture of *Sitophilus oryzae* was obtained from the infested stocks and was maintained on grains in 2-kg capacity glass jars covered with muslin cloth. Insect (*S. oryzae*) culture was maintained at the insect culture unit of the Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute, Mysuru. The test insects were cultured on wheat grains with controlled atmospheric temperature (27 ± 2°C), relative humidity (75 ± 5%), and 13:11 light–dark photoperiod conditions.

Tribolium castaneum

The collection of the stored grain insect pest was done from the insectary of the Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute. The *Tribolium castaneum* were cultured on wheat flour (Triticum aestivum) in a 2-kg capacity glass jar covered with muslin cloth. The insect culture of *Tribolium castaneum* was maintained at the

insect unit of the Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute, Mysore, Karnataka. The culture was maintained at controlled atmospheric temperature (27 ± 2°C), relative humidity (75 ± 5%), and 13:11 light-dark photoperiod condition.

Figure 1. Insect culture – *S. oryzae*, (Wheat grains), *T. castaneum* (wheat flour)

4.3 Collection and preparation of plant materials

Pimenta dioica, Cinnamomum verum, Eucalyptus globulus

Healthy, mature leaves of *Pimenta dioica*, *Cinnamomum verum,* and *Eucalyptus globulus* were collected from Paravur, Kerala, for essential oil extraction. Harvesting was done in the morning, when essential oil content is typically highest. Care was taken to gently pluck the leaves without damaging the plants, avoiding leaves that were discolored or damaged. The collected leaves were rinsed thoroughly under cool water to remove dust and debris. After cleaning, they were dried using a clean cloth or paper towel to prepare them for further processing. This careful selection and preparation ensured the leaves retained their natural properties, optimizing them for essential oil extraction.

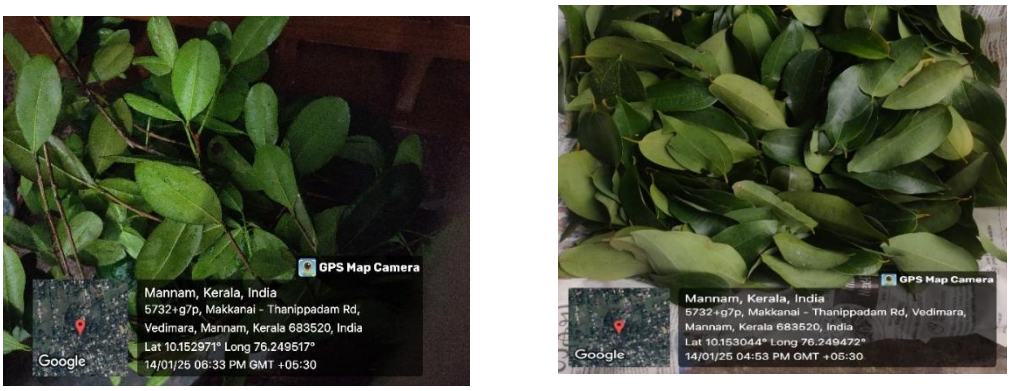

Figure 2. Aromatic plants – *Cinnamomum verum*(Ceylon cinnamon), *Pimenta dioica* (Allspice

4.4 Extraction and isolation of essential oil

Fresh and healthy leaves of *Pimenta dioica*, *Cinnamomum verum.* and *Eucalyptus globulus* were collected from Paravur, Kerala, during optimal growth conditions. The leaves were carefully selected to exclude any that were damaged, discolored, or infected. After collection, the plant

materials were thoroughly rinsed under a steady flow of tap water to remove surface dirt, dust, and other contaminants. Once cleaned, the leaves were air-dried at room temperature (25–27 °C) for two to three days, ensuring partial moisture removal while preserving the natural properties of the samples. The dried leaves were then cut into small uniform pieces, approximately 2–3 cm in length, using a Secateur. Precisely 1 kg of prepared plant material was weighed and combined with 4 L of distilled water in a distillation apparatus. The mixture underwent steam distillation at 60 °C using a Clevenger-type apparatus (Borosil, India) for three to four hours to extract the essential oils. Post-distillation, the oils were dried using anhydrous sodium sulfate (HiMedia, India) to remove any residual moisture. The purified essential oil was filtered and transferred into amber glass vials to protect it from light. The samples were refrigerated at 4 °C until further analysis and bioassay testing (Devi et al., 2021).

$$\text{Percentage yield} = (\text{ Volume of oil extracted} / \text{Weight of sample taken }) \times 100$$

Figure 3. Essential oil extraction using cleavenger apparatus

4.5 Analysis of chemical profile of essential oils from GCMS

For the GC-MS analysis, helium was utilized as the carrier gas, and the system featured an Elite-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) coupled with an Agilent GC-MS system (Norwalk, CT, USA). The ionization energy was configured at 70 eV for GC-MS detection. The injector temperature and the mass transfer line were both maintained at 250 °C. Helium was delivered at a consistent flow rate of 1 mL/min, with an injection volume of 0.5 μL per sample, prepared as a 1:100 dilution of the essential oil in hexane. The column temperature was programmed to start at 40 °C and held for 1 minute before increasing at a heating rate of 5 °C/min to 250 °C, where it was maintained for 20 minutes. This temperature program ensured optimal separation of volatile compounds. Compound identification was performed by analyzing mass spectra and retention indices (Kovats index) of the sample components. Matches were validated against the NIST mass spectral database, ensuring a relative abundance match criterion of over 40%. Reference standards were also used to confirm the identities of key compounds, ensuring accurate and reliable profiling of the essential oils.

Figure 4. Analysis of chemical profile of essential oils from GCMS

4.6 Fumigant toxicity bioassay for adults Insects

The fumigant activity of essential oils was assessed against adult insects of two stored grain pest species, following the protocol outlined by Rajashekar et al. (2016). Essential oil concentrations ranging from 50 to 100 µL/L air were tested to evaluate their toxic effects. Preliminary experiments were conducted to determine the effective dose range for the bioassays.

For each dose, 10 adult insects of each species were introduced into 1 L glass jars, which served as fumigation chambers. Filter paper discs impregnated with the essential oil were placed on porcelain plates positioned centrally within the jars. This setup ensured no direct contact between the insects and the filter paper while providing sufficient surface area for the evaporation of the oil. The required doses of essential oils were delivered using a gas-tight microsyringe, injected through a rubber septum fitted to the lid of the jars. Each dose and control treatment was replicated four times. Control jars were maintained under identical conditions without essential

oil application. The jars were incubated at 28 ± 2 °C and 70% relative humidity for an exposure duration of 24 hours.

After exposure, insects were transferred to clean vials containing food media to observe mortality. Insects were considered dead if they exhibited no movement upon gentle probing or exposure to mild heat under light. Mortality rates were recorded for each dose, providing insights into the fumigant efficacy of the essential oils against the pests.

Figure 5. Experimental setup for fumigant toxicity bioassay

4.7 Preparation of mixed age culture

The rearing of *Tribolium castaneum* and *Sitophilus oryzae* was carried out under controlled conditions. *Tribolium castaneum* was cultured on whole wheat flour supplemented with 5% dried yeast, while *S. oryzae* was reared exclusively on whole wheat grains. All insect cultures were maintained at a constant temperature of 25 ± 1 °C and relative humidity of 65 ± 10%.

Adults aged 1–2 weeks were collected from these cultures to establish mixed-age populations. Approximately 300 adults of *Tribolium castaneum* were introduced into 1 kg of the designated

culture medium in 2-liter glass jars. Similarly, 300 adults of *S. oryzae* were released into 1 kg of whole wheat grains in separate jars. The adults were allowed to breed for one week, after which they were removed to allow the subsequent development of immature stages. This process was repeated continuously to maintain active cultures.

Cultures of each species were maintained in six consecutive age groups (5–6, 4–5, 3–4, 2–3, 1–2, and 0–1 weeks old). These age groups were pooled together to create mixed-age populations, ensuring the presence of all developmental stages of the respective species. These mixed-age cultures were then used for toxicity assessments.

For fumigation experiments, 50 g portions of the mixed-age cultures were weighed and transferred into cloth bags measuring 20 × 14 cm. Each bag was placed in individual 0.85 L desiccators, which served as fumigation chambers for the bioassay tests.

4.8 Fumigant toxicity bioassay for mixed age culture

To evaluate the fumigant toxicity of essential oils, insects were exposed to varying doses of essential oils ranging from 42.5 to 425 µg/L for durations of 24 and 72 hours at a controlled temperature of 26 ± 2 °C. Each dose was tested with five replicates for both species, alongside an equal number of untreated control replicates. Gas-tight microsyringes were used to accurately inject the essential oil doses into the fumigation chambers.

After the exposure period, fumigation was terminated, and the insect-containing cloth bags were removed from the desiccators. The contents of each bag were carefully transferred into individual rearing bottles measuring 12 × 5 cm. These bottles were maintained under standard rearing conditions (temperature and humidity) for a subsequent observation period of eight weeks.

Emerging insects (*Sitophilus oryzae*) or surviving adults (*Tribolium castaneum*) were counted weekly for eight weeks to monitor post-exposure effects. Counts were also conducted in the untreated control groups to provide a baseline for comparison. The percentage of mortality was calculated by considering the survival or emergence rates in the control group as 100%.

In each bioassay, mortality was determined by probing insects lightly. Those showing no movement, even when exposed to light or mild heat, were classified as dead. This methodology provided precise insights into the fumigant activity of the essential oils against the test insect species.

Figure 6. Experimental setup for contact toxicity bioassay

4.9 Repellent activity of Essential oils

The repellent activity of essential oils (EOs) from Eucalyptus, Allspice, and Cinnamon was evaluated against *Sitophilus oryzae* adults using a filter paper disc method. Circular filter paper discs, 9 cm in diameter, were cut into two semicircles. For each essential oil, a test solution of 5 µL/L diluted with 300 µL/L acetone was prepared, with three replicates and one control per EO.

On one semicircle, 300 µL/L of the EO solution was evenly applied, while the other semicircle was treated with acetone alone as the control. After allowing the acetone to evaporate completely, the two semicircles were carefully joined with duct tape to form a complete disc. The treated filter papers were then placed in petri dishes.

Ten adult *S. oryzae* insects were released at the center of each disc, ensuring equal exposure to the treated and control sides. Observations were recorded to assess the distribution of insects, indicating the repellency of each EO.

Figure 7. Repellent activity of essential oils

4.10 Fumigant toxicity of combination of essential oils

Fumigant toxicity of a combination of essential oils (eucalyptus, allspice, and cinnamon) was conducted against rice weevil (*Sitophilus oryzae*) in 30 ml vials. 20 insects were introduced in each vial. Binary mixtures of EOs of Eucalyptus , Allspice , and Cinnamon were in the ratio of 75:25, 25:75, and 50:50, respectively. The essential oils were loaded on Whatman No. 1 filter paper (dimension 1 cm × 1 cm) and pasted on the lid of each desiccator. Four replicates are kept for each concentration. After 48 hours of treatment, the insects' mortality was noted.

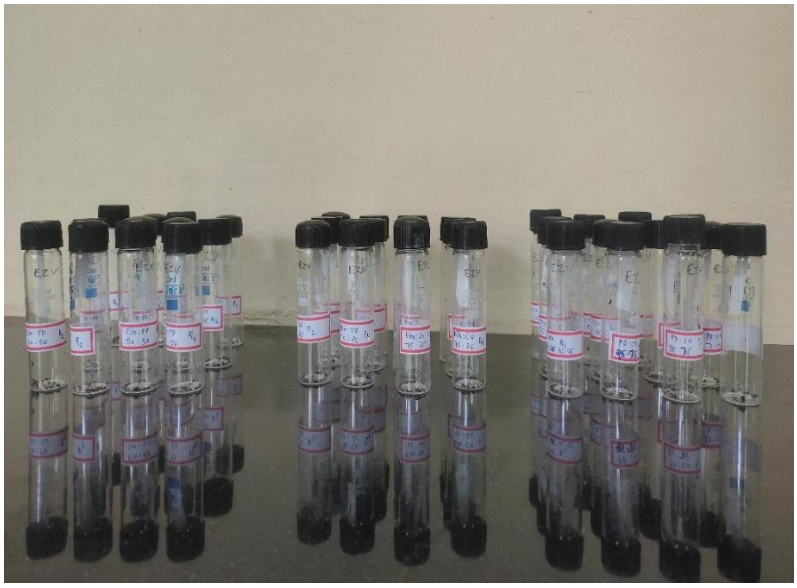

Figure 8. Experimental setup for fumigant toxicity bioassay of combination of Eos

4.11 Moisture Analysis

Eucalyptus, allspice, and cinnamon essential oils' repellent activities were assessed using the method of (McDonald et al.,1970). Two semicircles have been cut out of the 9 cm diameter filter paper discs. For each EO, 3 duplicates and 1 control were made, and 5 µl/L was diluted with 300 µl/L acetone. One side of the disc was evenly covered with 300 µl/L of acetone. The two semicircles were connected with duct tape when the solvent had evaporated, and they were subsequently put in the petri dishes. In the middle of each disk was a batch of ten mature insects

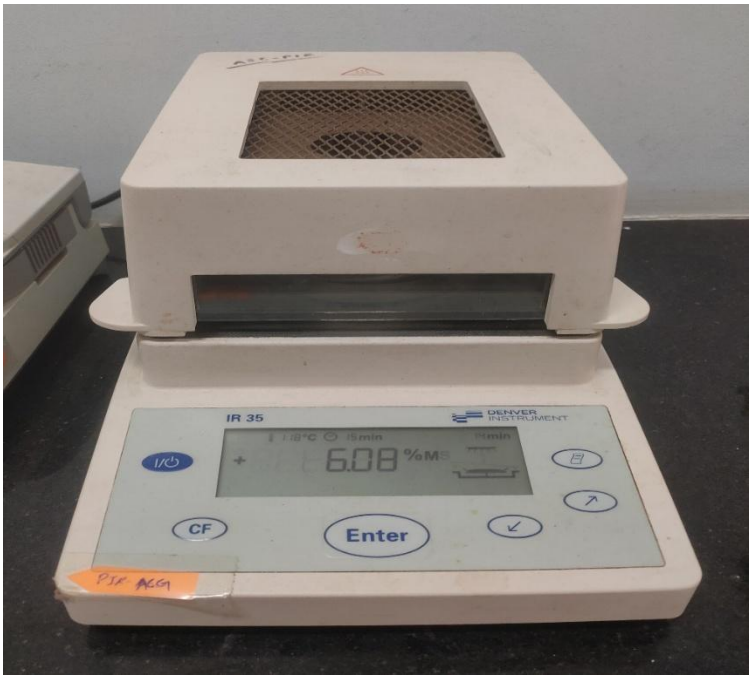

Figure 9. Moisture Analyzer

4.12 Protein estimation of wheat grains

The protein content in treated wheat grains was analyzed using an N-protein analyzer (NIR Analyzer) at CIFS, CSIR-CFTRI, Mysore, Karnataka. Cleaned and ground samples were prepared by weighing approximately 2 g of each sample on an analytical balance and placing them in tin containers for N-protein and N-brew analysis. The tin containers were securely sealed, labeled with tray and sample numbers, and recorded in the system. The prepared sample tray was then placed in the analyzer, which was calibrated before processing. After 6 hours, the results were displayed on the system and documented accordingly.

Figure 10. N-Protein analyzer

4.13 Statistical analysis

Percentage mortality was obtained by employing Abbott formula equation (1925) and values of LC 50 with associated confidence limits by probit analysis (Finney 1971) through application of Statplus 2007 software statistical package.

5. RESULTS

5.1 GC – MS analysis and chemical composition of three essential oils

Eucalyptus globulus

The leaves of *Eucalyptus globulus* yielded 25 ml of a yellow oil with an pleasant odour. The GC-MS analysis identified 42 compounds (Table 1; Fig 12). The major EO components were Eucalyptol (16.72%), α-Pinene (13.02%), 2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8- methyle (10.35%), Bicyclo[3.1.1]heptane, 6,6- dimethyl-2-methylene-, (1S)- (11.26%), 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8- (8.56%), o-Cymene (5.01%), D-Limonene (4.18%), 3-Cyclohexene-1-methanol, α,α,4- trimethyl-, (R)- (3.88%), 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8- (4.36%), Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methylethyl)- (3.45%).

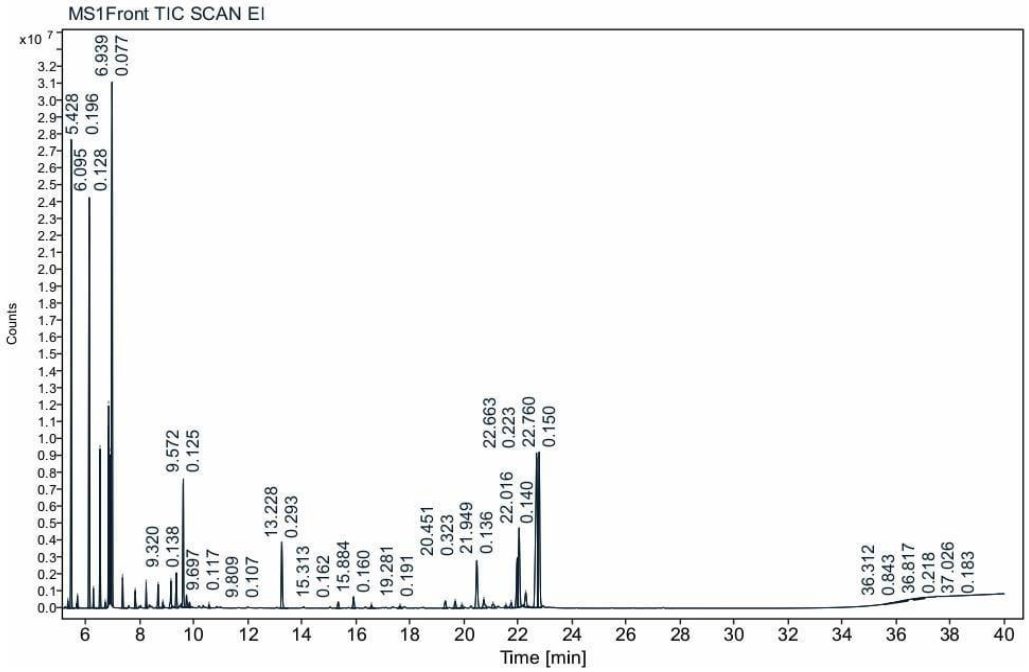

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Figure 12. GC - MS result of *Eucalyptus globulus*

Table 1. GC-MS result of *E. globulus*

Peak No:	RT	Component	RSI	RA
1	5.303	Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methylethyl)-	961	0.1690
2	5.428	α-Pinene	929	13.0278
3	5.656	Camphene	949	0.2682
4	6.095	Bicyclo[3.1.1]heptane, 6,6- dimethyl-2-methylene-, (1S)-	930	11.2628
5	6.247	β-Myrcene	911	0.4168
6	6.493	Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methylethyl)-	916	3.4506
7	6.682	Cyclohexene, 1-methyl-4-(1- methylethylidene)-	924	0.1352

8	6.809	o-Cymene	934	5.0178
9	6.884	D-Limonene	934	4.1821
10	6.939	Eucalyptol	965	16.7277
11	7.330	γ-Terpinene	947	0.6890
12	7.796	Cyclohexene, 3-methyl-6-(1- methylethylidene)-	941	0.4592
13	8.202	Fenchol	946	0.5898
14	8.643	Bicyclo[3.1.1]heptan-3-ol, 6,6- dimethyl-2-methylene-, [1S-(	947	0.6970
15	8.821	5-Isopropyl-2- methylbicyclo[3.1.0]hexan-2-ol #	819	0.1593
16	9.119	endo-Borneol	940	0.8791
17	9.320	Terpinen-4-ol	902	1.2110
18	9.572	3-Cyclohexene-1-methanol, α,α,4- trimethyl-, (R)-	922	3.8866
19	9.697	Bicyclo[3.1.1]hept-2-ene-2- methanol, 6,6-dimethyl	925	0.4294
20	9.809	Bicyclo[3.1.0]hexan-3-ol, 4- methylene-1-(1-methylethyl)-, (	908	0.1789
21	10.527	Bicyclo[3.1.0]hexan-3-ol, 4- methylene-1-(1-methylethyl)-, (	914	0.1268
22	13.228	α-Terpinyl acetate	952	2.5473
23	15.313	Caryophyllene	941	0.2782
24	15.884	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methyle	952	0.5371
25	16.548	Alloaromadendrene	951	0.1830
26	17.603	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,	888	0.1599
27	19.281	Cyclohexanemethanol, 4-ethenylα,α,4-trimethyl-3-(1-methyle	941	0.3664

28	19.646	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyc	954	0.3397
29	19.899	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclo	889	0.2120
30	20.451	(-)-GlobuloL	961	2.5815
31	20.706	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-,	916	0.5656
32	21.045	2-((4aS,8R,8aR)-4a,8-Dimethyl3,4,4a,5,6,7,8,8a-octahydr	874	0.3228
33	21.525	β-Guaiene	823	0.1592
34	21.720	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8- methyle	853	0.2888
35	21.949	2-((2R,8R,8aS)-8,8a-Dimethyl1,2,3,4,6,7,8,8a-octahydronaph	911	2.5486
36	22.016	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8-	948	4.3690
37	22.260	Hinesol	957	0.9053
38	22.663	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8- methyle	935	10.3537
39	22.760	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8-	896	8.5614
40	36.312	Cyclotrisiloxane, hexamethyl-	932	0.3927
41	36.817	Cyclotrisiloxane, hexamethyl-	925	0.2109
42	37.026	Cyclotrisiloxane, hexamethyl-	936	0.1528

Pimenta dioica

The leaves of *Pimenta dioica* yeilded 15 ml. The GC-MS analysis reported 28 components (Table 2; Fig 13) and the major compounds obtained were 3-Allyl-6-methoxyphenol (59.83%), Tris (tertbutyldimethylsilyloxy)arsane (12.76%), Limonene (7.46%), 2-Allylphenol (7.42%), 1-Octen-3-ol (2.14) and some of minor components are Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methyle (1.52%), (1S)-2,6,6- Trimethylbicyclo[3.1.1]hept-2-ene 5 (1.04%).

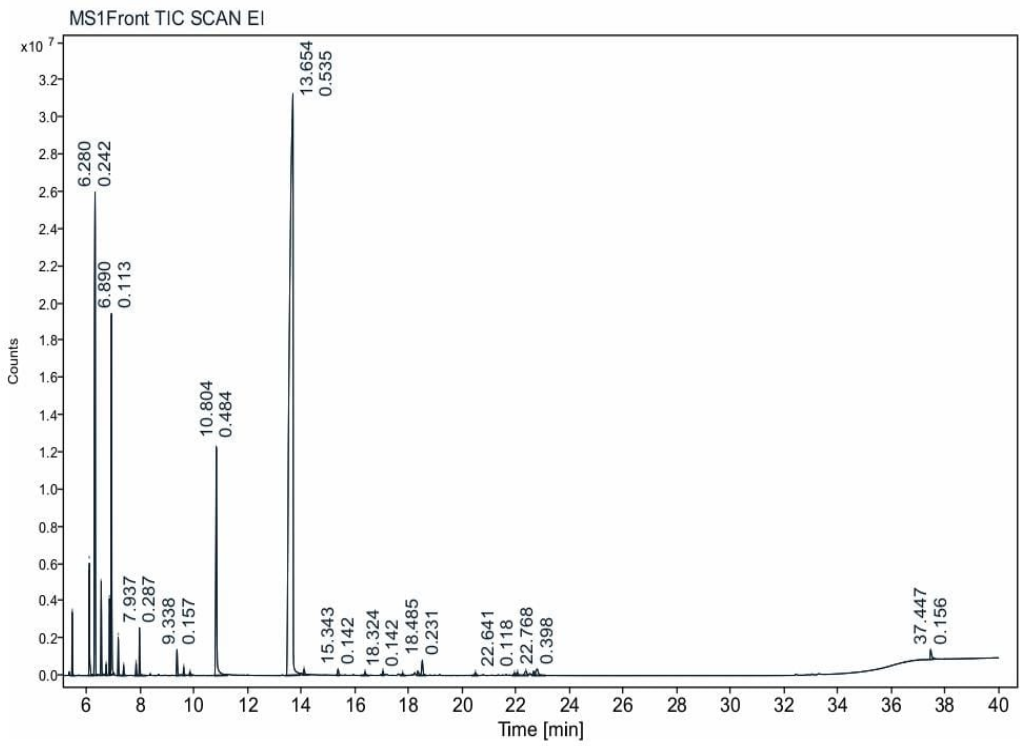


Figure 13. GC-MS result of *Pimenta dioica*

Table 2. GC-MS result of *Pimenta dioica*

Peak No:	RT	Component	RSI	RA
1	5.429	(1S)-2,6,6- Trimethylbicyclo[3.1.1]hept-2-ene 5	932	1.0463
2	6.064	1-Octen-3-ol	964	2.1472
3	6.280	Tris(tertbutyldimethylsilyloxy)arsane	714	12.7610
4	6.507	Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methyle	922	1.5289
5	6.694	Cyclohexene, 1-methyl-4-(1- methylethylidene)-	921	0.1829
6	6.817	Benzene, 1-methyl-3-(1- methylethyl)-	928	1.3107
7	6.89	Limonene	931	7.4674
8	7.143	β-Ocimene	950	0.6564
9	7.342	γ-Terpinene	940	0.1733

10	7.808	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	934	0.2440
11	7.937	Linalool	947	0.8676
12	9.338	Terpinen-4-ol	930	0.5880
13	9.585	L-α-Terpineol	916	0.2116
14	9.819	Decanal	851	0.1226
15	10.804	2-Allylphenol	888	7.4207
16	13.654	3-Allyl-6-methoxyphenol	949	59.8327
17	14.075	Copaene	880	0.2462
18	15.343	Caryophyllene	927	0.2276
19	16.350	1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z	892	0.1456
20	17.017	γ-Muurolene	950	0.1490
21	17.749	Naphthalene, 1,2,4a,5,6,8ahexahydro-4,7-dimethyl-1-(1-meth 1	939	0.1060
22	18.324	3-Cyclohexene-1-carboxaldehyde, 4-(4-methyl-3-pentenyl)-	868	0.1720
23	18.485	Cadina-1(6),4-diene,t rans-	879	0.6134
24	20.466	(-)-Globulol	885	0.1395
25	21.919	4a(2H)-Naphthalenol, 1,3,4,5,6,8a hexahydro-4,7-dimethyl-1-	888	0.1232
26	22.033	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,	885	0.1618
27	22.350	.tau.-Cadino	918	0.2760
28	22.641	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8- methyle	899	0.2016

Cinnamomum verum

The cinnamon EO yielded 20ml, in which the GC-MS reported 15 components (Table 3; fig 14). The major compounds identified were Methyleugenol (51%), (Z)-3-Phenylacrylaldehyde (14.29%), (Z)-3-Phenylacrylaldehyde (12.49%), Acetic acid, cinnamyl ester (10.31%), 3-Allyl-6-methoxyphenol (6.09%), The minor component were Caryophyllene (1.80).

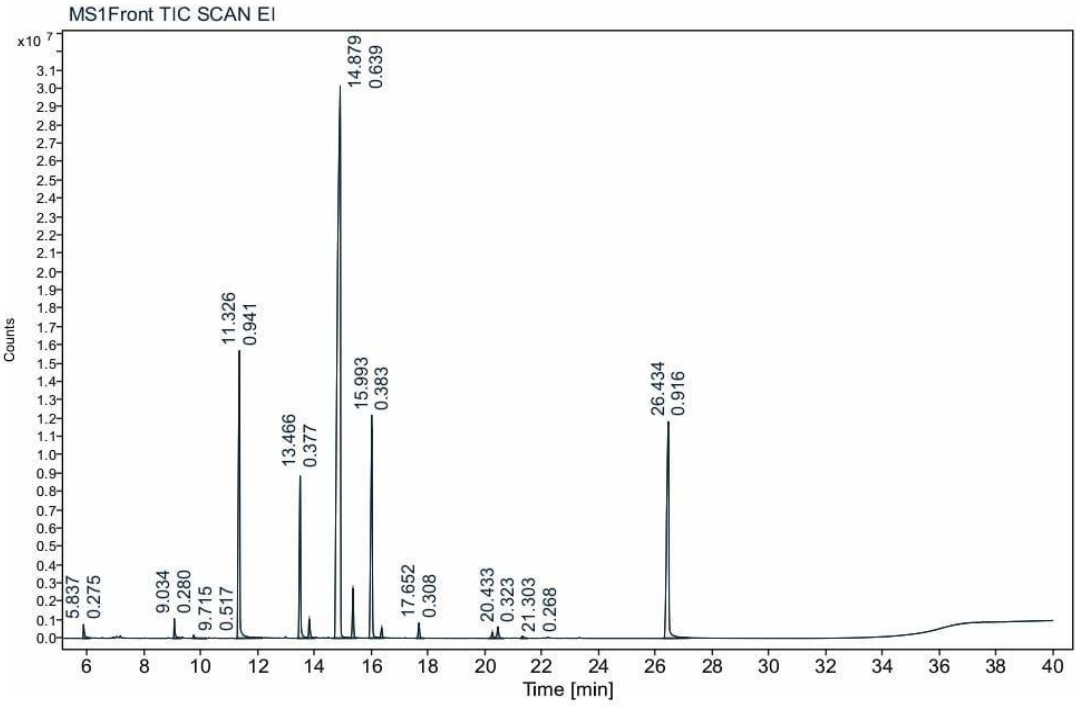


Figure 14. GC-MS result of *Cinnamomum verum*

3. GC-MS result of *Cinnamomum verum*

Peak No:	RT	Component	RSI	RA
1	5.837	Benzaldehyde	938	0.47
2	9.034	Benzenepropanal	914	0.652
3	9.715	Estragole	938	0.123
4	11.326	(Z)-3-Phenylacrylaldehyde	962	12.497
5	13.466	3-Allyl-6-methoxyphenol	950	6.098
6	13.79	3-Phenyl-1-propanol, acetate	919	0.7537
7	14.879	Methyleugenol	931	51.0050
8	15.330	Caryophyllene	957	1.8047
9	15.993	Acetic acid, cinnamyl ester	954	10.3103
10	16.338	1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z-	912	0.4102
11	17.652	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2	940	0.6210

12	20.236	(-)-Spathulenol	901	0.2678
13	20.433	Caryophyllene oxide	904	0.5708
14	21.303	(1R,3E,7E,11R)-1,5,5,8- Tetramethyl-12-oxabicyclo[9.1.0]dode	845	0.1128
15	26.434	Methyleugenol	917	14.2916

5.2 Fumigant toxicity bioassay of essential oils of adults of stored grain insects

The three essential oils were evaluated against *S. oryzae* adults and the lethal concentration (LC_{50} of Eucalyptus globulus, *Pimenta dioica* and *Cinnamomum verum* was 43 µl/L, 64 µl/L and 85 µl/L at 24 h exposure (Table). For *Tribolium castaneum* LC_{50} values for three Eos was 21 µl/L , 43 µl/L, 51 µl/L, 60 µl/L, 64 µl/L respectively. The mortality data was presented in the tables 4,5,6,7,8 and 9. Eucalyptus EO oils showed 100% mortality against *T.castaneum* at the concentrations of 21µl/L, 43 µl/L, 64 µl/L at 24 h exposure.

Table 4. Evaluation of fumigant toxicity of eucalyptus essential oil against *S. oryzae*

Eucalyptus Concentration	Mean ± SD
85 µl/L	100.00 ± 0.00 %
64 µl/L	90.00 ± 20.00 %
43 µl/L	68.75 ± 21.65 %

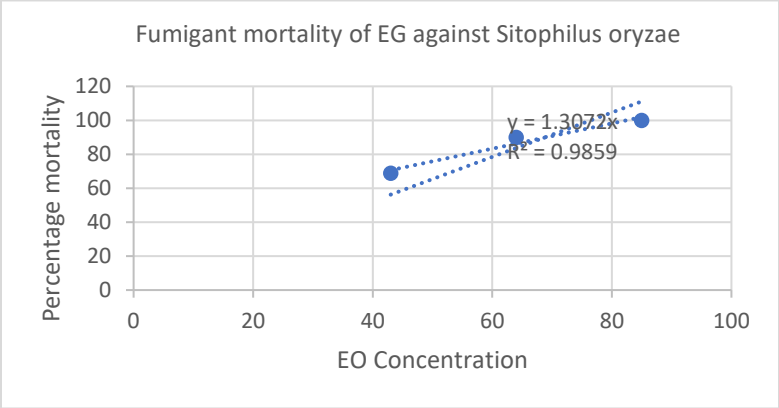


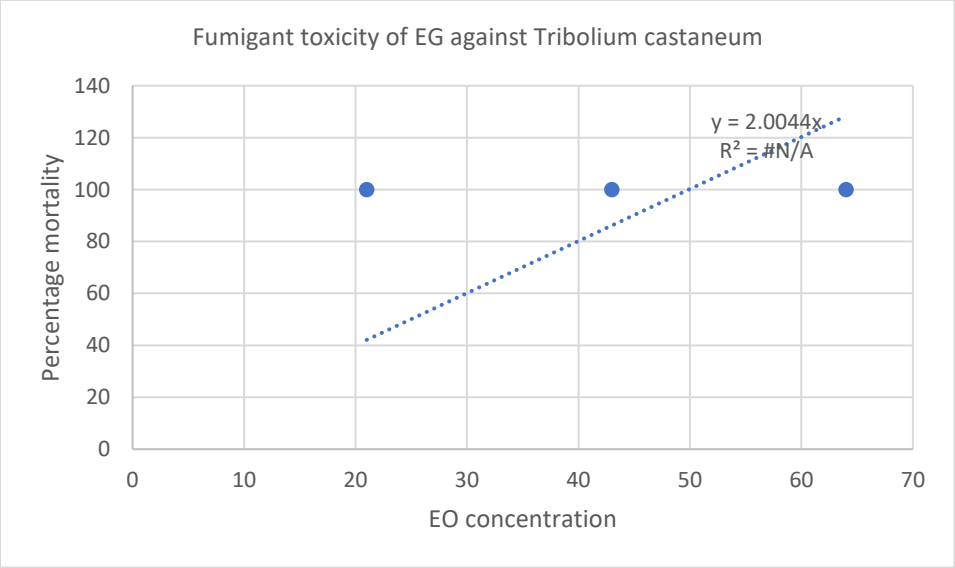
Figure 15. Fumigant mortality of EG against Sitophilus oryzae

LC_{50} = 38.2497

LC_{90} = 68.8494

Table 5. Evaluation of fumigant toxicity of eucalyptus essential oil against *T. castaneum*

Eucalyptus Concentration	Mean ± SD
21 µl/L	100.00 ± 0.00 %
43 µl/L	100.00 ± 0.00 %
64 µl/L	100.00 ± 0.00 %



LC50 = 24.9451

LC90 = 44.9012

Figure 16. Fumigant toxicity of EG against *Tribolium castaneum*

Table 6. Evaluation of fumigant toxicity of allspice essential oil against *S. oryzae*

Allspice Concentration	Mean ± SD
85 µl/L	100.00 ± 0.00 %
64 µl/L	86.25 ± 6.29 %
43 µl/L	57.50 ± 9.01 %

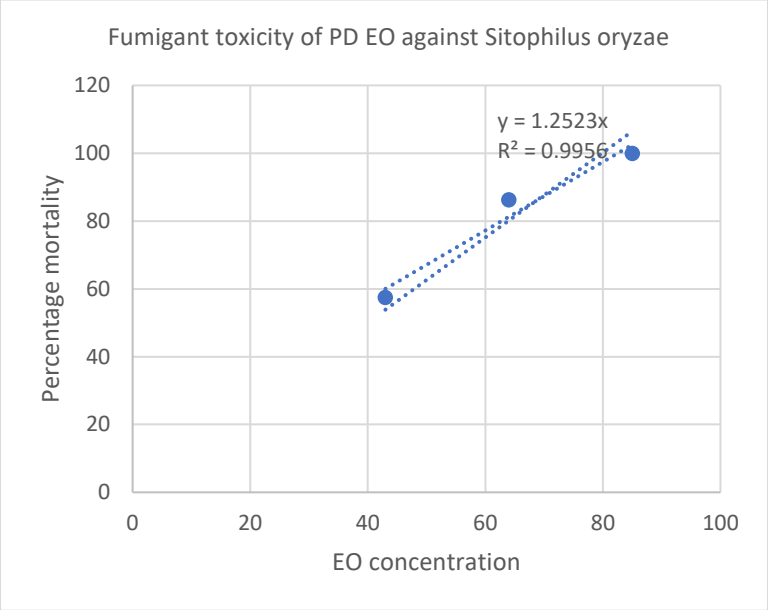


Figure 17. Fumigant toxicity of PD EO against Sitophilus oryzae

LC_{50} =39.92654

LC_{90} = 71.86776

Table 7. Evaluation of fumigant toxicity of allspice essential oil against *T. castaneum*

Concentration	Mean ± SD
60 µl/L	56.25 ± 28.44 %
51 µl/L	55.00 ± 27.39 %
43 µl/L	62.50 ± 6.45 %

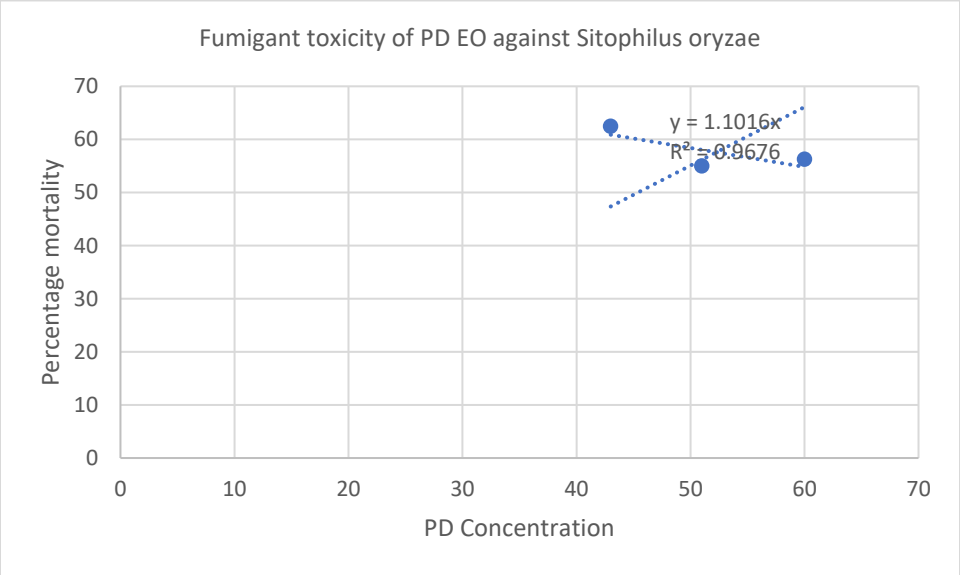


Figure 18. Fumigant toxicity of PD EO against Sitophilus oryzae

$LC_{50} = 45.38853$

$LC_{90} = 81.69935$

Table 8. Evaluation of fumigant toxicity of cinnamon essential oil against *S. oryzae*

Concentration	Mean ± SD
85 µl/L	22.50 ± 6.45 %
64 µl/L	23.75 ± 4.79 %
43 µl/L	23.75 ± 6.29 %

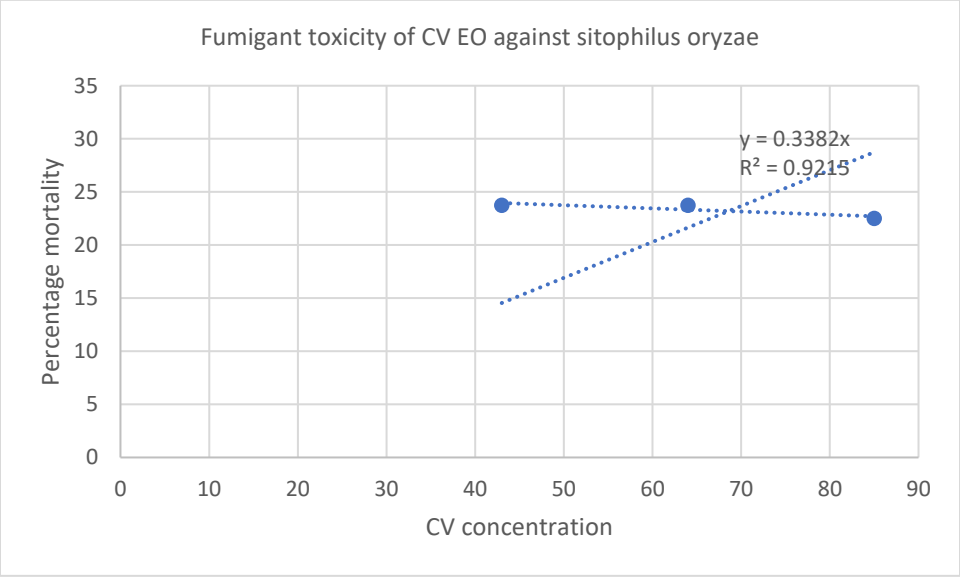


Figure 19. Fumigant toxicity of CV EO against sitophilus oryzae

LC_{50} = 147.8415

LC_{90} = 266.1147

Table 9. Evaluation of fumigant toxicity of cinnamon essential oil against *T. castaneum*

Concentration	Mean ± SD
127.5 µl/L	15.00 ± 4.08 %
85 µl/L	7.50 ± 8.54 %
43 µl/L	2.50 ± 2.89 %

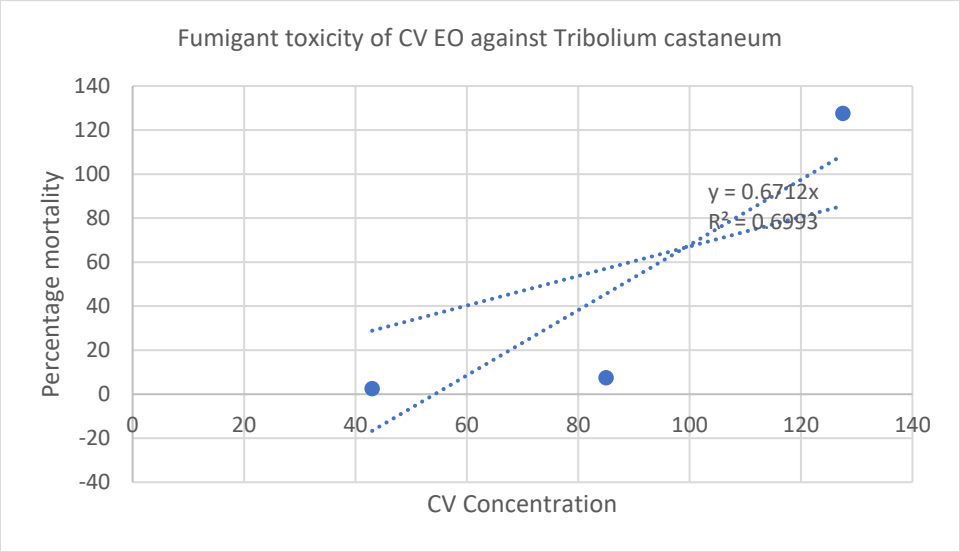


Figure 20. Fumigant toxicity of CV EO against *Tribolium castaneum*

LC_{50} = 74.49344

LC_{90} = 134.0882

5.3 Contact toxicity bioassay of essential oils of mixed age culture

Contact toxicity of Eucalyptus, Allspice and Cinnamon essential oils were evaluated against *S. oryzae* and *T. Castaneum* adults. The mortality data was presented in the tables 10, 11, 12 13, 14 and 15

Table 10. Evaluation of contact toxicity of eucalyptus essential oil against *S. oryzae*

Amount of Grains	Eucalyptus Concentration	% Mortality
50g	340 µl/L	100%
		97.56%
		100%
		100%
		99.39% ± 1.22
50g	170 µl/L	100%
		100%
		100%
		100%
		100% ± 0.00
50g	85 µl/L	100%
		100%
		100%
		100%
		100% ± 0.00

Table 11. Evaluation of contact toxicity of eucalyptus essential oil against *T. castaneum*

Amount of Flour	Eucalyptus Concentration	% Mortality
50g	340 µl/L	22.20%
		15.87%
		16.27%
		15.49%
		17.45% ± 2.86
50g	170 µl/L	1.58%
		13.88%
		12.50%
		10.10%
		9.55% ± 5.33
50g	85 µl/L	4.54%
		1.85%
		11.76%
		13.46%
		7.90% ± 5.27

Table 12. Evaluation of contact toxicity of allspice essential oil against *S. oryzae*

Amount of Grains	Allspice Concentration	% Mortality
50g	340 µl/L	41.66%
		72.72%
		64.28%
		58.82%
		59.37% ± 11.89
50g	170 µl/L	9.75%
		36.36%
		42.10%
		27.86%
		29.01% ± 13.48
50g	85 µl/L	53.57%
		10.00%
		12.24%
		7.69%
		20.87% ± 20.58

Table 13. Evaluation of contact toxicity of allspice essential oil against *T. castaneum*

Amount of Flour	Allspice Concentration	% Mortality
50g	425 µl/L	18.91%
		13.26%
		23.72%
		28.94%
		21.21% ± 6.40
50g	340 µl/L	18.18%
		6.84%
		12.90%
		26.58%
		16.12% ± 7.69
50g	255 µl/L	4.21%
		8.23%
		10.76%
		21.73%
		11.23% ± 7.34

Table 14. Evaluation of contact toxicity of Cinnamon essential oil against *S. oryzae*

Amount of Grains	Cinnamon Concentration	% Mortality
50g	170 µl/L	16.32%
		24.44%
		25.71%
		30.00%
50g	85 µl/L	33.33%
		24.13%
		29.72%
		22.22%
		27.35% ± 4.95
50g	43 µl/L	44.11%
		13.63%
		15.00%
		7.14%
		19.97% ± 13.97

Table 15. Evaluation of contact toxicity of Cinnamon essential oil against *T. castaneum*

Amount of Flour	Cinnamon Concentration	% Mortality
50g	425 µl/L	11.76%
		2.22%
		5.88%
		2.56%
		5.61% ± 3.84
50g	340 µl/L	3.57%
		11.11%
		0.00%
		0.00%
		3.67% ± 4.61
50g	255 µl/L	0.00%
		1.63%
		2.17%
		0.00%
		0.95% ± 1.01

5.4 Repellent activity of *Pimenta dioica* essential oil against *S. oryzae*

Allspice EO shows repellent activity towards *S. oryzae* at 15, 30 1 h ,2 h, 3 h and 4 h.

Figure 21. Repellent activity of *Pimenta dioica* essential oils against Sitophilus oryzae

5.5 Fumigant toxicity of combination of essential oils against *Sitophilus oryzae*

Composition of the constituents strongly influenced the bioefficacy of essential oil blends against insects of stored product origin. Among blends studied, a blend of (EG:CV) showed highest insecticidal efficacy with a mean of 95.00 ± 4.08% mortality. Of all the ratio investigated, combinations of *Pimenta dioica* and *Cinnamomum verum* recorded minimum mortality; achieved 12.50 ± 12.58% . Additionally, a 75:25 ratio of *Eucalyptus globulus* to *Pimenta dioica* (EG:PD) exhibited maximum insecticidal activity (85.00 ± 10.41%). The synergistic mixes (Trisyono and Whalon 1999) permit the application of lower doses, ideally due to comparatively

higher insecticidal activity, among the many interactions shown in EO mixtures. In contrast to traditional pesticides, this ensures lower management expenses, environmental hazards, and the emergence of resistance (Hummelbrunner and Isman 2001; Tak and Isman 2015; de Oliveira et al. 2017).

Table 16. Fumigant toxicity of combination of essential oils against *Sitophilus oryzae*

EO Concentration	Mean ± SD
EG : CV	95.00 ± 4.08 %
EG : CV	16.25 ± 15.14 %
EG : CV	46.25 ± 26.27 %
EG : PD	85.00 ± 10.41 %
EG : PD	63.75 ± 9.01 %
EG : PD	42.50 ± 23.70 %
PD : CV	22.50 ± 11.18 %
PD : CV	17.50 ± 9.57 %
PD : CV	12.50 ± 12.58 %

5.6 Effect of moisture during fumigation

Moisture analysis of fresh grains was carried out and values are shown in Table 17. Moisture % of fresh wheat and wheat flour where 10.12 and 9.27% which correlates with previous reports.

Table 17. Moisture content of wheat grains treated with three different essential oils.

Sample	Mean Moisture (%)
EG 340 µl/L	5.79
EG 170 µl/L	5.59
EG 85 µl/L	6.04
PD 340 µl/L	6.46
PD 170 µl/L	6.47
PD 85 µl/L	6.39
CV 170 µl/L	6.49
CV 85 µl/L	6.17
CV 43 µl/L	5.98

Table 18. Moisture content of wheat flour treated with three different essential oils.

Sample	**Mean Moisture (%)**
EG 340 µl/L	11.43
EG 170 µl/L	11.12
EG 85 µl/L	11.44
PD 340 µl/L	13.16
PD 170 µl/L	13.32
PD 85 µl/L	13.18
CV 170 µl/L	11.61
CV 85 µl/L	11.65
CV 43 µl/L	11.18

5.7 Protein analysis of treated grains

The protein content was the highest in infected wheat (14.46%), followed by PD-treated wheat (12.85%) and CV-treated wheat (11.92%). The protein content (11.93%) of fresh wheat was on par with the CV treatment. The EG-treated wheat had the lowest protein content (8.95%).Bioactive compounds such as eucalyptol and α-pinene, which have been reported to exist in eucalyptus oil, can potentially influence protein degradation or retention of nutrients within stored grain (Reddy et al., 2009). Yet, the relatively higher protein level in PD and CV-treated wheat suggests that the oils could exert a protective effect against insect-mediated degradation due to their strong antibacterial and insecticidal activities (Isman, 2000; Nerio et al., 2010). The insect biomass present, potentially causing artificially high levels of nitrogen, or the accumulation of nitrogenous waste metabolites of insects could be responsible for the elevated protein content in infested wheat (Trematerra & Fleurat-Lessard, 2015).

Table 19. Protein analysis of EO treated grains

Sample Name	Sample weight (g)	Protein factor	Nitrogen	Protein (%)
EG	39	6.25	1.431227326	8.945170403
PD	34.2	6.25	2.056097269	12.85060787
CV	44.6	6.25	1.907680154	11.92300129
Infested wheat	62.6	6.25	2.312898397	14.45561504
Fresh wheat	54.5	6.25	1.909441948	11.93401241

6. DISCUSSION

In post-harvest management, the application of EO is regarded as a substitute for managing most stored grain insects (Rajendran and Sriranjini,2008). Essential oils and their constituents have been employed as probable fumigants to control stored grain insect pests (Rajashekar et al., 2012, Rajendran and Sriranjini, 2008, Shaaya and Kostyukovsky, 2011). Biofumigants are advantageous in terms of offering new modes of action against insects which can minimize the risk of cross-resistance along with providing new leads for the design of target-specific molecules (Liu et al., 2010, Rajashekar et al., 2012). Essential oils (EOs) are extracted from a wide range of aromatic herbs and have widespread application in traditional medicine in the fight against pest infestation (Isman et al. 2011). These EOs may have a higher potential than grain protectants such as chemical pesticides in terms of efficiency, economic value and storages (Weaver and Subramanyam 2000; Chu et al. 2012; Gueye et al. 2012) since the application of synthetic pesticides can cause resistance and is potentially health-hazardous (Champ and Dyte 1976; Subramanyam and Hagstrum 1995; White and Leesch 1995).The United States Food and Drug Administration (FDA) identified botanical pesticides (essential oils) as safer than synthetic pesticides (Regnault-Roger et al.2012). The fragrant EOs have been extensively studied for their fumigant, contact and repellent activities because of their high volatility for the management of stored product pests (Isman 2000; Bakkali et al. 2008; Nerio et al.2010). Hence, the employment of EOs is an alternative to chemical insecticides for environment and food chain protection (Casida 2012).

In the present study, results of GC-MS analysis revealed that the EO components from the leaves of *E.globulus* has 42 compounds. Eucalyptol(16.72%),α-Pinene(13.02%), 2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methyle (10.35%), Bicyclo[3.1.1]heptane,

6,6-dimethyl-2-methylene-,(1S)-(11.26%),2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8- (8.56%), o-Cymene (5.01%), and D-Limonene (4.18%) were the predominant EO components detected by GC-MS analysis. 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydroα,α,4a,8- (4.36%), 3-cyclohexene-1-methanol, α,α,4-trimethyl-, (R)- (3.88%), and 4-methyl1-(1-methylethyl)- (3.45%) are the minor compounds isolated. The GC-MS result of essential oil of P.dioica reported 28 compounds and its major compounds identified were 3-Allyl-6-methoxyphenol (59.83%), Tris (tertbutyldimethylsilyloxy)arsane (12.76%), Limonene (7.46%), 2-Allylphenol (7.42%), 1-Octen-3-ol (2.14), Bicyclo[3.1.0]hex-2-ene, 4-methyl1-(1-methyle (1.52%), and (1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene 5 (1.04%) were the major compounds in allspice essential oil. The GC-MS result of C. verum showed 15 compounds of which major compounds were Methyleugenol (51%), Methyleugenol (14.29%), (Z)-3-Phenylacrylaldehyde (12.49%), Acetic acid, cinnamyl ester (10.31%), 3-Allyl-6-methoxyphenol (6.09%), Caryophyllene (1.80%).

In the study conducted, adult *S. oryzae* are significantly impaired by eucalyptus essential oil. 100% total mortality was obtained in all replicates at the level at 21 µl/L, 43 µl/L and 64 µl/L on red flour beetle indicating the oil's high efficacy. This is further supported by other research findings which indicated eucalyptus essential oil contains high fumigant toxicity to pests of stored commodities (Lee et al., 2001). The mortality rates declined at lower concentrations: 90.00 ± 20.00% death was obtained at 64 µl/L, while 68.75 ± 21.65% death was obtained at 43 µl/L. This dose response is in agreement with other studies that have reported higher eucalyptus oil concentrations led to greater S. oryzae mortality (Lee et al., 2001). The main compound that contributes to fumigant toxicity is 1,8-cineole, which makes up a large percentage of eucalyptus

oil. Lee et al. (2001) report that this molecule is one of the major contributions towards the insecticidal activity of eucalyptus oil.

The essential oil of allspice was weakly toxic to adults of *S. oryzae* by contact, but not its fumigant toxicity. The mortality rate at the highest concentration tested of 340 µl/L was 59.37% ± 11.89% on average. This accorded with earlier research that found that the LD_{50} value of contact toxicity of allspice oil on *S. oryzae* was 75.1 ± 3.08 µg/mm². Mortality rates dropped further to 29.01% ± 13.48% and 20.87% ± 20.58% at lower dosages of 170 µl/L and 85 µl/L, respectively. Essential oil of allspice (Pimenta dioica) (AEO) was tested for contact and fumigant toxicity against adult *T. castaneum*, one of the stored-product pests. The results indicated that AEO has relatively low contact toxicity and moderate fumigant toxicity against the species. AEO was effective to a certain degree at different doses in fumigation tests. The total mortality rate at 60 µL/L was 56.25% ± 28.44%, varying between 30% and 95% for the repeats. Likewise, representative mortalities at 51 µL/L and 43 µL/L were 55.00% ± 27.39% and 62.50% ± 6.45%, respectively. The results indicate a dose response that is dependent to some extent but extremely variable. Taking the same into consideration, from similar studies, AEO's fumigant LC_{50} against adult *T. castaneum* is 19.1 ± 0.43 µg/L of air, representing moderate fumigant.

According to the current study, *S. oryzae* adults is highly damaged by cinnamon essential oil. Increasing concentrations caused increased mortality: 85 µl/L caused 22.50% mortality, 64 µl/L caused 23.75% mortality, and 43 µl/L caused 23.75% mortality. These results are consistent with earlier research on the efficacy of cinnamon oil as an insect fumigant against stored commodity insects. For example, Abd El-Salam (2010) realized 90% mortality of S. oryzae through *Cinnamomum zeylanicum* essential oil exposure for 24 hours at 8.0 µl/50 ml air concentration. It has been reported that (E)-cinnamaldehyde, the major active component of cinnamon oil, is a

very effective insecticide. Lee et al. (2009) observed, in vapor-phase bioassays, that (E)-cinnamaldehyde and structurally related compounds were very toxic to S. oryzae, indicating that these compounds are useful as fumigants.

The eucalyptus essential oil showed strong contact toxicity to S. oryzae and fumigant toxicity. Complete mortality (100%) was observed in all replicates against concentrations of 170 µl/L and 85 µl/L. The oil was an effective contact insecticide as it showed a mortality of 99.39% ± 1.22% at the highest concentration tested (340 µl/L). These results are consistent with other studies that documented contact toxicity of eucalyptus oil to the stored-product insects (Lee et al., 2001). Efficiency of the oil when applied by points of contact to its potential as a natural substitute for eucalyptus oil to synthetic insecticides in the management of infestations by S. oryzae. Eucalyptol (16.72%), which is a major component of eucalyptus oil, is the main compound that contributes to fumigant. The chemical has been found to be one of the main contributors to the insecticidal activity of eucalyptus oil. Eucalyptus essential oil showed comparatively weaker contact toxicity against *Tribolium castaneum* compared to its fumigant toxicity. The mortality was 17.45% ± 2.86% at the highest concentration tested of 340 µl/L and reflects low efficiency as a contact insecticide. This is in keeping with earlier studies showing that eucalyptus oil exhibits lower contact toxicity to T. castaneum. Mortality reduced to 86.25% ± 6.29% and 57.50% ± 9.01% at decreasing dosages of 64 µl/L and 43 µl/L, respectively, reflecting a dose-dependent effect.

The contact toxicity experiments revealed that AEO was not as effective in killing T. castaneum. It had mortality ranges of 13.26%-28.94% at a high concentration test value of 425 µL/L, having a mean mortality of 21.21% ± 6.40%. The lower test values of 340 µL/L and 255 µL/L were recorded with average mortalities of 16.12% ± 7.69% and 11.23% ± 7.34%, respectively. These

results concur with the earlier studies which showed that AEO has a contact LD_{50} value of 81.6 ± 2.04 µg/mm² against *T. castaneum*, which implies fairly low contact toxicity.

The chemical structure of AEO, containing its components including 1,8-cineole, methyl eugenol, and eugenol, may be responsible for its poor fumigant activity. In a number of investigations, these compounds have been found to be connected with insecticidal activities. The comparatively lower contact toxicity realized, however, leaves open the possibility that AEO acts more potently against *T. castaneum* as a fumigant compared to its role as a contact insecticide.

As per Haddi et al. (2020), Isman (2020), and Jumbo et al. (2022), plant-based biorational compounds—like extracts, essential oils, and phytochemicals—have been evaluated as a good alternative to manage pests of storages durable products due to as they show less risky behavior toward species other than their target. Since essential oils (EOs) and their blended combinations consist of a broad range of chemical components, EOs have attracted more attention from the scientific community (de Oliveira et al. 2017). Among the various interactions observed in EO blends, the synergistic blends (Trisyono and Whalon 1999) allow for the application of lower dosages, preferably because of relatively higher insecticidal activity. Thus, ensuring lower management costs, environmental risk, and development of resistance compared to conventional pesticides (Hummelbrunner and Isman 2001; Tak and Isman 2015; de Oliveira et al. 2019). The result showed the binary and individual combination effect (Synergistic, Antagonistic or additive) of eucalyptus, allspice and cinnamon essential oils against S. oryzae. Proportions of the constituents greatly affected the bioefficacy of essential oil combinations against insects from stored products. *Eucalyptus globulus* and *Cinnamum verum* in a proportion (EG:CV) exhibited greatest insecticidal activity among the mixtures investigated with a mean of 95.00 ± 4.08%

mortality. This work replicates previous investigations demonstrating that E. globulus oil is highly fumigant toxic, due primarily to high levels of the eucalyptol (1,8-cineole) and α-pinene present, which are compounds that possess neurotoxic properties against insects (Batish et al., 2008; Nerio et al., 2010). The essential oil chemical cinnamonaldehyde, identified as having an insecticidal action and for inhibiting the enzymatic action of insects, could be the reason for C. verum's observed synergistic activity (Chang et al., 2009; Cheng et al., 2009). In contrast, mortality was much lower when the EG:CV ratio was reversed (16.25 ± 15.14%), suggesting that a greater proportion of *C. verum* essential oil may not coexist or even counteract E. globulus action in certain proportions. Conversely, among all the ratios studied, blends of *Pimenta dioica* and *Cinnamomum verum* had the lowest mortality of 12.50 ± 12.58% mortality. Whereas individual components of the two oils have been previously reported to be insecticidal, the data indicate a paucity of synergistic action and possibly antagonist effects when they are blended. Volatility differences or interaction effects that decrease the overall bioavailability of active ingredients could be the reason for the poor efficacy (Isman, 2000; Regnault-Roger et al., 2012).

Generally, these findings show how important the exact combination ratios are to defining the efficacy of EO blends. The proportion of ingredients greatly determines the outcome, and even oils with proven insecticidal activity will not necessarily interact synergistically. This reinforces how critical empirical testing is in developing botanical pesticides.

All treated samples within this study had moisture levels ranging from 5.59% to 6.49%, which indicates that treatments using essential oils at varying doses had not significantly altered the moisture content of the grains. Among the treatments, grains treated with Eucalyptus globulus contained the lowest mean moisture content (170 µl/L; 5.59%), while grains treated with *Cinnamomum verum* contained the highest (170 µl/L; 6.49%). These findings are consistent

with previous research indicating that fumigation with essential oils maintained grain quality during storage by having no detectable impact on the moisture content of treated grains (Ayvaz et al., 2010; Rajendran & Sriranjini, 2008). The moisture content ranged from 11.12% to 13.32% for all of the treatments. EG 170 µL/L was the sample with the lowest moisture content (11.12%), and PD 170 µL/L was the sample with the highest (13.32%). Because it lowers the likelihood of microbiological development and insect infestation, wheat flour with less than 14% moisture is generally considered safe for storage (Kent, 1983; Oluwamukomi et al., 2011).

The present results indicate that all the EO-treated flour samples remained within this acceptable range, which means that the application of essential oils did not adversely affect the fitness of the flour for storage. Conversely, the water content in *Pimenta dioica* EO-treated wheat flour was slightly higher (mean range: 13.16–13.32%). This can be attributed to the physicochemical nature of the oil or its potential interaction with wheat components, which may lead to moisture hold-up, although still within acceptable levels. The differences were not, however, significant enough to negatively affect flour stability. Since EOs of Eucalyptus globulus and *Cinnamomum verum* are volatile and hygroscopic, they could possibly control moisture. Essential oil treatment tended to keep the moisture levels lower (mean values around 11.1–11.6%). Essential oil treatment can possibly stabilize moisture during storage by acting as moisture barriers and reducing water activity, as suggested by Mohammed et al. (2021), who reported similar trends.

These findings are in agreement with previous studies that noted that essential oils do not significantly enhance the water content of grains or flour and can even contribute to maintaining optimum levels during storage (Kordali et al., 2006; Channa et al., 2022). One of the critical factors governing the nutritional content of wheat, particularly for human and animal consumption, is its protein content. The percentage protein in wheat samples exposed to

essential oils of Eucalyptus globulus (EG), *Pimenta dioica* (PD), and *Cinnamomum verum* (CV) were computed and compared to fresh (control) and infested wheat samples in the present study.

The protein percentage of fresh wheat was 11.93% (Shewry and Hey, 2015), similar to previously documented percentages ranging between 10% and 15%, varying based on variety as well as environment of growth. The level of protein present in infested wheat was distinctly greater (14.46%). This increase is consistent with evidence showing a concentration effect due to loss of moisture and carbohydrate degradation triggered by infestation by insects, particularly Sitophilus oryzae and Tribolium castaneum, which relatively increases the proportion of protein (Abebe et al., 2020).

Yet, as insect infestation tends to result in a decrease in essential amino acids and quality in general, the nutritional quality may not necessarily increase (Bamaiyi et al., 2012).

The protein content in the samples treated with essential oils was different: PD (12.85%) > CV (11.92%) > EG (8.95%). The greater proportion of protein in PD and CV-treated grains increases the likelihood that these essential oils better preserved grain quality compared to EG. High antioxidant and antibacterial activity of cinnamon essential oils (*Cinnamomum verum*) and allspice essential oils (Pimenta dioica) has been shown in previous studies to be able to prevent microbial spoilage and retain macronutrients, such as proteins (Singh et al., 2021; Misharina et al., 2009). The reduced protein level in the EG-treated sample, however, could indicate partial degradation or weaker protection against storage-related factors, although Eucalyptus globulus oil has been reported to possess bioactivity, especially as a fumigant and insect repellent (Batish et al., 2008).

Based on these findings, essential oils—particularly those obtained from PD and CV—can potentially serve as natural preservatives to ensure the nutritional integrity and safety of stored grains.

7. CONCLUSION

In this study, essential oils were extracted from eucalyptus, allspice, and cinnamon leaves collected from Malipuram, Ernakulam, Kerala. The extracted essential oils were tested against *Sitophilus oryzae* and *Tribolium castaneum* adults using fumigant and contact toxicity bioassays. Comparative evaluations demonstrated that *Tribolium castaneum* was more susceptible than *S. oryzae* in fumigant toxicity bioassays. Among the essential oils, eucalyptus oil exhibited the highest fumigant toxicity against both insect species. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified eucalyptol (16.72%) as the major compound in eucalyptus oil, suggesting it as a potential active ingredient. In allspice oil, chavibetol (59.83%) was identified as the dominant compound with insecticidal properties, while methyleugenol (51%) was the primary compound in cinnamon oil. The essential oils showed greater insecticidal activity than crude extracts against *S. oryzae* and *T. castaneum*. Given their efficiency, essential oils present a promising alternative for controlling stored grain insect pests.

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