BIOLOGICAL INVESTIGATION AND CHEMICAL PROFILE OF *VITEX NEGUNDO* AGAINST TWO MAJOR STORED GRAIN INSECT PESTS

A dissertation submitted by

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In partial fulfilment of the Degree of

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IN

FOOD PROCESSING TECHNOLOGY

Under the Guidance of

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I hereby certify that this dissertation entitled **"Biological investigation and chemical profile of *Vitex negundo* against two major stored grain insect pests"** submitted by **Ms. Aparna Raj (Reg No. VM23FPT005)** 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.

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DECLARATION

I hereby declare that, the report on the project entitled **"Biological investigation and chemical profile of *Vitex negundo* against two major stored grain insect pests"** submitted by **Ms. Aparna Raj (Reg No. VM23FPT005)** to **St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam, Kerala**, for the partial fulfilment of the requirement for the award of degree of **Master of Vocational Studies in Food Processing Technology** is the record of the original work carried out by me under the guidance of **Dr. Rajashekar Y**, Principal Scientist, Department of Food Protectants and Infestation Control (FPIC), CSIR-CFTRI, Mysuru, Karnataka. I further declare that; the results of present study have not formed the basis for the award of any other degree to any present candidate of any university during the period of my study.

Place: Mysuru

Date: Aparna Raj

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APARNA RAJ

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LIST OF ABBREVIATIONS

%	-	percentage
ml	-	milli liter
L	-	Linnaeus
LC	-	Lethal Concentration
+	-	Plus
–	-	Minus
GC-MS	-	Gas Chromatography-
Mass Spectrometry		
°C	-	degree Celsius
±	-	plus, or minus

g	-	gram
kg	-	kilo gram
=	-	equal to
/	-	divided by
No.	-	number
cm	-	centi meter
µl	-	micro liter
µl/L	-	micro liter per liter
h	-	hours
UV	-	Ultraviolet
R_f	-	retention factor

ABSTRACT

The present study aims to develop a safe alternative source for synthetic pesticides for storage and to increase shelf life of cereals. Protection of stored grains during post-harvest management remain as a great challenge. It is a prevalent threat to global food security and quality of stored grains. The study focuses on the plant *Vitex negundo*—a medicinally important plant as a bioinsecticide. It was evaluated against two major grain pests, *Sitophilus oryzae* (rice weevil) and *Tribolium castaneum* (red flour beetle). Extracts were obtained from four different solvents-Hexane, Ethyl acetate, Methanol and Acetone were tested for their insecticidal activity under controlled laboratory conditions. Different parameters such as mortality rate, change in moisture, protein and fat content of the stored grains were studied. The plant extract exhibited significant insecticidal activity, with notable differences observed based on solvent type and extract concentration. In addition, Gas Chromatography-Mass Spectrometry (GC-MS) analysis were conducted to identify the bioactive compounds present in the plant material. The chemical profiling revealed the presence of several compounds with potential insecticidal, repellent, and antifeedant properties. The use of plant-derived bioinsecticides like *V. negundo* offers a promising, eco-friendly approach to enhancing grain storage safety, maintaining grain quality, and minimizing chemical residues in the food supply chain.

1. INTRODUCTION

Cereals are among the most essential staple foods worldwide, playing a pivotal role in human nutrition by providing the energy required for daily activities (Bansal et al., 2016). Wheat (*Triticum aestivum L.*) ranks as the second most cultivated cereal crop in India and is also the second-largest contributor to global wheat production. In the 2016–2017 agricultural season, India achieved a record wheat production of 97.44 million tons from an area of 30.72 million hectares (Jaiswal and J.P,2018). The Food and Agriculture Organization (FAO) projected wheat production to reach 113 million tons by 2025, and in 2024, production was recorded at 112.9 million tons—a substantial increase due to above-average yields and extensive cultivation areas. Wheat remains a significant cereal globally, with over 219 million hectares cultivated in 2020 (Ashiq et al., 2022).

Population growth, projected to surpass 9.1 billion by 2050, raises concerns about food security and the potential scarcity of staple foods if proper storage and management practices are not implemented (Yang et al., 2023). FAO reported that in 2018 alone, approximately 2.685 million tons of cereals were lost due to various forms of contamination and damage throughout harvest and post-harvest processes.

Wheat, often referred to as the "Golden Grain" and the "King of Cereals" due to its nutritional value and global consumption, is categorized into winter and spring varieties based on the season of cultivation. Grain hardness further classifies it into hard, medium, and soft wheat, each suited for specific uses. For instance, hard wheat is rich in gluten and ideal for bread-making, while soft wheat is used for cakes and biscuits. Medium-strong wheat is commonly used for staple foods like chapati and roti (Dhanada S et al., 2017). Wheat's nutritional composition, particularly its protein content (ranging from 8–20%), varies based on factors like variety, geographic region, temperature, and water availability. It is also a rich source of starch, making it a high-energy food (Szuba-Trznadel et al., 2024).

Storage losses, often caused by insect infestations, are a significant concern in maintaining grain quality and food security. FAO estimates indicate that 17% of stored grain yield is lost, with 10% attributed to insect damage and an additional 7% due to other pests like mites and rodents. Approximately 80% of plant-based food originates from cereals like wheat, rice,maize, and grains are particularly susceptible to damage during storage in tropical and temperate regions. Primary pests, such as the rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*), penetrate and infest intact grains, while secondary pests, like the flat grain beetle feeds on broken kernels or damaged grain (Stathas et al., 2023; Dufera et al., 2020).

The introduction of synthetic pesticides in the 1960s was initially effective in mitigating pest-related losses but later led to issues like pest resistance and environmental damage (Al-Harbi et al., 2016). Fumigation plays a major role in insect pest elimination in stored products (Rajendran & Sriranjini,2008). Consequently, there is a growing interest in natural alternatives such as plant-based biofumigants. These natural substances, known for their insecticidal, repellent, and growth-inhibiting properties, offer safer and eco-friendly solutions. Biofumigants derived from plants are less harmful to humans and the environment and do not leave pesticide residues. They have been shown to effectively manage pests without compromising seed viability, cooking quality, or milling properties, making them a cost-effective and sustainable alternative to chemical pesticides (Kathirvelu and Raja, 2015; Sahu et al., 2021; Singh et al.,).

1.1 Post-Harvest Loss and the Role of Biofumigants in Food Security

The journey from harvest to consumption involves significant investments of time and resources. However, a considerable portion of agricultural produce is lost post-harvest due to multiple factors, including improper handling during harvesting, processing, and storage. Major contributors to these losses are spoilage caused by microbiological, physical, and insect or rodent activity (Prusky, 2011). Such losses not only impact food security but also pose challenges to the economic growth of nations. Reducing post-harvest losses is critical to ensuring food availability, safety, and economic stability. Infested produce often suffers from decreased consumer acceptability, reduced nutritional value, and compromised edibility.

1.2 The Limitations of Synthetic Pesticides

Synthetic pesticides and fumigants have historically played a significant role in controlling post-harvest pests. Since the 1950s, chemicals such as methyl bromide, phosphine, and sulfuryl fluoride have been widely used to eliminate insects at all life stages in stored cereals and pulses. However, over time, insects have developed resistance to these chemicals, particularly phosphine, which is a major issue in countries like Australia and India (Kathirvelu and Raja, 2015)

The increased resistance has necessitated the use of higher doses of synthetic fumigants, leading to several adverse consequences, including residual toxicity in food, environmental pollution, and negative effects on human health. The accumulation of these chemicals in the food chain has raised significant concerns, prompting the ban of certain chemical pesticides. These challenges have highlighted the urgent need for eco-friendly alternatives that are biodegradable, selective, and safe for humans and the environment.

1.3 The Promise of Biopesticides

Biopesticides have emerged as a promising alternative to synthetic pesticides, offering several advantages. Unlike chemical pesticides, biopesticides do not leave harmful residues, pose minimal risks during application, and are safer for both consumers and the environment. Numerous studies have demonstrated the insecticidal, antifeedant, repellent, and oviposition-inhibiting properties of plant-derived biofumigants. These natural alternatives are cost-effective, readily available, and have negligible or no adverse effects on seed germination, cooking quality, or milling properties (Kathirvelu and Raja, 2015).

1.4 The Role of Cereals in Food Security

Cereals are staple foods for much of the global population and play a vital role in food security, particularly in agriculturally driven economies like India. However, without proper storage and preservation techniques, cereal production may fail to meet the growing demands of the population. Developing natural and safer biofumigants to extend the shelf life of cereals such as wheat is essential for ensuring food security. Biofumigants, derived from plant extracts, offer targeted pest control without harming humans or the environment. Edible plant-based compounds represent safe candidates for use as repellents, fumigants, or contact pesticides (Nikolaou et al., 2021).

1.5 Mechanisms of Plant-Based Biofumigants

Plants produce various bioactive compounds with insecticidal properties, which have been extensively studied for their potential in pest management. Essential oils, allelochemicals, and secondary metabolites such as terpenoids, monoterpenoids, and polyphenols are known for their repellent and insecticidal effects. Monoterpenoids, for example, function by inhibiting the acetylcholinesterase enzyme in insects, leading to neurotoxic effects. These compounds not only cause behavioural changes in pests but also disrupt their physiological functions, making them highly effective in pest control (Rajashekar et al., 2014).

Minimizing post-harvest losses is a critical step in addressing global food security challenges. While synthetic pesticides have historically been effective in pest control, their environmental and health-related drawbacks necessitate the exploration of sustainable alternatives. Biopesticides, derived from plant-based compounds, offer a safe, eco-friendly, and cost-effective solution to managing stored grain pests. By harnessing the insecticidal properties of natural biofumigants, it is possible to protect food commodities, ensure their nutritional integrity, and safeguard the environment.

2.REVIEW OF LITERATURE

Infestation of stored grains is a pressing issue that affects the preservation of food grains and related products. It not only results in significant quantitative losses but also compromises the quality of the commodities. The presence of insect remnants, such as wings, legs, and larvae, degrades the integrity of the stored grains. Moreover, infestations create favorable conditions for the growth of molds, including aflatoxins—a type of mycotoxin known to pose serious health risks to humans. Numerous studies have highlighted elevated mycotoxin levels in grains like maize, almonds, peanuts, sweet potatoes, wheat, and rice (Stathas et al., 2023). Insects at various developmental stages, including larvae, pupae, and adults, pose a substantial threat to the storage of food products (Trivedi et al., 2018). Pest infestations have been shown to alter the nutritional composition of agricultural produce, with the extent of these changes influenced by factors such as the type of insect, the nature of the damage, the host plant, the stage of pest growth, the severity of infestation, and the control methods implemented. Key nutritional changes observed include reductions in fiber and essential amino acids, alterations in carbohydrate and fat content, and an increase in protein levels (Ouaarous et al., 2025).

With the health concerns relating to use of synthetic pesticides, various studies and experiments were being conducted all over the world to find an alternative source for man-made pesticides. Recent investigations point outs to the fact that compounds derived from plants not only has insect-repellent activity but can be incorporated into pest management due to presence of bioactive compounds which results in fumigant and contact toxicity of various stored grain pests. During past few decades, scientific investigations on biological activities of plant compounds were actively carried out (Isman, 2000). The chemical composition of the active compounds in a plant can vary according to various factors such as geographic origin, genetic diversity, ambient circumstances, seasonal variations, plant age, harvesting time, and extraction techniques (Sabira et., al)

Table 1: Primary and Secondary insect pests, their mechanism of action, feeding mechanism and degree of effect

Pest Type	Insect Species	Mechanism of Damage	Feeding Mechanism	Degree of Effect	Reference
Primary Pest	*Sitophilus zeamais* (Maize weevil)	Larvae develop inside whole grains, feeding internally	Endophagous; bore into and feed within intact grains	High – causes direct grain weight loss and quality degradation	Arthur (1996)
Primary Pest	*Sitophilus oryzae* (Rice weevil)	Infests whole grains; adult and larva feeding causes grain hollowness	Internal feeder: oviposition in kernel, larva consumes from inside	High – structural damage and contamination	Rees (2004)
Primary Pest	*Rhyzopertha dominica* (Lesser grain borer)	Adults and larvae bore into kernels, reducing grain to powder	Internal borer and feeder; larva causes major internal damage	High – both quantitative and qualitative loss	Subramanyam & Hagstrum (1995)
Secondary Pest	*Tribolium castaneum* (Red flour	Feeds on broken grains, flour, and processed	External feeder; scavenger on already	Medium – quality loss, causes unpleasant	Hill (2002)

	beetle)	products	damaged grains	odour	
Secondary Pest	*Oryzaephilus surinamensis* (Saw-toothed grain beetle)	Feeds on broken grains and milled products	External chewer; doesn't damage whole grains	Low to Medium – affects market value	Rees (2004)
Secondary Pest	*Lasioderma serricorne* (Cigarette beetle)	Larvae bore through packaging and processed food products	External feeder; mainly affects processed commodities	Medium – packaging damage and contamination	Hinton (1945)

Plants are a valuable source of natural pesticides and have been increasingly utilized in managing stored pests. Research has demonstrated their effectiveness as biofumigants. For instance, a study by Rahman et al. (2021) investigated the insecticidal properties of Sundarbans mangrove plants. The research tested extracts from five plant species against two insect species, *Sitophilus oryzae* and *Sitophilus zeamais*, using the direct contact feeding deterrent wafer disc method. Results showed significant mortality rates of 80–100% when the bark extracts of *A. corniculatum*, *E. agallocha*, and *H. fomes* were applied to both insect species. Further fractionation of the extracts was conducted, and each fraction was tested for insecticidal activity. Among them, fraction 4 exhibited the highest insecticidal effectiveness, with lethal concentration 50% (LC50) values of 0.5, 1.0, and 1.5 mg/disc for *A. corniculatum*, *E. agallocha*, and *H. fomes*, respectively. To identify the bioactive compounds responsible for the observed effects, the active fraction underwent analysis using liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS). This analysis revealed two known compounds—isorhamnetin 3-O-rutinoside (m/z 625.17630) and paspaline (m/z 422.25346)—along with several unidentified peaks, indicating potential new bioactive substances (Rahman et al., 2021).

The phenolic compound 2,4 di-tert-butylphenol is a major component of violate or essential oils in plants and are toxic to test insects. It was found in various bacteria and plants such as *Phaeodactylum tricornutum*, *Marchantia polymorpha*, *Osmunda regalis* and *Adiantum venustum* (Zhao et al., 2020). 1-Docosene is a plant metabolite having anti-bacterial properties and are isolated from various plants such as *Urena lobata*, common name-Caesar weed (Keke et al., 2023). Eicosyl trifluoroacetate were isolated from the plant material *Eranthemum pulchellum* and are well known for their bioactive, anti-microbial and anti-fungal properties. (Darmwal et al., 2023). E-15 Heptadecenal were found to have activity on food borne pathogens and insect pests and were isolated from ethyl acetate extract of *Monochaetia kansensis* (Yogeswari et al., 2012). Tetracosan 10yl acetate is a volatile plant metabolite and were isolated from *Adinandra hongiaoensis* (Pham et al., 2024)

Khoobdel in 2022 demonstrated that lectin, a protein compound found in plant *Polygonum persicaria* (PPA). The insects feeding the diet containing PPA caused the mortality of the insect species with a LC50 (Lethal Concentration to kill 50% of insects). The results also concludes that the compound affects the digestive system of the insect *Sitophilus oryzae*, leading to oxidative stress in *S. oryzae* and that most adults were killed at diet containing 8 % PPA. A positive result was obtained from PPA concentrations and insect mortality. The consumption of PPA treated grains have affected the digestive system of the insect *Sitophilus oryzae*. Enzymes such as alpha-amylase and alpha-glucosidase showed lower activities. There was a visible increase in the esterase activity of treated insects.

Karthivelu et al., 2015 have worked on 25 plant species namely *Tagetes erecta ,Coriandrum sativum, Vitex negundo, Cymbopogon nardus Spreng , Allium sativum ,Cinnamomum verum , Ocimum canum , Murraya koenigii,Piper nigrum , Nicotiana tobacum, Citrus limon Jatropha curcas, Artemisia vulgaris, Aegle marmelos, Neerium oleander , Cuminum cyminum ,Mentha piperita, Eucalyptus globulus Latrill , Brassica juncea, Ocimum bacillum , Adathoda vasica, Acorus calamus , Azadiracta indica A.Juss , Calotropis gigantia.* and *Pongamia glabra* . The highest mortality for *Sitophilus oryzae* were obtained from the plant materials, *A. vulgaris* (66.33%) followed by *E. globulus* (64.67 %) and *M. piperita* (63 %) mortality rates.

The Oryzae, (2006) study reveals and explored the insecticidal effects of six plant-derived materials against the rice weevil (*Sitophilus oryzae*). The materials tested included bakain drupes (*Melia azedarach*), habulas leaves (*Myrtus communis*), mint leaves (*Mentha longifolia*), bakain leaves, harmal shoots and seeds (*Peganum harmala*), and lemongrass roots (*Cymbopogon citratus*). Among these, the extract from bakain leaves exhibited the highest repellency rate at 74%. For insect mortality, bakain drupes showed the most significant results, achieving an 82% mortality rate by the sixth day.

Singh et al., 2023 derived plant extracts from *Dillenia indica* (Dilleniaceae). It had been evaluated against three coleopterans stored products insects – *Sitophilus oryzae, Rhyzopertha dominica* and *Tribolium castaneum.* They evaluated both fumigant and contact toxicity against three insect pests. Fraction III were found to show the most insecticidal activity by inhibiting the activity of acetylcholinesterase (AChE) enzyme at concentration 88.57 µg/ml for *Sitophilus* , 97.07 µg/ml for *Tribolium* and 66.31 µg/ml for *Rhizopertha.*By using GC-MS, three major bioactive compounds namely, 6-Hydroxy-4,4,7a trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one, 1,2-Benzisothiazol-3(2H) one, and Benzothiazole, 2-(2-hydroxyethylthio) were identified. This study is a proof for understanding the importance of investigating the potential of plant compounds for insecticidal actions.

Natural plant products are increasing in demand as biopesticides due to their rich source of agrochemicals which are also environment friendly due to its biodegradability unlike synthetic pesticides. The working mechanism is also similar to that of synthetic chemicals. (Mugao et al., 2020). Various studies and research have been done on plant-derived compounds to tackle the problem of storage grains. Research indicates that the highest activity was observed with essential oils from the Lamiaceae family (68 combinations), followed by Asteraceae and Rutaceae (33 combinations each), and Myrtaceae (20 combinations). Most studies targeted *Coleoptera* species (232 combinations), with fewer focusing on *Lepidoptera* (13 combinations) and *Psocoptera* (10 combinations). The plants collected for biopesticides were collected from different geographical conditions and shows variations accordingly. Thus, extracts and essential oils of same plant from different geographical areas can show different results for different insect species (Campolo et al., 2018)

One of the mechanisms of action of plant extract is anti-feeding mechanism. Anti-feedants work by prevention of insects from consuming the grain causing them to starve and subsequently leads to death. It is included in Integrated Pest Management for effective storage of food commodities. Major advantage of it lies in its non-toxic nature, are biodegradable and eco-friendly. *Vitex negundo* is an aromatic shrub, found throughout the greater part of India. The plant is well-known for its medicinal properties. It includes anti-inflammatory, antiulcer, larvicidal and antiasthmatic. The aromatic bioactive components in *Vitex negundo* can be used against stored grain pests *Sitophilus oryzae* and *Tribolium castaneum* (Haridasan et al., 2017)

The effect of stored grain pests on nutritional quality of food were also studied by many Scientists. According to the investigations, the loss of nutritional components such as protein is dependent on the pest infesting the cereals. For an instance, *Trogoderma granarium* is an insect species that feed on wheat germ and bran, so they cause reduction of proteins in wheat. On the other hand, insects such as *Rhyzopertha dominica* feeds on endosperm of wheat and *Trogoderma granarium* feeds on germ and causes significant loss of lipids in wheat. *Tribolium castaneum*, most destructive secondary pest in wheat flour causes overall deterioration of the food commodity. The major effects caused by *Tribolium* includes increase in moisture content of wheat, colour change which makes the food undesirable to use, increase in insect fragments, the concentration of uric acid, the microbial growth, and the prevalence of aflatoxins. The infestations result in decreased levels of essential amino acid, methionine (38.9%). Non-essential amino acids such as alanine and tyrosine in wheat were also observed. Carbohydrate content in the crop is estimated by the parameter called Glycemic Index (GI). Higher GI content is harmful for human health. The carbohydrate content of the infested cereal is also dependent on the type of pest infesting the crop. Infestation of *C. cephalonica*, *E. kuehniella*, and *T. confusum* reduced the amount of carbs in wheat flour. Whereas insects *S. zeamais*, *P. truncatus*, and *Callosobruchus maculatus* in stored maize were observed to have decrease in carbohydrate levels and an increase in protein content of the crop. Cereal crops such as wheat are sources of fat (lipid) content. Cereals contains about 10 % of lipid content and are found in germ layer of the crop and few found in bran and endosperm. Due to this reason, endosperm feeders like *Rhyzopertha dominica* cause less loss of lipid content and germ feeders like *Trogoderma granarium* cause higher loss in lipid content of the cereal (Stathas et al., 2023)

Table 2: Common insect pets, affected food products and their effect on food quality

Insect Pest	Affected Food/Product	Effect on Food Quality	Reference
Red Flour Beetle (*Tribolium castaneum*)	Flour,cereals, spices	Contamination with body parts and secretions leading to foul odour and taste	Hagstrum & Subramanyam, 2006
Rice weevil(*Sitophilus oryzae*)	Whole grains (rice, wheat, maize)	Hollows out grains, reduces weight,quality,contamination with frass	Gullan & Cranston, 2014
Indian Meal Moth (*Plodia interpunctella*)	Cereals,dried fruits, nuts, chocolate	contamination with frass and cast skins & larvae produces silk web	Rees, 2004
Fruit fly(*Drosophila spp.*)	Fruit and vegetable	Increased spoilage,microbial contamination, visible larvae	White & Elson-Harris, 1992
Aphids (*Aphidoidea*)	Fruit and vegetable	Retards growth, transmits plant viruses	Blackman & Eastop, 2000
Cowpea Weevil (*Callosobruchus maculatus*)	Legumes(e.g. cowpea, lentils)	Reduced nutritional quality and germination potential	Credland et al., 1998

3.OBJECTIVES

- To prepare organic extracts from leaves of *Vitex negundo*.
- To evaluate the insecticidal activity of various organic extracts against *Sitophilus oryzae* and *Tribolium castaneum*.
- To identify the chemical composition of the active fraction of *Vitex negundo*.
- To assess the insecticidal efficacy of the active fraction derived from *Vitex negundo*.
- To analyse food safety parameters in relation to stored grain insects.

4.MATERIALS AND METHODS

4.1 Chemicals

The solvents used in this study included n-hexane, ethyl acetate, acetone, and methanol, which were procured from Sriram Distributors in Mysuru. The n-hexane used was of HPLC grade to ensure high purity and reliable results. Additionally, chloroform and silica gel were employed for various experimental procedures, providing essential support for the extraction, separation, and analysis of the samples.

4.2 Materials

Five kilogram of fresh *Vitex negundo* leaves was collected from Malipuram in Ernakulam, Kerala.

4.3 Apparatus and techniques used:

Soxhlet apparatus

Rotary evaporator

TLC

Column Chromatography

GCMS

Moisture Analyzer

Fat Analysis

n-Protein Analyzer

4.4 Culturing of Stored grain insects

4.4.1 *Sitophilus oryzae (Rice Weevil)*

The *Sitophilus oryzae* culture was reared on wheat and obtained from infested stocks at the Food Protectants and Infestation Control (FPIC) Department, CSIR-Central Food Technological Research Institute (CFTRI), Mysuru. The culture was maintained in controlled conditions at 30°C and 75% relative humidity. To prevent cross-contamination and ensure proper aeration, the jars were labelled and covered with muslin cloth. The insectary conditions were carefully designed to mimic the natural environment of the insects. Newly emerged insects from the culture were selected for experimental studies, ensuring consistency and reliability in the results.

Figure 1. Insect culture of *Sitophilus oryzae* (Wheat)

4.4.2 *Tribolium castaneum (Red Flour Beetle)*

The culture of *Tribolium castaneum* was maintained on wheat flour supplemented with 2% yeast to ensure optimal growth and development. Mixed-aged insect cultures were sourced from infested stocks provided by the Food Protectants and Infestation Control (FPIC) Department of CSIR-Central Food Technological Research Institute (CFTRI), Mysuru. The cultures were kept under controlled conditions at 30°C and 75% relative humidity. To prevent cross-contamination and maintain proper aeration, jars were carefully labelled and covered with muslin cloth. The insectary environment was designed to closely replicate natural conditions, ensuring the insects' typical behaviour and development. For experimental purposes, newly emerged insects from the culture were selected, providing consistent and reliable specimens for accurate results.

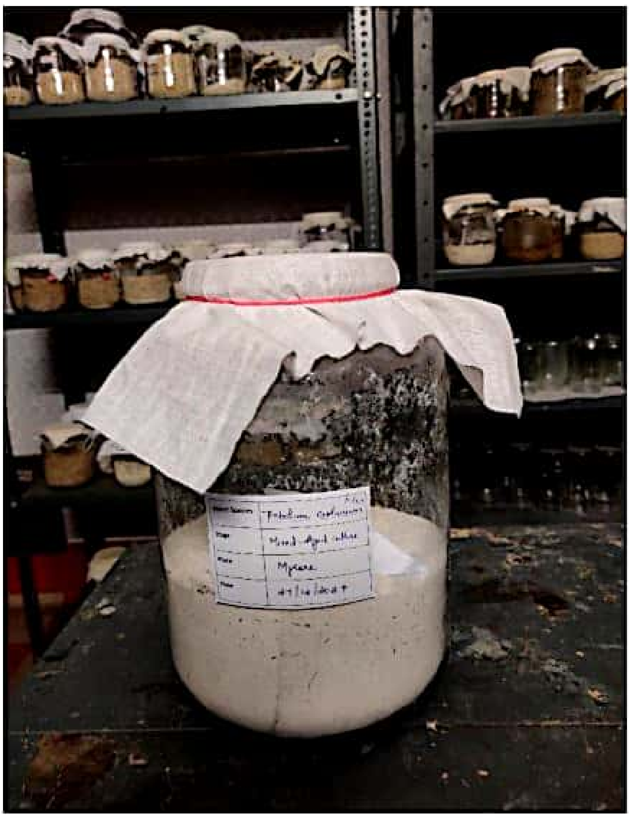

Figure 2. Insect culture of *Tribolium castaneum* (Wheat flour)

4.5 Preparation of crude organic extracts

Fresh and healthy leaves of *Vitex negundo* were collected from Malipuram, Vypin, Ernakulam, Kerala. The leaves were washed, cut into smaller pieces, and air-dried under shade for three days to preserve their bioactive properties. Once dried, the leaves were finely powdered. For extraction, 50 grams of the powdered leaves were placed in a thimble and subjected to sequential extraction using a Soxhlet apparatus. A series of solvents with increasing polarity—hexane, ethyl acetate, acetone, and methanol—were employed for the process. A total of 850 ml of each solvent was used at a temperature maintained 7–8°C below its boiling point to prevent thermal degradation of the bioactive compounds. The extraction process was carried out for six hours for each solvent. The solvent-extract mixture was collected in a round-bottom flask, and the yield was calculated using the formula:

Percentage yield = (Weight of the extract / Total Weight of the sample) $\times 100$

Figure 3. Leaves of *Vitex negundo*

Figure 4. Extraction using Soxhlet Apparatus

4.6 Concentrate of Crude Extract

The crude extract was concentrate using a rotary evaporator to separate the solvent from the bioactive compounds. The cooling system of the evaporator was prepared with a water and glycol mixture in a 1:1 ratio, which was circulated continuously throughout the solvent recovery process to maintain efficiency. The chilling temperature of the cooling water was set to 4°C, while the water bath temperature was maintained at 50°C. A vacuum pressure of 153 Pa was applied, and the apparatus operated at a rotation speed of 100 rpm. Each solvent was allowed to reach its boiling point, vaporizing during the process. The vaporized solvent was condensed by the chilled water and collected in a collector flask. This process ensured effective separation of the solvent from the extract, leaving behind the crude bioactive compounds in the evaporation flask. The resulting crude extract was then used for subsequent analysis and experimentation.

Figure 5. Concentrate of plant crude extract using rotary evaporator

4.7 Fumigant toxicity bioassay of Extract

To assess fumigant toxicity, the crude extract was dissolved in 20 ml of acetone to create a test solution. Desiccators with an internal volume of 850 ml were used as fumigation chambers. Whatman No. 1 filter paper (125 mm in diameter) was treated with the prepared solution by evenly loading the extract onto the filter paper. The treated filter paper was securely attached to the septum of the desiccator to ensure consistent vapor release (Rajashekar et al., 2016).Three different concentrations of the crude extract were prepared to evaluate the dose-dependent fumigant activity. The fumigation experiments were conducted under controlled conditions, with each desiccator housing the test insects in the absence of food. The exposure period for all treatments was standardized at 24 hours. A control setup was maintained for each experiment, where no treatment was applied, to compare and validate the results. The same procedure was repeated for solvent extracts obtained using hexane, acetone, and methanol at varying concentrations. Following the 24-hour exposure period, the mortality of the test insects was observed visually and recorded. This method enabled a comparative analysis of the fumigant efficacy of extracts derived from different solvents

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Figure 6. Experimental setup for fumigant toxicity bioassay

4.8 Contact toxicity by using filter paper diffusion method

The contact toxicity of the extracts was evaluated against *Sitophilus oryzae* and *Tribolium castaneum* using the filter paper diffusion method, as described by Rajashekar et al. (2010). A stock solution was prepared by mixing 1000 µl of the crude extract with 9 ml of acetone. From this stock, 1000 µl of the solution was evenly applied onto Whatman filter paper discs in four replicates for each test concentration. The filter papers, once treated, were allowed to dry completely to ensure the evaporation of the solvent.

After drying, the treated filter papers were placed in sterile petri dishes with a diameter of 9 cm, providing a surface area of 63.5 cm^2. Control setups were also prepared using acetone alone without the crude extract to account for solvent effects. Twenty freshly emerged adult insects were introduced into each petri dish for testing.

The petri dishes were sealed and maintained under controlled conditions to observe the effect of the extract. After a 24-hour exposure period, the mortality of insects was recorded. To quantify the dosage for each treatment, the dry weight of the crude extract applied was calculated and divided by the surface area of the petri dish. This ensured standardized and reproducible results across all experiments. The data provided insights into the contact toxicity potential of the extracts.

Figure 7. Experimental set up for contact toxicity bioassay

4.9 Contact toxicity with Food

To evaluate the contact toxicity of plant extracts on adult *Sitophilus oryzae*, a bioassay was conducted using treated wheat grains. Each 50 mL glass vial was filled with 10 g of wheat grains, which were thoroughly mixed with the respective plant extract solutions. The solvent in the mixtures was allowed to evaporate completely at room temperature to eliminate any potential residual effects. Once the solvent had fully dried, twenty newly emerged adult *S. oryzae* were introduced into each vial. Two replicates were prepared for each treatment to ensure the reliability of the results. A control group was also established using the same procedure, but the wheat grains were treated with solvent alone, serving as a baseline to account for any mortality caused by the solvent. Mortality of the insects was observed and recorded at intervals of 24-, 48-, and 72-hours post-treatment. Insects were considered dead if they exhibited no response to gentle probing with a fine brush. This method provided a clear and measurable assessment of the contact toxicity of the plant extracts while ensuring minimal interference from the solvent itself.

Percentage mortality was corrected using Abbott's formula [Abbott, 1925]:

Corrected Mortality (%) = [(Mortality in Treatment − Mortality in Control) / (100 − Mortality in Control)] × 100

Figure 8. Experimental set up for contact toxicity with food

4.10 Preliminary components detection by thin layer chromatography

Analysis of components was performed using Thin-layer chromatography (TLC). Silica gel 60 F254 TLC plates were used. The plates were cut into uniform sheets with 10 cm length and 5 cm width. The sample was loaded at the centre, 2 cm above the base of the plate. TLC was carried out with different solvents such as Ethyl acetate, Chloroform and methanol in the ration 5:4:1.and hexane, ethyl acetate, chloroform and methanol in the ratio 2:3:4:1. The plates were examined under UV 254nm for visualization of bands.

R_f value = Distance travelled from the original point to the spot / Distance travelled by solvent from the original point to front line

Figure 9. Experimental set up for TLC

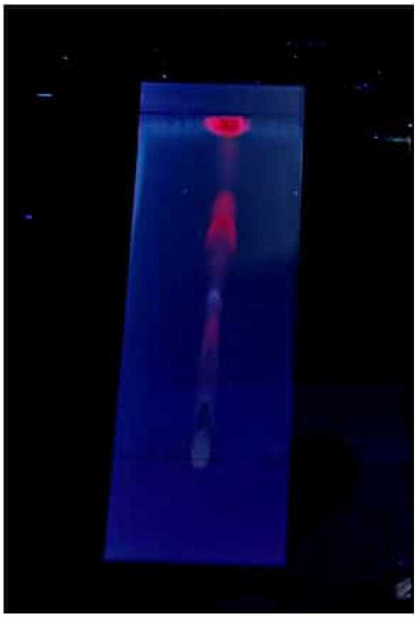

Figure 10. Visualization of band under UV

4.11 Purification of bioactive fractions from *Vitex negundo*

Column chromatography was performed in a glass column with a surface area of 62.8 cm^2, using silica gel as the stationary phase and hexane as the initial solvent. Five millilitres (1.2 g) of ethyl acetate extract were mixed into a slurry with silica gel and hexane, which was then carefully loaded onto the column. The process employed a gradient elution technique, using a series of 12 solvent mixtures with varying polarities to separate the components into distinct fractions. The elution sequence began with 100% hexane, followed by solvent mixtures in the following ratios: 50:50 and 25:75 hexane and ethyl acetate, 100% ethyl acetate, 75:25, 50:50, and 25:75 ethyl acetate and chloroform, 100% chloroform, 75:25, 50:50, and 25:75 chloroform and methanol, and finally 100% methanol. Each combination aimed to elute compounds of differing polarity to achieve optimal separation.

The eluents were collected in 12 separate conical flasks, corresponding to each solvent mixture. Once collected, the solvents in the fractions were evaporated using a rotary evaporator, leaving behind the concentrated extracts. Each extract was subsequently dissolved in a known volume of acetone and subjected to screening for insecticidal activity. This method allowed for the systematic isolation and evaluation of bioactive components from the ethyl acetate extract.

Figure 11. Column Chromatography

Figure 12. Collected fractions

4.12 GC-MS Analysis of Active fraction from *Vitex negundo*

The chemical constituents of active fractions from *V. negundo* were analysed using GC-MS. The GC-MS results was achieved using Agilent 8890 system, including an Agilent 5977 MSD. The analysis was conducted using a HP-5MS fused silica capillary column (30m x 250 µm x 0.25 µm). A 70 eV ionization energy was applied, which is standard for electron ionization in GC-MS, allowing the generation of ions for mass spectrometric detection. To guarantee efficient vaporization and transfer of the extract sample, both the injector and mass transfer line were held at 250°C. A flow rate of 1 mL/min was maintained for helium as the carrier gas, with a 0.5 μL injection volume and a 1:100 dilution of the sample in hexane. The temperature of the GC column was first set at 40°C for 1 minute, then increased at 5°C per minute to 250°C, where it remained for 20 minutes. This temperature program is commonly applied for separating vaporizing and semi-vaporizing compounds in the sample. By comparing the mass spectra of the extract compounds with those in the 2017 NIST GC-MS Libraries, the chemical components were identified. This comparison allows for precise identification of the compounds based on their distinct mass spectral profiles.

Figure 13. GC-MS System

4.13 Moisture Analysis

A moisture analyzer was employed to quantify the level of moisture contained in specimens of fresh, infected, and treated wheat as well as in wheat flour. To ensure equal drying, every sample was accurately weighed and spread evenly on the sample tray. Following that, the tray was placed in the moisture analyzer, where it was permitted to dry for fifteen minutes in carefully controlled conditions. Depending on weight reduction during drying, the device itself recorded automatically the final moisture content (%) after completion. This method provided an instantaneous and accurate method for estimation of the moisture content, necessary to assess samples' stability and quality under diverse treatment conditions.

Figure 14. Determination of moisture content using Moisture Analyzer

4.14 Fat Analysis

The traditional soxhlet extraction technique was employed to determine the crude fat content in both fresh and infected wheat grain samples. To calculate the uniform particle size and for efficient solvent penetration, the materials were powdered fine prior to extraction. A cellulose thimble containing approximately a known weight of powdered material was placed inside the Soxhlet extractor. Since petroleum ether is a good solvent for lipids, it was selected as the solvent for extraction. To ensure that all the lipids were extracted, the extraction was carried out for ten hours at a controlled temperature of 50 to 60 °C. The solvent was evaporated after extraction, and the weight of the fat residue left behind was measured. A percentage of the initial sample weight was employed to express the fat content.

Figure 15. Experimental set up for Fat Analysis

4.15 Protein Estimation

A Dumas combustion method-based N-protein analyser was utilized to determine the protein content in fresh, infested, and treated wheat grain samples. A known weight of each sample (30-50 mg) was accurately measured with an analytical balance prior to loading into sample trays with distinct labels. Then the samples were injected into the protein analyser, where they were automatically combusted prior to being detected and analysed for gases. The entire process of analysis took around six hours. The equipment used to automatically convert the nitrogen content to crude protein using a standard conversion factor, which is often 6.25 in wheat. These results were given as a percentage of the sample's dry weight.

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Figure 16. Protein estimation using n-Protein Analyzer

4.16 Statistical analysis

By using the Abbot formula equation (1925), percentage mortality was calculated while the LC_{50} values with their respective confidence limits were calculated by Probit analysis using Statplus 2007 software statistical programme (Devi et al.,2021)

5. RESULTS

5.1 Yield of extracts

The organic extracts of *Vitex negundo* were obtained through Soxhlet extraction, employing a sequential use of solvents with increasing polarity. The highest yield was observed with methanol, which produced 10.2 g of extract, followed by acetone with 8.2 g, hexane with 6.8 g, and ethyl acetate with 3.8 g, as presented in Table 3. This gradient extraction approach ensured the recovery of a broad range of bioactive compounds, from nonpolar to highly polar molecules. The variation in yield highlights the solubility differences of plant constituents across the solvents, emphasizing the methanol extract's efficiency in isolating a greater quantity of polar compounds.

Table 3 : Yield of Plant extract

Sl. No.	Plant Material	Solvents	Percentage yield
1	*Vitex negundo*	Hexane	6.8
		Ethyl Acetate	3.8
		Acetone	8.2
		Methanol	10.2

5.2 Fumigant toxicity bioassay of Ethyl acetate extract of *Vitex negundo*

The ethyl acetate (EA) extracts from *Vitex negundo* exhibited strong fumigant activity against *Sitophilus oryzae* and *Tribolium castaneum*. The lethal concentration required to kill 50% of the insect population (LC_{50}) for the EA extract was 11.7 µL/L for *S. oryzae*, 12.6 µL/L for *T.*

castaneum where in case of LC_{90} which increased to 21.8 µL/L and 22.68 µL/L respectively, after a 24-hour exposure period, as shown in Table 4 & 5. These findings indicate a notable decrease in the effectiveness of the extract with prolonged exposure, suggesting that the fumigant activity is time dependent. The results underline the potential of *V. negundo* EA extracts as an effective natural fumigant for pest control, with significant implications for stored grain protection.

Table 4. Mortality of *S. oryzae* adults due to the fumigant toxicity of ethyl acetate extract of *Vitex negundo*

Extract Concentration	Mean ± SD
20 µl/L	100 ± 0.00
15 µl/L	72.5 ± 11.46
10 µl/L	0 ± 0.00

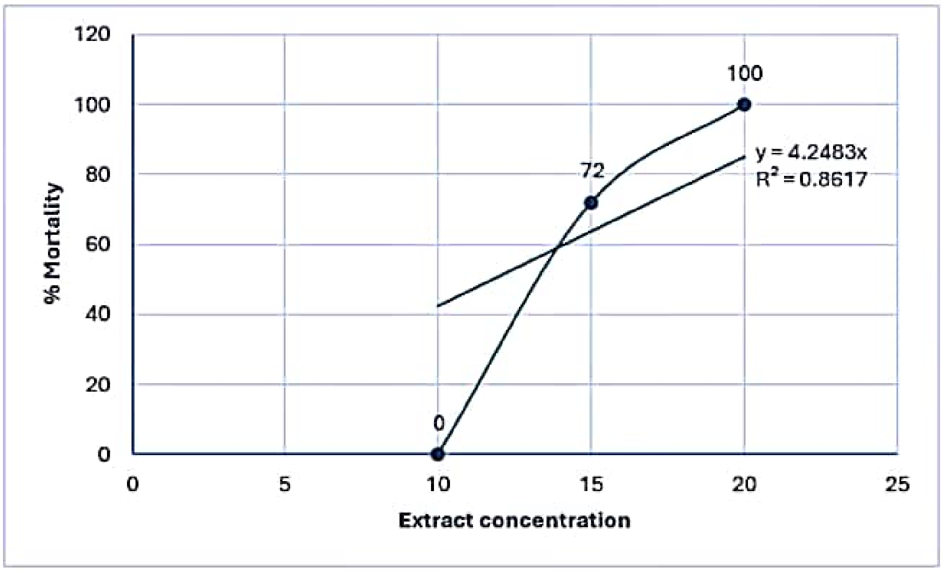


Figure 17. Fumigant toxicity of EA extract of *Vitex negundo* against *S. oryzae* at concentrations 20 µl/L,15 µl/L and 10 µl/L

Table 5. Mortality of *Tribolium castaneum* adults due to the fumigant toxicity of ethyl acetate extract of *Vitex negundo*

Extract Concentration	Mean ± SD
20 µl/L	93.75 ± 7.50
15 µl/L	57.50 ± 15.55
10 µl/L	26.25 ± 22.13
5 µl/L (22.3 µl)	0.00 ± 0.00

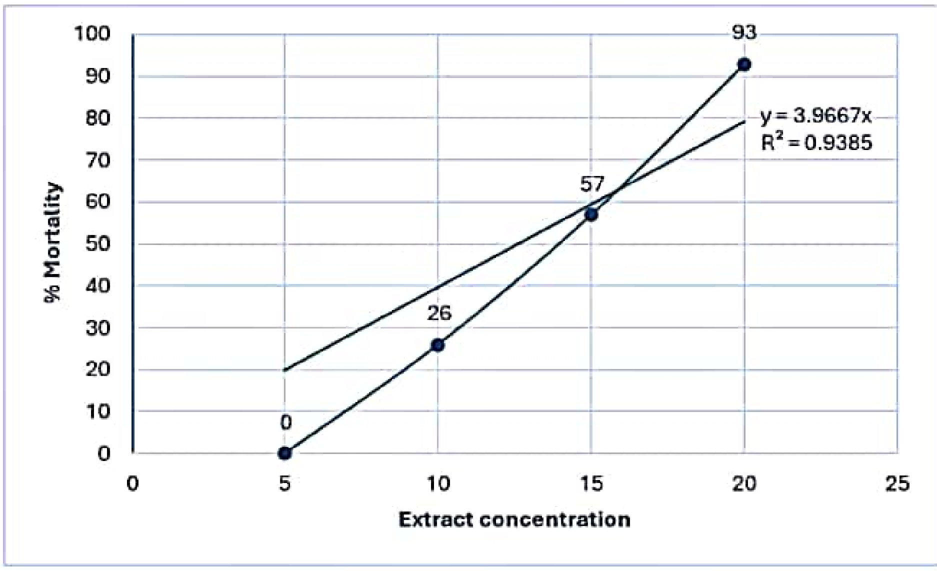


Figure 18. Fumigant toxicity of EA extract of *Vitex negundo* against *T.castaneum* at concentrations 20 µl/L,15 µl/L,10 µl/L and 5 µl/L

Table 6. LC_{50} & LC_{90} Values of EA extracts against *S.oryzae* and *T.castaneum*

Plant Extract	Insect	LC_{50} Value	LC_{90} Value
EA extract of *Vitex*	*Sitophilus oryzae*	11.76941	21.18494
negundo	*Tribolium castaneum*	12.60494	22.68888

5.3 Fumigant toxicity bioassay of Methanol extract of *Vitex negundo*

The fumigant toxicity of the methanol extract from *Vitex negundo* was assessed against *Sitophilus oryzae* and *Tribolium* adults. Fumigation for *Sitophilus* adults was conducted at concentrations of 40 µl/L, 70 µl/L, and 75 µl/L (Table 7). Similarly, for *Tribolium* adults, fumigation was performed at the same concentrations (40 µl/L, 70 µl/L, and 75 µl/L) following a 24-h exposure period (Table 8). The effectiveness of the fumigation was evaluated based on the mortality rate of the insect adults at each concentration, providing insight into the potential of *Vitex negundo* as a fumigant against these pests.

Table 7. Mortality of *Sitophilus oryzae* adults due to the fumigant toxicity of Methanol extract of *Vitex negundo*

Extract Concentration	Mean ± SD
75 µl/L	100 ± 0.00
70 µl/L	81.25 ± 15.41
40 µl/L	0 ± 0.00

Table 8. Mortality of *Tribolium castaneum* adults due to the fumigant toxicity of Methanol extract of *Vitex negundo*

Extract Concentration	Mean ± SD
75 µl/L	90.0 ± 9.35
70 µl/L	63.75 ± 19.80
40 µl/L	2.5 ± 4.33

5.4 Fumigant toxicity bioassay of Acetone extract of *Vitex negundo*

The fumigant toxicity of the acetone extract from *Vitex negundo* was assessed against *Sitophilus oryzae* and *Tribolium castaneum* adults. Fumigation for *Sitophilus* adults was conducted at concentrations of 45 µl/L, 40 µl/L, and 25 µl/L (Table 9). Similarly, for *Tribolium* adults, fumigation was performed at the same concentrations (405µl/L, 40 µl/L, and 25 µl/L) following a 24-h exposure period (Table 10). The effectiveness of the fumigation was evaluated based on the mortality rate of the insect adults at each concentration, providing insight into the potential of *Vitex negundo* as a fumigant against these pests.

Table 9. Mortality of *Sitophilus oryzae* adults due to the fumigant toxicity of Acetone extract of *Vitex negundo*

Extract Concentration	Mean ± SD
45 µl/L	100 ± 0.00
40 µl/L	81.25 ± 18.83
25 µl/L	1.25 ± 2.17

Table 10. Mortality of *Tribolium castaneum* adults due to the fumigant toxicity of Acetone extract of *Vitex negundo*

Extract Concentration	Mean ± SD
45 µl/L	77.5 ± 24.87
40 µl/L	63.75 ± 12.93
35 µl/L	0 ± 0.00

5.5 Fumigant toxicity bioassay of Hexane extract of *Vitex negundo*

The fumigant toxicity of the hexane extract from *Vitex negundo* was assessed against *Sitophilus oryzae* and *Tribolium castaneum* adults. Fumigation for *Sitophilus* adults was conducted at concentrations of 40 µl/L, 35 µl/L, and 25 µl/L (Table 11). Similarly, for *Tribolium* adults, fumigation was performed at the same concentrations (40 µl/L, 35 µl/L, and 20 µl/L) following a 24-h exposure period (Table 12). The effectiveness of the fumigation was evaluated based on the mortality rate of the insect adults at each concentration, providing insight into the potential of *Vitex negundo* as a fumigant against these pests.

Table 11. Mortality of *Sitophilus oryzae* adults due to the fumigant toxicity of Hexane extract of *Vitex negundo*

Extract Concentration (µl/L)	Mean ± SD
40	100 ± 0.00
35	65 ± 13.69
25	0 ± 0.00

Table 12. Mortality of *Tribolium castaneum* adults due to the fumigant toxicity of Hexane extract of *Vitex negundo*

Extract Concentration (µl/L)	Mean ± SD
40	95 ± 6.12
35	65 ± 15.81
20	0 ± 0.00

5.6 Contact Toxicity

No death was recorded among any of the treated insect populations following a 24-hour contact exposure to the tested extract. This indicates that, in the test environments, the extract was not displaying acute contact toxicity. During the period of observation, all test insects of the treatment and control groups remained alive, meaning that there was no direct injurious effect due to contact exposure at the dosages examined.

5.7 Insect Mortality Due to Different Extracts of *Vitex negundo*

Table 13. Contact toxicity of extract of *Vitex negundo* against *Sitophilus oryzae*

Extract Type	Concentration (%)	Mean No. of Dead Insects ± SD
Hexane Extract	0.2	0.00 ± 0.00
EA Extract	0.2	0.00 ± 0.00
Methanol Extract	0.4	0.00 ± 0.00
Acetone Extract	0.3	0.00 ± 0.00

5.8 Contact Toxicity bioassay with food

Four solvent extracts of *Vitex negundo*—hexane, ethyl acetate, methanol, and acetone extracts—were used in contact toxicity tests, however none of them significantly harmed the test insects. For every concentration and time period examined, there was no mortality. According to these results, in the circumstances of the current investigation, the solvent extracts of *Vitex negundo* have very little or no contact toxicity.

Hexane extract

Table 14. Contact Toxicity bioassay with food of Hexane extract

Time (hours)	Mean % Mortality ± SD
24	0.00 ± 0.00
48	0.00 ± 0.00
72	18.75 ± 6.29

Ethyl acetate extract

Table 15. Contact Toxicity bioassay with food of EA extract

Time (hours)	Mean % Mortality ± SD
24	0.00 ± 0.00
48	0.00 ± 0.00
72	22.50 ± 11.90

Methanol extract

Table 16. Contact Toxicity bioassay with food of Methanol extract

Time (hours)	Mean % Mortality ± SD
24	0.00 ± 0.00
48	0.00 ± 0.00
72	25.00 ± 9.13

Acetone Extract

Table 17.Contact Toxicity bioassay with food of Acetone extract

Time (hours)	Mean % Mortality ± SD
24	0.00 ± 0.00
48	0.00 ± 0.00
72	13.75 ± 6.29

5.9 Fumigant Toxicity of Ethyl Acetate Fractions

Fraction 9 showed the most promising insecticidal activity out of the twelve fractions that were extracted from *Vitex negundo*. The fraction showed higher mortality rate compared other fractions. Fraction 9 was chosen for additional examination using Gas Chromatography-Mass Spectrometry (GC-MS) in order to determine the possible bioactive compounds in charge of the observed insecticidal effects because of its high level of bioactivity. It is anticipated that the thorough chemical profile will provide insight into the components that make up this fraction's efficacy.

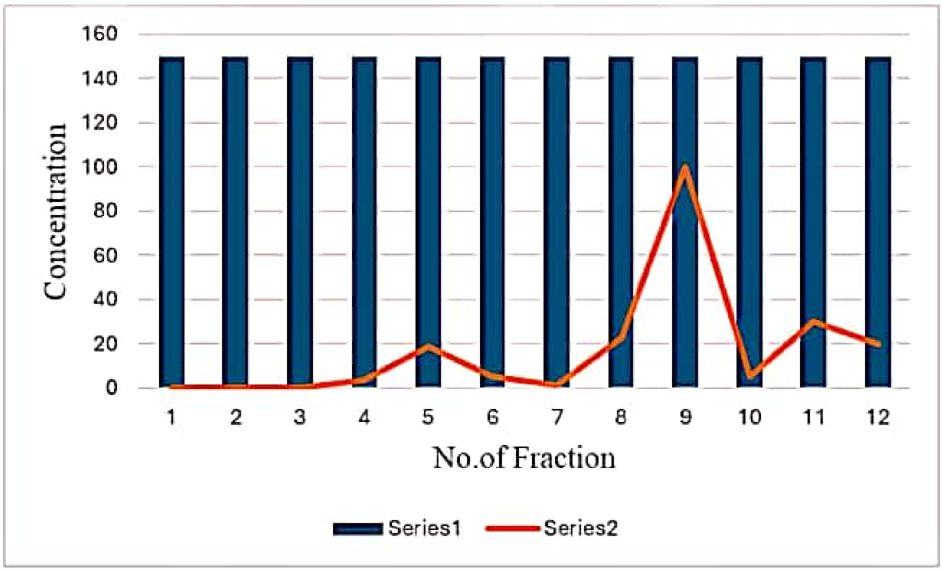


Figure 19. Fraction 9 showing the highest fumigant toxicity against *Sitophilus oryzae*

5.10 GC-MS Result of active fraction of *Vitex negundo*

5.10.1 Chemical compounds present in the active fraction of EA extract of *Vitex negundo*

The GC-MS analysis identified 21 compounds accounting for 91.674% of the total fraction composition (Table 18 ; figure 20). The major compounds are 2,4-Di-tert-butylphenol(15.80 %), 1-Docosene (15.82), Eicosyl trifluoroacetate (15.76%), E-15-Heptadecenal (13.98%), Tetracosan-10-yl acetate(12.59%) that all likely contributed to the insecticidal activity through different mechanism of action. 2,4-Di-tert-butylphenol a key ingredient in volatile or essential oils, and it is very harmful to practically every tested organism (Zhao et al.,2020).1-Docosene is a plant metabolite and were previously derived from the plant leaf ethanolic extract of *Mangifera indica* (Adeyinka et al.,2022). It has also been reported in *Acorus calamus* and *Vitex negundo* through GC-MS analysis, indicating its presence as a bioactive hydrocarbon component (Kumar et al., 2016; Rekha et al., 2020). Eicosyl trifluoroacetate were reported in *Eranthemum pulchellum*, a tropical evergreen shrub by Darmwal et al.,2023 . E-15-Heptadecenal is an unsaturated aldehyde, found in volatile profiles and was previously derived from the roots of *Bacopa monnieri* (Malini, S., & Eganathan, P 2013). Tetracosan-10-yl acetate is a volatile plant material and were obtained from *Persicaria hydropiper* and were effect against targeted pests(Jena et al., 2022).The difference observed in the chemical composition of the extract may be attributed to the environment for plant growth, genetic factors, geographical differences and ecological variations such as soil, climatic conditions, place of cultivation and experimental conditions (Devi et al.,2021)

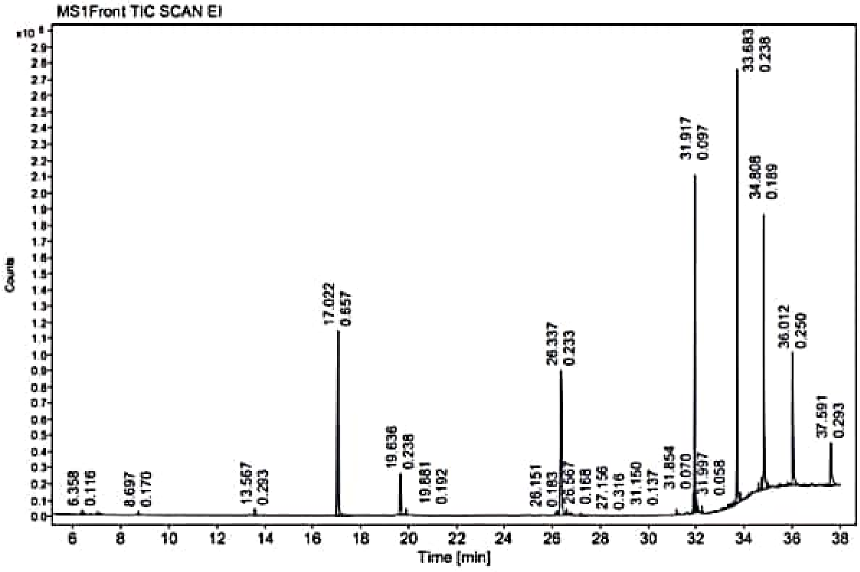
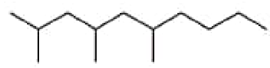
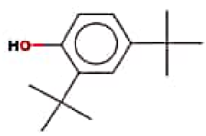
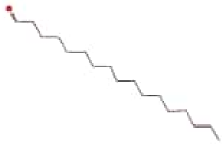
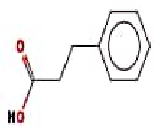
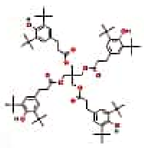
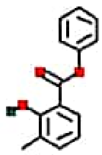
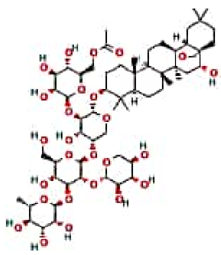


Figure 20: GC-MS chromatogram of fraction-9 ethyl acetate *Vitex negundo* extract

Table 18. GC-MS result of Fraction 9 of EA extract of *Vitex negundo*

Peak no:	RT	Bioactive Compound	Molecular Structure	Molecular Formula	RSI	RA
1	6.358	Decane, 2,4,6-trimethyl-		$C_{13}H_{28}$	874	0.2074
2	8.697	Dodecane		$C_{12}H_{26}$	830	0.2731
3	13.567	Tetradecane		$C_{14}H_{30}$	850	0.5815
4	17.022	2,4-Di-tert-butylphenol		$C_{14}H_{22}O$	924	15.8000

5	19.636	Dichloroacetic acid, 4-hexadecyl ester		$C_{18}H_{34}Cl_2O_2$	924	3.7549
6	19.881	Hexadecane		$C_{16}H_{34}$	841	0.6393
7	26.151	E-7-Octadecene		$C_{18}H_{36}$	858	0.7577
8	26.337	E-15-Heptadecenal		$C_{17}H_{32}O$	939	13.9837
9	26.567	Octadecane		$C_{18}H_{38}$	825	0.6782
10	26.736	Acetic acid, chloro-, octadecyl ester		$C_{20}H_{39}ClO_2$	751	0.3267
11	27.156	4-Trifluoroacetoxypentadecane		$C_{17}H_{31}F_3O_2$	785	0.3145
12	31.150	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy	 	$C_9H_{10}O_2$ $C_{73}H_{108}O_{12}$	832	0.3879
13	31.854	5-Eicosene, (E)-		$C_{20}H_{40}$	868	1.1151
14	31.917	Eicosyl trifluoroacetate		$C_{22}H_{41}F_3O_2$	911	15.7616
15	31.99	Eicosane		$C_{20}H_{42}$	843	0.5682

	7					
16	32.06 0	Acetic acid, chloro-, octadecyl ester		$C_{20}H_{39}ClO_2$	787	0.2397
17	32.22 6	9-octadecenoic acid		$C_{18}H_{34}O_2$	734	0.4050
18	33.68 3	1-Docosene		$C_{22}H_{44}$	897	15.8202
19	34.14 6	Benzoic acid, 2-hydroxy-3-methyl-phenyl ester		$C_{14}H_{12}O_3$	701	0.3088
20	34.80 8	Tetracosan-10-yl acetate		$C_{60}H_{98}O_{26}$	941	12.5957
21	36.01 2	17-Pentatriacontene		$C_{35}H_{70}$	781	7.3622

5.11 Moisture Content

There were significant differences in the average moisture content of the wheat and wheat flour samples under fresh, infected, and solvent-treated conditions. The moisture content of fresh wheat (10.23%) and fresh wheat flour (9.46%) was within normal storage limits.

Infestation, however, resulted in higher moisture levels in both wheat (12.01%) and wheat flour (11.67%). This is probably because insects enhanced metabolic activity and related microbial development raise humidity in storage settings. Acetone-treated wheat had the lowest moisture content (9.05%) among the solvent-treated samples, possibly as a result of its drying action and volatility. In contrast, relatively higher moisture levels were the outcome of treatments with ethyl acetate (12.05%), methanol (11.92%), and hexane (11.45%). These variations highlight how infestation and treatment techniques affect grain moisture, which has a direct bearing on storage stability.

Table 19. Moisture Content of Fresh, infested and treated wheat and wheat flour

Sample	Mean Moisture Content (%)
Fresh Wheat	10.23 %
Infested Wheat	12.01 %
Fresh Wheat Flour	9.46 %
Infested Wheat Flour	11.67 %
EA Treated Wheat	12.05 %
Hexane Treated Wheat	11.45 %
Acetone Treated Wheat	9.05 %
Methanol Treated Wheat	11.92 %

5.12 Protein Content

In fresh, infected, and ethyl acetate (EA) extract-treated wheat, the protein content varied significantly. Protein content in fresh wheat was 11.93%, which is in conformity with the expected value for wheat grain. The protein content in infested wheat was significantly higher at 13.63%. The occurrence of insect biomass, i.e., insect excreta, larvae, and body parts, could be responsible for this increase in nitrogen and thus calculated protein. This can increase the protein levels by adding more nitrogenous compounds. Interestingly, compared to infested samples without treatment, EA-treated infested wheat had slightly lower protein content (13.20%).

Table 20. Protein Content of Fresh, infested and EA treated wheat sample

Sample Name	Sample Weight (mg)	Protein factor	Nitrogen	Protein (%)
Fresh Wheat	54.5	6.25	1.909441948	11.93401241
Infested Wheat	39.1	6.25	2.180988073	13.63117504
Ethyl Acetate extract Treated Wheat (Infested)	69.5	6.25	2.112720966	13.20450592

5.13 Fat Content

With the Soxhlet extraction technique, the crude fat content of infested and fresh wheat samples was determined. Infested wheat contained a much higher fat content of 0.1946 g, while fresh wheat yielded 0.1648 g of fat per 10 g sample. The presence of insect residues or metabolic products, which could be adding more lipid material, could be responsible for the increase in fat observed in the infested sample. Based on these findings, infestation has the potential to cause contamination that misleadingly increases the fat level of grains that have been stored along with changing their nutritional composition.

Table 21. Fat content of Fresh and Infested Wheat

Sample	Wt. of empty RB Flask	Wt. of Sample	Wt. of RB+Fat	Wt. Of Fat
Fresh Wheat	122.0626 g	10 g	122.2274 g	0.1648
Infested Wheat	127.7233 g	10 g	127.9179 g	0.1946

6. DISCUSSION

The use of synthetic pesticides for pest management has been a standard practice in agriculture for decades. Among the various chemical agents employed, methyl bromide and aluminium phosphide are two of the most widely used fumigants. These compounds have been extensively used to manage stored grain pests, which are responsible for significant post-harvest losses in grains such as wheat, rice, and corn. While these chemicals have proven effective in controlling pest populations, they also pose considerable risks to the environment, non-target organisms, and even human health. Furthermore, there is growing concern about the development of pest resistance to these chemicals due to prolonged exposure, which diminishes their effectiveness over time.

The environmental hazards associated with methyl bromide have led to its eventual ban in many countries. Methyl bromide has been identified as a major contributor to the depletion of the ozone layer, which shields the Earth from harmful ultraviolet radiation. The adverse effects of ozone layer depletion are well-documented and include an increase in skin cancers, cataracts, and other health issues, as well as disruptions to ecosystems. (Rajashekar et al., 2014).) As a result, international regulations, such as the Montreal Protocol, have mandated the phase-out of methyl bromide use, especially in developing countries. Moreover, research indicates that prolonged exposure to these chemicals can lead to the development of resistance in pest species, further complicating pest management efforts.

The need for alternative pest control methods has never been more urgent. Given the drawbacks of synthetic chemicals, researchers and agricultural experts have turned their attention to the development of more sustainable, eco-friendly solutions. Biopesticides, which are derived from natural materials such as plants, bacteria, fungi, and other biological agents, have emerged as a promising alternative. Biopesticides are biodegradable, easily available, and often cost-effective. Furthermore, they are generally considered safe for humans and non-target organisms, making them a desirable option for sustainable pest management. Despite the promise of biopesticides, however, only a limited number of plant species have been actively developed and commercialized for use in pest control, indicating that further research is necessary to fully harness the potential of plant-based solutions (Acheuk et al., 2022).

In the present study, the potential of *Vitex negundo* (commonly known as the Chinese chaste tree) was investigated as a biopesticide for the control of two major stored grain pests: *Sitophilus oryzae* (rice weevil) and *Tribolium castaneum* (red flour beetle). These pests are responsible for significant damage to stored grains and controlling them is critical for ensuring food security. *Vitex negundo* is known for its bioactive compounds, which exhibit a wide range of biological activities, including insecticidal, antifungal, and antimicrobial properties. In this study, bioactive compounds were extracted from the leaves of *Vitex negundo* using various solvents, including ethyl acetate, hexane, methanol, and acetone. The resulting extracts were tested for fumigant toxicity against the two insect species at different concentrations.

The fumigation trials were conducted at concentrations of 20, 15, 10, and 5 µl/L for both insect species. The results showed that the fumigant activity varied depending on the solvent used and the insect species tested. The ethyl acetate extract of *Vitex negundo* exhibited the highest toxicity, with 100% mortality of *Sitophilus oryzae* at the highest concentration of 20 µl/L. In contrast, the mortality rate for *Tribolium castaneum* was lower, indicating a species-specific difference in susceptibility. This finding is consistent with previous studies that have demonstrated varying degrees of resistance between different pest species to plant-based insecticides. For example, a study by Shawer et al. (2022) found that *Sitophilus oryzae* was more susceptible to plant extracts from *Simmondsia chinensis* and *Rosmarinus officinalis* than *Tribolium castaneum*. The fumigant toxicity of other solvent extracts, such as methanol, acetone, and hexane, was also evaluated, with the ethyl acetate extract showing superior efficacy.

To better understand the effectiveness of the plant extracts, the study also estimated the LC_{50} and LC_{90} values for the different solvents. These values represent the concentrations of the extracts required to kill 50% and 90% of the insect population, respectively. The results revealed that *Sitophilus oryzae* was significantly more susceptible to the plant extracts than *Tribolium castaneum*. This difference in susceptibility could be attributed to several factors, including variations in insect physiology, behavioural responses, and resistance mechanisms. Previous research has shown that *Sitophilus oryzae* is more vulnerable to lipophilic and volatile compounds compared to *Tribolium castaneum*, which may have developed more robust detoxification systems and greater resistance to certain bioactive compounds.

Among the twelve fractions of ethyl acetate *V.negundo* extract, fraction nine shown the highest mortality rate. The bioactive compounds present fraction 9 were identified using gas chromatography-mass spectrometry (GC-MS) analysis. Several compounds with known insecticidal properties were detected, including 2,4-di-tert-butylphenol, eicosyl trifluoroacetate, 1-docosene, E-15-heptadecenal, and tetracosan-10-yl acetate. 2,4-di-tert-butylphenol, for instance, is known for its antioxidant and antibacterial properties, which disrupt enzymatic activity and cellular membranes in pests and microorganisms (Cheng et al., 2011). The long-chain alkene, docosene, has been identified as a component of insect cuticular hydrocarbons and is believed to play a role in insect defence and communication systems (Howard & Blomquist, 2005). Eicosyl trifluoroacetate, a fatty acid ester, has demonstrated larvicidal and repellent activities, while tetracosan-10-yl acetate could act by disrupting the insect's respiratory system or cuticle, causing desiccation or metabolic stress. These compounds likely contribute to the insecticidal activity observed in the fumigation trials (Kumar et al., 2010)

The mechanism of action of these bioactive compounds is complex and multifaceted. Insects exposed to these compounds may experience disruptions in their feeding, reproduction, and survival. Some compounds may act as antifeedants, deterring insects from consuming treated grains, while others may interfere with their physiological processes, such as respiration, metabolism, or reproduction. The findings of this study support the hypothesis that *Vitex negundo* contains potent bioactive compounds that can be used as an eco-friendly alternative to synthetic pesticides.

In addition to its insecticidal properties, *Vitex negundo* may also contribute to the preservation of grain quality by reducing moisture content and altering the biochemical composition of infested grains. The moisture content in grains and their products strongly impacts their vulnerability to microbial spoilage, infestation by insects, and total shelf-life (Sánchez-Mariño et al., 2020). The moisture content of wheat grains and flour was measured before and after treatment with the plant extracts. Fresh wheat had a moisture content of 10.23%, while infested wheat had a higher moisture content of 12.01%. This increase in moisture is likely due to insect metabolic activity, as insects release moisture through respiration and also damage the grain surface, allowing moisture to penetrate from the environment. (Kučerová & Stejskal, 2008). Interestingly, wheat treated with acetone had the lowest moisture content, likely due to the solvent's volatile nature and drying effect. In contrast, wheat treated with hexane, ethyl acetate, and methanol exhibited varying moisture levels, with ethyl acetate showing the highest moisture retention. These differences underscore the impact of both infestation and treatment methods on grain moisture, which directly influences storage stability and spoilage risk (Navarro & Donahaye, 2005).

The data agrees with previous studies that indicated that infestation by insects leads to physical and biochemical changes, including pollution, reduction in nutritional value, and enhanced wetness (Ali et al., 2020; Kuerová & Stejskal, 2008)

The protein and fat content of the wheat samples were also analysed to assess the impact of infestation and treatment on grain quality. Infested wheat had a higher protein content (13.63%) compared to fresh wheat (11.93%), likely due to metabolic changes induced by insect activity. The fat content of infested wheat was also slightly higher than that of fresh wheat, suggesting that insect infestation alters the biochemical composition of the grain. Treatment with ethyl acetate extract resulted in a slight reduction in protein content, indicating that the insecticidal properties of the extract may have minimized some of the metabolic alterations associated with infestation (Koul et al., 2008; Mishra &Tripathi,2011). This finding suggests that botanical extracts not only reduce insect populations but also help maintain the nutritional quality of stored grains. The effect of evaporation loss or microbial breakdown on protein content, which may impact the quantity of nitrogen within grains that are stored (Bhargava & Meena, 2009; Sauer, 1992).

The findings of this study have important implications for pest management in stored grains. The use of *Vitex negundo* extracts as a biopesticide could offer an eco-friendly and sustainable alternative to synthetic pesticides, helping to reduce the environmental impact of pest control while maintaining grain quality. Furthermore, the study highlights the need for further research into the development of plant-based pest control systems, particularly those that target specific pests and are safe for humans and non-target organisms. By incorporating biopesticides into integrated pest management (IPM) strategies, farmers can reduce reliance on chemical pesticides and improve food security.

In conclusion, the results of this study demonstrate the potential of *Vitex negundo* as an effective biopesticide for the control of stored grain pests. The bioactive compounds present in the plant extracts exhibit significant fumigant toxicity against *Sitophilus oryzae* and *Tribolium castaneum*, with varying degrees of efficacy depending on the solvent used. The insecticidal activity of *Vitex negundo* may be attributed to the presence of bioactive compounds such as 2,4-di-tert-butylphenol, docosene, and eicosyl trifluoroacetate, which disrupt insect physiology and behaviour. Additionally, the plant extracts help to preserve grain quality by reducing moisture content and minimizing metabolic changes in infested grains. These findings provide valuable insights into the development of eco-friendly pest control solutions that can be used to protect stored grains and improve food security.

7. CONCLUSION

In the present study, crude extracts were prepared from *Vitex negundo*, a plant collected from Malipuram, Kerala. These extracts were evaluated for their potential to control two major stored grain pests, *Sitophilus oryzae* (rice weevil) and *Tribolium castaneum* (red flour beetle), using both fumigant and contact toxicity bioassays. The fumigation bioassay results revealed a clear difference in susceptibility between the two insect species, with *S. oryzae* showing greater sensitivity to the crude extracts than *T. castaneum*. Among the various solvents used for extraction, ethyl acetate proved to be the most effective, exhibiting significant fumigant toxicity against both *S. oryzae* and *T. castaneum* adults.

Further analysis using Gas Chromatography-Mass Spectrometry (GC-MS) identified several bioactive compounds from the *Vitex negundo* extract, which may contribute to its insecticidal properties. The major compounds identified included tetracosan-10-yl acetate, 2,4-di-tert-butylphenol, eicosyl trifluoroacetate, and 1-docosene. These compounds are known for their biological activities, including insecticidal and repellent effects. For instance, 2,4-di-tert-butylphenol is recognized for its antioxidant properties, while 1-docosene, a long-chain alkene, has been associated with insect defense mechanisms and may contribute to the toxicity observed in the bioassays.

The study also evaluated the biochemical effects of insect infestation on wheat grains, beyond just mortality rates. The fat content of fresh and infested wheat was measured to assess the biochemical changes caused by pest activity. Infestation led to an increase in fat content, which suggests that insect activity disrupts the grain's biochemical composition. This alteration in fat content can affect the nutritional quality, shelf life, and marketability of the grains, highlighting the importance of pest management in maintaining the overall quality of stored grains.

These findings underscore the need for effective pest control strategies that not only reduce insect populations but also preserve the nutritional integrity of stored grains. The long-term effects of plant-derived insecticides, their influence on other quality parameters such as protein and moisture, and their integration into practical storage systems warrant further investigation. Ultimately, this study supports broader efforts toward improving post-harvest management practices and advancing sustainable storage and farming methods.

8. REFERENCE

Abbott, C. E. (1925). The relative importance of vision and the chemical sense in Anax larvae. *Psyche: A Journal of Entomology*, *32*(6), 315-316.

Acheuk, F., Basiouni, S., Shehata, A. A., Dick, K., Hajri, H., Lasram, S., Yilmaz, M., Emekci, M., Tsiamis, G., Spona-Friedl, M., May-Simera, H., Eisenreich, W., & Ntougias, S. (2022). Status and Prospects of Botanical Biopesticides in Europe and MediterraneanCountries. *Biomolecules*, *12*(2),311. https://doi.org/10.3390/biom12020311

Adeyinka, O. A., Bankole, I. A. S., & John, R. (2022). Gc-Ms Bioprospecting of Phytochemicals in The Ethanolic Extract of Fresh Mangifera indica Leaves. *Journal of Chemical Society of Nigeria*, *47*(4).

Al-Harbi, N. A., Al Attar, N. M., Hikal, D. M., Mohamed, S. E., Abdel Latef, A. A. H., Ibrahim, A. A., & Abdein, M. A. (2016). Evaluation of Insecticidal Effects of Plants Essential Oils Extracted from Basil, Black Seeds and Lavender against Sitophilus oryzae. Plants (Basel, Switzerland), 10(5), 829. https://doi.org/10.3390/plants10050829

Ali, M. Y., Khan, F. A., Jan, M. T., & Naeem, M. (2020). Effect of insect infestation on moisture content and nutritional quality of stored wheat grains. *Journal of Stored Products and Postharvest Research,* *11*(3), 24–29. https://doi.org/10.5897/JSPPR2020.0304

Arthur, F. H. (1996). Grain protectants: current status and prospects for the future. *Journal of Stored Products Research, 32*(4), 293–302. https://doi.org/10.1016/0022-474X(96)00033-9

Ashiq, S., Edwards, S., Watson, A., & Back, M. (2022). Biofumigation for the Management of Fusarium graminearum in a Wheat-Maize Rotation. Pathogens (Basel, Switzerland), 11(12), 1427. https://doi.org/10.3390/pathogens11121427

Bansal, M., Sharma, M., Kanwar, P., & Goyal, A. (2016). Recent advances in proteomics of cereals. Biotechnology & genetic engineering reviews, 32(1-2), 1–17. https://doi.org/10.1080/02648725.2016.1272357

Bhargava, M. C., & Meena, B. L. (2009). *Pests of stored grain and their management*. Agrotech Publishing Academy.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops: An identification and information guide* (2nd ed.). Wiley.

Campolo, O., Giunti, G., Russo, A., Palmeri, V., & Zappalà, L. (2018). Essential oils in stored product insect pest control. *Journal of Food Quality*, *2018*(1), 6906105.

Credland, P. F., Armitage, D. M., Bell, C. H., Cogan, P. M., & Highley, E. (Eds.). (1998). *Proceedings of the 7th International Working Conference on Stored-product Protection*. CAB International.

Darmwal, Jyoti & Dhuni, Manoj & Joshi, Pushpa & Rawat, Neelam & Koshyari, Vandana & Kathuria, Shubham. (2023). GC-MS AND FTIR ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN DIFFERENT EXTRACTS OF ERANTHEMUM PULCHELLUM ANDREWS.

Devi, T. B., Raina, V., Sahoo, D., & Rajashekar, Y. (2021). Chemical composition and fumigant toxicity of the essential oil from Tithonia diversifolia (Hemsl.) A. Grey against two major stored grain insect pests. *Journal of Plant Diseases and Protection*, *128*, 607-615.

Dhanavath, S., & Prasada Rao, U. J. S. (2017). Nutritional and Nutraceutical Properties of Triticum dicoccum Wheat and Its Health Benefits: An Overview. *Journal of food science*, *82*(10), 2243–2250. https://doi.org/10.1111/1750-3841.13844

Dufera, L. T., & Jimma, E. (2020). Management of mycotoxin in post-harvest food chain of durable crops. *Management*, *100*, 12-20.

Food and Agriculture Organization (FAO). (1995). *Manual on simple methods of meat preservation*. FAO Animal Production and Health Paper 79. https://www.fao.org/3/t0561e/T0561E00.htm

Gullan, P. J., & Cranston, P. S. (2014). *The insects: An outline of entomology* (5th ed.). Wiley-Blackwell.

Hagstrum, D. W., & Subramanyam, B. (2006). *Fundamentals of stored-product entomology*. AACC International.

Haridasan, P., Gokuldas, M., & Ajaykumar, A. P. (2017). Antifeedant effects of *Vitex negundo* L. leaf extracts on the stored product pest, *Tribolium castaneum* H. (Coleoptera: Tenebrionidae). *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*(3), 17-22.

Cheng, S. S., Liu, J. Y., Hsui, Y. R., & Chang, S. T. (2011). Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of *Cinnamomum osmophloeum*. *Bioresource Technology*, 102(1), 127–133. https://doi.org/10.1016/j.biortech.2010.06.139

Hill, D. S. (2002). *Pests of stored foodstuffs and their control*. Springer. https://doi.org/10.1007/978-1-4471-0081-3

Hinton, H. E. (1945). *A monograph of the beetles associated with stored products* (Vol. 1). British Museum (Natural History)

Howard, R. W., & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, 50, 371–393. https://doi.org/10.1146/annurev.ento.50.071803.130359

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop protection*, *19*(8-10), 603-608.

Jaiswal, J. P. (2018). Inheritance of resistance to rice weevil (Sitophilus oryzae L.) in bread wheat (Triticum aestivum L.). Electronic Journal of Plant Breeding, 9(3), 1083-1093.

Jena, M., Annamalai, M., Basana-Gowda, G., Muthiah, C., Patil, N., Rath, P. C., & Adak, T. (2022). Toxicity of water pepper, Persicaria hydropiper (L.) extracts against Nilaparvata lugens (Stål) and non-targeted effect on earthworm. *Industrial Crops and Products*, *187*, 115309.

Kathirvelu, C., & Raja, R. S. (2015). Efficacy of selected plant extracts as insecticidal fumigant against certain stored grain insect pests under laboratory conditions.

Keke, C. O., Nsofor, W. N., Kumabia, F. K. R., Iloabuchi, G. C., Ejiofor, J. C., & Osuagwu, O. L. (2023). GCMS and FTIR analysis of ethanol and methanol leave extract of Urena lobata (Caesar weed) for bioactive phytochemical constituents. *Journal of Drug Delivery and Therapeutics*, *13*(1), 99-115.

Khoobdel, M., Rahimi, V., Ebadollahi, A., & Krutmuang, P. (2022). Evaluation of the Potential of a Lectin Extracted from *Polygonumpersicaria* L. as a Biorational Agent against *Sitophilusoryzae* L. *Molecules (Basel, Switzerland)*, *27*(3), 793. https://doi.org/10.3390/molecules27030793

Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Essential oils as green pesticides: Potential and constraints. *Biopesticides International, 4*(1), 63–84.

Kučerová, Z., & Stejskal, V. (2008). Moisture content and its influence on the distribution of Sitophilus granarius in wheat grain bulks. *Journal of Pest Science, 81*(2), 89–96. https://doi.org/10.1007/s10340-007-0188-9

Kumar, A., Bhatt, D. C., & Tripathi, R. (2016). GC–MS analysis and antimicrobial activity of essential oil from rhizomes of *Acorus calamus* L. *Pharmacognosy Journal, 8*(5), 432–436. https://doi.org/10.5530/pj.2016.5.17

Kumar, S., Wahab, N., & Warikoo, R. (2010). Larvicidal efficacy of essential oils of *Cymbopogon citratus* and *Syzygium aromaticum* against malaria vector *Anopheles stephensi. Asian Pacific Journal of Tropical Medicine*, 3(7), 491–495. https://doi.org/10.1016/S1995-7645(10)60118-4

Malini, S., & Eganathan, P. (2013). GC-MS analysis of chemical composition of in vivo plant, in vitro and elicited roots of Bacopa monnieri (L.) Pennell. *Analytical Chemistry Letters*, *3*(5-6), 380-388.

Mishra, B. B., & Tripathi, S. P. (2011). Repellency and toxicity of essential oils from plant extracts against the red flour beetle, *Tribolium castaneum. Applied Entomology and Zoology,* *46*(1),103–109. https://doi.org/10.1007/s13355-010-0014-8

Mugao, L. G., Muturi, P. W., Gichimu, B. M., & Njoroge, E. K. (2020). In vitro control of Phytophthora infestans and Alternaria solani using crude extracts and essential oils from selected plants. *International Journal of Agronomy*, *2020*(1), 8845692.

Navarro, S., & Donahaye, E. (2005). Innovative environmentally friendly technologies to maintain quality of durable agricultural produce. *Acta Horticulturae*, 694, 33–46. https://doi.org/10.17660/ActaHortic.2005.694.2

Nikolaou, P., Marciniak, P., Adamski, Z., & Ntalli, N. (2021). Controlling Stored Products' Pests with Plant Secondary Metabolites: A Review. *Agriculture*, *11*(9), 879.

ORYZAE, S. (2006). Effects of six plant extracts on rice weevil Sitophilus oryzae L. in the stored wheat grains. *Journal of Agricultural and Biological Science*, *1*(4), 1-5.

Ouaarous, M., El Fakhouri, K., Taarji, N., Baouchi, A., Amri, M., Ramdani, C., Sobeh, M., Mesfioui, A., & El Bouhssini, M. (2025). Impact of Field Insect Pests on Seed and Nutritional Quality of Some Important Crops: A Comprehensive Review. *ACS omega*, *10*(9), 8779–8792. https://doi.org/10.1021/acsomega.4c08982

Pham, T. V., Nguyen, T. D., Ngo, T. T. T., Nguyen, H. T. D., Nguyen, N. A., Nguyen, Q. H., ... & Hop, V. (2024). Phytochemistry, antibacterial and antioxidant properties from Adinandra hongiaoensis andPolyspora huongiana (Theaceae). *HORIZON*, *11*(4), 1072-1077.

Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future prospects. *Food security*, *3*, 463-474. DOI 10.1007/s12571-011-0147-y

Rahman, M. A., Paul, R. R., Biswas, C., Akter, H., Rouf, R., Nath, S., Shilpi, J. A., Nahar, L., Sarker, S. D., & Uddin, S. J. (2021). Pesticidal Activity of Sundarban Mangrove Plant Extracts against *Sitophilus* Pests and Identification of Active Constituents Using LC-MS. *Advances in pharmacological and pharmaceutical sciences*, *2021*, 1540336. https://doi.org/10.1155/2021/1540336

Rajashekar, Y., Bakthavatsalam, N., & Shivanandappa, T. (2012). Botanicals as grain protectants. *Psyche: A Journal of Entomology*, *2012*(1), 646740.

Rajashekar, Y., Gunasekaran, N., & Shivanandappa, T. (2010). Insecticidal activity of the root extract of Decalepis hamiltonii against stored-product insect pests and its application in grain protection. *Journal of food science and technology*, *47*, 310-314.

Rajashekar, Y., Bakthavatsalam, N., & Shivanandappa, T. (2016). Botanicals as grain protectants. In *Sustainable Management of Arthropod Pests of Tropical Fruit Crops* (pp. 121–136). Springer. https://doi.org/10.1007/978-3-319-24397-5_7

Rajashekar, Y., Bakthavatsalam, N., & Shivanandappa, T. (2010). Contact and fumigant toxicity of selected plant oils and their combinations against the stored grain pest Callosobruchus maculatus. International Journal of Pest Management, 56(3), 207–213. https://doi.org/10.1080/09670871003674974

Rajashekar, Y., Raghavendra, A., & Bakthavatsalam, N. (2014). Acetylcholinesterase inhibition by biofumigant (Coumaran) from leaves of Lantana camara in stored grain and household insect pests. BioMed research international, 2014, 187019. https://doi.org/10.1155/2014/187019

Rajendran, S., & Sriranjini, V. (2008). Plant products as fumigants for stored-product insect control. *Journal of stored products Research*, *44*(2), 126-135.

Rees, D. (2004). *Insects of stored products*. CSIRO Publishing. https://doi.org/10.1071/9780643090277

Rees, D. (2004). *Insects of stored products*. CSIRO Publishing.

Rekha, R., Srinivasan, K., & Arumugam, M. (2020). Phytochemical screening and GC–MS profiling of bioactive compounds in leaf extract of *Vitex negundo* L. *Journal of Pharmacognosy and Phytochemistry,* *9*(4), 1194–1199. https://www.phytojournal.com/archives/2020/vol9issue4/PartS/9-4-163-476.pdf

Sabira, K. S. F., Bandara, H. M. M. O., Prasannath, V., & Prasannath, K. Use of Botanical Pesticides in Crop Protection: Current Status in Sri Lanka. In *Young Scientist Forum (YSF) National Science and Technology Commission Sri Lanka* (p. 302).

Sahu, U., Ibrahim, S. S., & Ezhil Vendan, S. (2021). Persistence and ingestion characteristics of phytochemical volatiles as bio-fumigants in Sitophilus oryzae adults. Ecotoxicology and environmental safety, 210, 111877. https://doi.org/10.1016/j.ecoenv.2020.111877

Sánchez-Mariño, J., Aponte, A. A., & Barbosa-Cánovas, G. V. (2020). Impact of moisture content on the quality of stored cereal grains: A review. *Food Engineering Reviews, 12*(2), 109–123. https://doi.org/10.1007/s12393-019-09195-z

Sauer, D. B. (1992). *Storage of cereal grains and their products* (4th ed.). American Association of Cereal Chemists.

Saxena, R. C., Dixit, O. P., & Harstfield, T. H. (1992). Insecticidal action of neem (*Azadirachta indica*) seed oil against the lesser grain borer (*Rhyzopertha dominica*). *Journal of Stored Products Research*, 28(4), 257–260. https://doi.org/10.1016/0022-474X(92)90024-W

Shawer, R., El-Shazly, M. M., Khider, A. M., Baeshen, R. S., Hikal, W. M., & Kordy, A. M. (2022). Botanical Oils Isolated from *Simmondsia chinensis* and *Rosmarinus officinalis* Cultivated in Northern Egypt: Chemical Composition and Insecticidal

Activity against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). *Molecules (Basel, Switzerland)*, *27*(14), 4383. https://doi.org/10.3390/molecules27144383

Singh, K. D., Koijam, A. S., Bharali, R., & Rajashekar, Y. (2023). Insecticidal and biochemical effects of *Dillenia indica* L. leaves against three major stored grain insect pests. *Frontiers in plant science*, *14*, 1135946. https://doi.org/10.3389/fpls.2023.1135946

Singh, K. D., Mobolade, A. J., Bharali, R., Sahoo, D., & Rajashekar, Y. (2021). Main plant volatiles as stored grain pest management approach: A review. *Journal of Agriculture and Food Research*, *4*, 100127.

Singh, N. B., Devi, M. L., Biona, T., Sharma, N., Das, S., Chakravorty, J., ... & Rajashekar, Y. (2023). Phytochemical Composition and Antimicrobial Activity of Essential Oil from the Leaves of Artemisia vulgaris L. *Molecules*, *28*(5), 2279.

Stathas, I. G., Sakellaridis, A. C., Papadelli, M., Kapolos, J., Papadimitriou, K., & Stathas, G. J. (2023). The Effects of Insect Infestation on Stored Agricultural Products and the Quality of Food. *Foods (Basel, Switzerland)*, *12*(10), 2046. https://doi.org/10.3390/foods12102046

Subramanyam, B., & Hagstrum, D. W. (1995). *Integrated management of insects in stored products*. CRC Press. https://doi.org/10.1201/9781003064557

Szuba-Trznadel, A., Gałka, B., Kamińska, J., Jama-Rodzeńska, A., Król, Z., Jarki, D., & Fuchs, B. (2024). Diversity of chemical composition and nutritional value in grain from selected winter wheat cultivars grown in south-western Poland. *Scientific reports*, *14*(1), 2630. https://doi.org/10.1038/s41598-024-53094-0

Trivedi, A., Nayak, N., & Kumar, J. (2018). Recent advances and review on use of botanicals from medicinal and aromatic plants in stored grain pest management. *Journal of Entomology and Zoology Studies*, *6*(3), 295-300.

White, I. M., & Elson-Harris, M. M. (1992). *Fruit flies of economic significance: Their identification and bionomics*. CAB International.

Yang, H., Li, Y., Xin, L., Teng, S. W., Pang, S., Zhao, H., Cao, Y., & Zhou, X. (2023). MCSNet+: Enhanced Convolutional Neural Network for Detection and Classification of *Tribolium* and *Sitophilus* Sibling Species in Actual Wheat Storage Environments. *Foods (Basel, Switzerland)*, *12*(19), 3653. https://doi.org/10.3390/foods12193653

Yogeswari, S., Ramalakshmi, S., Neelavathy, R., & Muthumary, J. Y. (2012). Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. Global Journal of Pharmacology, 6(2), 65-71.

Zhao, F., Wang, P., Lucardi, R. D., Su, Z., & Li, S. (2020). Natural Sources and Bioactivities of 2,4-Di-Tert-Butylphenol and Its Analogs. *Toxins*, *12*(1), 35. https://doi.org/10.3390/toxins12010035