"Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava"

Dissertation Work Report

Submitted in the partial fulfilment of the degree

of

MASTER OF VOCATION

in

FOOD PROCESSING TECHNOLOGY

Submitted by

SAVYA SURESH

(VM23FPT017)

Under the guidance of

Dr. PARTHIBAN P

Scientist

Food Protectants and Infestation Control Department

CSIR - Central Food Technological Research Institute

Mysuru – 570 020

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ಸಿ.ಎಸ್.ಐ.ಆರ್–ಕೇಂದ್ರೀಯ ಆಹಾರ ತಂತ್ರಜ್ಞಾನ ಸಂಶೋಧನಾಲಯ, ಮೈಸೂರು – 570020, ಭಾರತ
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CSIR - Central Food Technological Research Institute, Mysuru - 570 020, India
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This is to certify that the dissertation work entitled **"Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava"**, submitted by **Ms. SAVYA SURESH (VM23FPT017)** during the period of December 2024 to April 2025, in partial fulfilment for the award of the degree of MASTER OF VOCATION IN FOOD PROCESSING TECHNOLOGY from St. Teresa's College, Ernakulam, Kerala, is the result of an investigation carried out by her in the Department of Food Protectants and Infestation Control Laboratory, at CSIR – Central Food Technological Research Institute, Mysuru, Under the guidance of **Dr. Parthiban P**, Scientist, FPIC, CSIR-CFTRI, Mysuru.

P. Parthiban 17/4/25

Dr. Parthiban P
Guide
Scientist
F P I C Department
CSIR-Central Food Technological Research Institute
MYSORE - 570 020

Sandeep M

Dr. Sandeep N Mudliar
Chief Scientist and Head
FPIC Dept., CSIR-CFTRI, Mysuru
ಮುಖ್ಯಸ್ಥರು / प्रमुख / Head
ಆಹಾರ ಸಂರಕ್ಷಕಗಳು ಹಾಗೂ ಕೀಟನಿಯಂತ್ರಣ
खाद्य संरक्षक एवं कीट नियंत्रण
Food Protectants & Infestation Control

Revathy 17/4/25

Dr. Revathy Baskaran
Chief Scientist and Head
HRD Dept., CSIR-CFTRI, Mysuru
डा. रेवती भास्करन / Dr. Revathy Baskaran
प्रमुख / HEAD
मानव संसाधन विकास विभाग
Human Resource Development
सीएसआईआर-सीएफटीआरआई / CSIR-CFTRI
मैसूरु / Mysuru-570 020

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SAVYA SURESH

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ERNAKULAM-682 011

Dr. ALPHONSA VIJAYA JOSEPH
PRINCIPAL
ST. TERESA'S COLLEGE
Autonomous
ERNAKULAM

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This is to certify that the dissertation work entitled **"Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava"** is a bonafide work done by **MS. SAVYA SURESH (Reg.no.VM23FPT017)**, student of St. Teresa's College (Autonomous), Ernakulam, in partial fulfillment of the degree of MASTER OF VOCATION IN FOOD PROCESSING TECHNOLOGY. This dissertation work is carried out under the guidance and supervision of **Dr. Parthiban P**, Scientist, Department of Food Protectants and Infestation Control (FPIC), CSIR-CFTRI, Mysuru, Karnataka during the period of December 2024 to April 2025.

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DECLARATION

I, **SAVYA SURESH,** declare the dissertation work entitled **"Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava"** submitted by me in the partial fulfilment of the degree of M.Voc. (Food Processing Technology) in St. Teresa's College, Ernakulam, Kerala - work carried out by me during the period December 2024 to April 2025 under the supervision and guidance of **Dr. Parthiban P,** Scientist, Department of Food Protectants and Infestation Control, CSIR- Central Food Technological Research Institute, Mysuru. The results represented in this thesis have not been submitted to any other university or institute for the award of any degree or diploma.

SAVYA SURESH

Date: 17.04.2025

Place: CSIR-CFTRI, Mysuru

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SAVYA SURESH

Abstract

Abstract

ABSTRACT

This study investigates the efficacy of silica particles as a treatment to control *Tribolium castaneum* (red flour beetle) infestations in *Avena sativa* (oats) and Rava (semolina), both commonly stored food products. The effect of silica treatment on some key physical grain properties, such as moisture, bulk density, particle density, and porosity, was analyzed. Results indicated that these parameters were quite unaffected by silica treatment and, therefore, were good for maintaining grain quality.

The experiment was done by analyzing survival, dead, and mortality rates of *T. castaneum* at different concentrations of silica ranging from 10,000 to 50,000 ppm. Results showed a very strong inverse correlation between silica concentration and beetle survival, with beetle mortality rates seen to rise dramatically with increasing concentrations. Between concentrations of 10000-50000 ppm, 100% mortality was achieved within 3-6 days of exposure. Further, also less damage was visible and weight loss was greatly less in silica-treated oats and Rava, thereby proving the protectiveness of treatment.

Scanning Electron Microscopy (SEM) of the silica-treated insects showed severe cuticular damage, indicating physical abrasion and desiccation as the primary means of action resulting in *T. castaneum* death. Residue analysis via Energy Dispersive X-ray (EDX) spectroscopy confirmed that residues of silica were negligible and removable by a single wash, rendering the method safe and convenient. Thereby, this study endorses silica particles as an environmentally sustainable and non-toxic alternative to chemical insecticides for the preservation of stored oats and Rava against *T. castaneum* infestations.

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Introduction

CHAPTER I

INTRODUCTION

Oats (*Avena sativa* L.) are unique among cereal crops due to their rich nutrient profile, benefiting human consumption, animal feed, healthcare, and cosmetics (Butt et al., 2008; Varma et al., 2016). Cultivated for over 2,000 years (Sang et al., 2017), oats are one of the oldest known crops (Lásztity, 1998) and were domesticated later than cereals like wheat and barley (Marshall and Sorrells, 1992). Oats contain carbohydrates, soluble dietary fiber, balanced proteins, lipids, vitamins, and minerals (Joyce et al., 2019). A key component is oat beta-glucan (OBG), a soluble fiber that helps reduce cholesterol and prevent diabetes. Its concentration varies with storage, processing, and cultivation conditions. Typically, oats have 13% to 20% protein, with about 30% coming from the embryo. They also contain trace antioxidants such as phenolic compounds and sterols, which may offer health benefits (Paudel et al., 2021).

Semolina is a coarse flour made from durum wheat, commonly known as Rava or sooji in India. It is a key ingredient in various savory dishes like upma and Rava dosa, as well as sweet treats such as halwa and Rava Kesari. Semolina is milled from the endosperm of durum wheat, resulting in a high-protein, yellow flour that contains gluten, ideal for making pasta and couscous (Rashid et al., 2022). In addition to its culinary uses, semolina is also being explored for health foods. For example, combining semolina with Asparagus racemosus root powder has shown potential for enhancing fiber and calcium absorption (Meena et al., 2024). Recent studies have also highlighted its ability to produce resistant starch, beneficial for digestion (Sharma et al., 2023).

Post-harvest losses in India considerably impact the agricultural sector, threatening economic stability and food security. These losses occur due to poor infrastructure, inefficient handling, and pest infestations. Basavaraja et al. (2007) noted significant losses in Karnataka, while Grover and Singh (2013) emphasized the ongoing issue in Punjab. Addressing these challenges requires modernization, efficient supply chains, and robust policies to minimize losses and ensure food availability. Pest infestation in agricultural storage poses

significant risks, causing both quantitative and qualitative losses to grains, pulses, and processed commodities. These pests thrive in optimal conditions, multiplying rapidly and damaging food by deteriorating nutritional value, generating off odors, and contaminating with excreta and body parts. Additionally, contaminated products can harbor harmful microbes, posing health risks to consumers (Mohan et al., 2023).

Stored grain insect pests invade food supplies in public warehouses and farms, with their populations growing due to inadequate storage facilities and climate control. Therefore, exploring more effective technologies to reduce these populations is crucial (Rajendran et al., 2020). Insects significantly challenge global grain storage, leading to economic losses (Fields, 2006). While chemical insecticides have been the traditional method of control (Salem et al., 2007), issues such as pesticide residue concerns, resistance, and environmental impact highlight the need for new approaches (Udo, 2005; Fields, 2006; Mahdi and Rahman, 2008). Processed staple meals (PSM) purchased in bulk are frequently stored, making them vulnerable to pests like the red flour beetle, T*ribolium castaneum* (Herbst). This pest, a common threat to stored grain, is well-studied in entomology due to its extensively sequenced genome (Rösner et al., 2020). *T. castaneum* primarily feeds on stored flour and milled cereal products, and its susceptibility increases when grains are threshed before consumption (Campbell and Runnion, 2003; Tanzubil, 1991).

The red flour beetle, is a significant pest of stored products and has been widely used in ecological studies due to its ability to thrive on a wide variety of substrates (Campbell and Runnion, 2003). According to Hagstrum (1973), the red flour beetle is highly active in food areas, such as grain and flour, and easily disperses as an adult (Ziegler, 1976). Howe (1962) noted that female *Tribolium* adults have a long lifespan and lay eggs almost continuously. It has been reported that *T. castaneum* is extremely prolific, capable of producing millions of offspring throughout its life (Haines, 1991). The quality of the diet significantly influences the growth and development of *T. castaneum* (Apert, 1987). The complete life cycle of *T. castaneum* includes four stages: egg, larva, pupa, and adult. Under optimal conditions, this lifecycle can last from two to three weeks, characterized by intense feeding and rapid growth. The larvae are

white, segmented, and slender. The pupal stage typically lasts between five and nine days and involves several internal transformations before the adult emerges. The duration of each developmental phase is affected by environmental factors such as temperature, relative humidity, and food availability (Sokoloff, 1974; Park et al., 1966; Campbell and Runnion, 2003). As the global population increases, the demand for grains and other agricultural products is rising. Addressing losses from stored product insect pests is crucial to meet this demand. Inadequate warehousing and damage caused by stored grain pests during storage, shipping, and transportation pose significant challenges, particularly in South-East Asia and other developing regions (Upadhyay and Ahmad, 2011; Talukder et al., 2004; Dubey et al., 2008;).

Tribolium castaneum is responsible for the highest economic losses and deterioration of food quality among stored products. According to Ali et al. (2016), after 30 to 90 days of infestation, *T. castaneum* causes the most damage to wheat, followed by rice and maize. Wheat losses can be 1.5 to 6 times greater than those of other similar cereals. Traditionally, chemical insecticides have been used for pest management; however, *T. castaneum* has rapidly developed resistance to many classes of pesticides, complicating pest control efforts (Venkatesan et al., 2022). Various pest management strategies are being considered solutions in response to these challenges. One approach is using inert dusts, such as amorphous silica powders. These powders adhere to the insect's body and penetrate it, damaging the cuticular wax layer and ultimately leading to death from dehydration. Athanassiou et al. (2005) suggest that finer particle sizes of silica powders are more effective in killing *T. castaneum* and inhibiting the development of its progeny (Ajayi and Rahman, 2006).

Diatomaceous earth (DE), a form of amorphous silica, is emerging as a cost-effective alternative to synthetic insecticides for managing stored-product pests like *T. castaneum*. Unlike chemical insecticides, silica works through physical mechanisms, making it less likely to cause resistance. Research by Manivannan and Subramanyam (2023) showed that two types of amorphous silica powders significantly increased mortality rates in *T. castaneum* upon contact. This suggests that silica could effectively replace chemical insecticides. Combining DE with other control agents has also proven beneficial. Rizwan et

al. (2019) found that DE alone caused high mortality rates in *T. castaneum*, particularly when used with other chemicals, indicating a synergistic effect suitable for integrated pest management. Food-grade silica, especially silica nanoparticles (SiNPs), has gained attention for its desiccant properties, which disrupt the protective waxy layer of insects, leading to dehydration and death (Cáceres et al., 2019). SiNPs can also enhance plant resistance against pests and attract natural predators through increased volatile emissions (Saw et al., 2023).

Silica is the most abundant oxide in nature, followed by oxygen (Matichenkov and Calvert, 2002). Bernstein and Carpi (2015) note that silica sand forms from the weathering of feldspar and quartz-bearing rocks. This versatile mineral finds applications in technology and agriculture, particularly in extracting silica nanoparticles from agricultural wastes like rice husk and wheat straw (Kouadri et al., 2023). For this study, we will use pure synthetic silica forms, such as amorphous powders like colloidal silica and silica gels. Silica gels serve as flavoring additives in the food industry and are safe due to their non-toxic amorphous nature. Additionally, porous sol-gel silica microspheres are valuable for encapsulating chemicals for controlled release, and sol-gel silica glasses are being explored for food packaging (Kasaai, 2015).

Based on the above situation necessitated, the preparation of this project is to "Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava" with the following objectives.

- To evaluate the efficacy of silica powder against *T. castaneum* in food commodities such as Rava and Oats (*Avena sativa* L.).
- To evaluate the analysis of residual on silica powder treated oats and Rava.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Post-harvest losses caused by stored grain pest

Postharvest loss refers to the reduction in quantity and quality of food from harvest to consumption. Quality losses affect nutrient content and edibility and are more common in developed countries, while quantity losses are prevalent in underdeveloped nations (Kader, 2002; Kiaya and Victor, 2014). Key causes of postharvest losses include biological factors like microbial attacks and pest infestations. Significant pests affecting stored grains include weevils, lesser grain borers, flour beetles, and Indian meal moths (Muez Berhe et al., 2022). Infestations can lead to economic losses and deteriorate food quality, with major increases in pest populations due to poor environmental controls and warehousing practices (Upadhyay et al., 2011). Globally, postharvest losses from stored insects range from 9% in developed countries to over 20% in developing ones. There is an increasing interest in alternatives to conventional insecticides due to regulatory losses, insect resistance, and consumer demand for pesticide-free products (Philips and Throne, 2010).

Tropical climates are particularly conducive to insect proliferation, complicating food storage. Some pests infest crops shortly after harvest, while others may remain dormant in grain residues until the next harvest. These issues can increase the risks of famine and malnutrition, especially in subsistence-focused food production. To enhance food security, it's essential to reduce dependence on pesticides and utilize a mix of cultural, biological, and physical control methods (Nairobi and Kenya, 2011).

Food commodities and storage losses

Stored food grains face significant risks from insect infestations, which can result in losses of 5% to 30% of global agricultural yields. The red flour beetle (*Tribolium castaneum*) is a major pest, affecting grain, flour, peas, nuts, dried fruits, and spices, with a preference for milled grain products. In warm climates, this insect can reproduce year-round, leading to considerable storage losses. Cereals and

legumes are vital components of diets worldwide, providing essential calories and proteins, as well as income and employment for rural communities (Duranti, 2006; Rajashekar et al., 2016). In tropical and subtropical regions, grain storage is common, primarily for food reserves and seed supply. Major crops include kidney beans, mung beans, rice, and sorghum, with food grain production occupying 80% of fertile land in India (Jacobs Mobolade et al., 2019).

Wheat (*Triticum aestivum*), supporting about 35% of the global population, is the most traded crop, with over 766 million tons produced annually. It consists mainly of complex carbohydrates (74-77%) and proteins (11-15%), contributing to efforts against hunger and malnutrition. However, various insect pests significantly impact wheat grain quality and market value, leading to losses of up to 70% in storage due to infestations. These pests can contact the grains during harvesting, transportation, and storage, creating substantial economic losses (Negbenebor et al., 2024).

Temperature and humidity significantly influence the feeding behavior and weight loss of *T. castaneum* on various commodities. Majeed et al. (2016) reported that increasing the temperature from 28 to 32 degrees' Celsius doubles the weight loss in sorghum, while a further increase to 35 degrees Celsius decreases loss by 1.76 times. Daniels (1956) found that a 2% increase in moisture content in whole wheat leads to a 1.6 times increase in insect damage during the first 60 days, with lower increases over the next periods.

Ali et al. (2016) showed that *T. castaneum* caused the most weight loss in wheat compared to maize and rice. After 30 days, wheat weight loss was 1.5 times that of rice, and 6.0 times that of maize. Among the three, semolina was identified as the most favorable food source for *T. castaneum*, yielding an average of 28.7 eggs per female, while cracked wheat and broken maize resulted in much lower fecundity.

Semolina is of particular interest because infestations can lead to unpleasant streaks in noodles. These insects also produce quinones that can affect the odor and flavor of food, posing potential health risks (Stejskal and Hubert, 2006). Large infestations contribute to negative changes in food quality, such as increased fat acidity and reduced thiamine levels (Venkatrao et al., 1960; Smith et al., 1971).

Demographic studies suggest that while semolina promotes population growth, cracked wheat and cracked maize only support survival (Skourti et al., 2020). 2020)

Biotic and abiotic factors affecting safe storage

Food grain losses vary depending on ecological conditions and are influenced by multiple factors. Key physical elements include temperature and moisture, while biological factors encompass insects, rodents, mites, birds, and grain metabolism. Chemical factors involve food breakdown and pesticides, along with engineering aspects related to storage structures. The main causes of food grain loss are moisture, temperature, respiration, and the presence of pests. Abiotic factors affecting storage include temperature and moisture content, while abiotic stresses like drought, waterlogging, extreme temperatures, salinity, and mineral toxicity can hinder seed quality and yield. "Biotic stresses" refer to organisms that harm crops through various processes, leading to the degradation of stored grains due to toxins and undesirable metabolites. The environment affects the type of biotic stress and the crop's ability to withstand it. (Gull et al., 2019).

Stored grain insect pests may be categorized as major or minor pests, depending on the degree of damage. Depending on how they feed, they can be separated into external and interior feeders: -

Primary feeders: Undamaged storage grains are susceptible to harm and reproduction from the main insect problem. Internal feeders: Their larvae entirely feed inside the kernel or stored material. External feeders: The larvae and adults feed on the kernel from outside.

Secondary feeders: Some of these would be insects that would develop following infestation by pests that feed on cut or broken seeds.

Biology and life cycle of red flour beetle

Tribolium beetles are among the most common insect pests affecting stored grains and commodities (Ahmed, 2002). These beetles cause an estimated 5 to 10 percent damage to stored products globally, with damage rates reaching about 50 percent in tropical regions and averaging between 10 and 40 percent worldwide. Factors such as environmental conditions in storage facilities, pest management

methods, and bulk sanitization practices all contribute to an increased likelihood of insect infestation (Bachrouch et al., 2010).

The species *T. castaneum* attacks a wide range of food products, including chocolate, spices, wheat, cereals, meal, crackers, beans, pasta, cake, dry pet food, dried flowers, nuts, seeds, and preserved food items. They also feed on other insect specimens (Weston and Rattlingourd, 2000). The primary food source for these beetles consists of grains that have been rendered unfit for human consumption due to decomposition caused by other insects (Li and Arbogast, 1991).

Deshwal et al. (2020) noted that the red flour beetle, *T. castaneum* (Coleoptera: Tenebrionidae), is the most significant pest found in flour mills, with a worldwide distribution. Its main sources of nutrition include cereals, flour, starches, fruit nuts, millet, and prepared cereal dishes. The beetles primarily consume broken grains, which create dust. Heavy insect excretions result in a putrid, sour odor in infected flour. Newly hatched larvae measure 1 mm in length and are yellowish-white in color. As they mature, they develop a reddish-yellow coloration, and pupation occurs within the flour. The pupae are yellowish and hairy, with the pupal stage lasting between five to nine days. The entire life cycle from egg to adult takes about 26 to 30 days during the summer. Both larvae and adults contribute to the damage caused, with the most significant harm occurring during the hot and humid monsoon season. Larvae are typically found concealed within food items, while the adults, though active creatures, usually hide in flour. A heavy infestation can turn flour gray and moldy, emitting a strong, unpleasant odor, rendering it unfit for human consumption.

According to Smith and Whitman (1992), the red flour beetle is of Indo-Australian origin and can survive winters in sheltered environments, particularly those with central heating (Tripathi et al., 2001). *T. castaneum* undergoes a typical holometabolous life cycle, metamorphosing through several larval stages. It can tolerate temperatures between 22 and 32 °C for embryonic development, requiring three days at 32 °C and seven days at 25 °C for development. The duration for an egg to develop into an adult decrease as temperature increases—from 74 days at 22.5 °C to approximately 23 days at 32 °C—making it suitable for extensive genetic research (Chafino et al., 2019; Sokoloff, 1974).

Taxonomy of *T. castaneum*

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Coleoptera
- Family: Tenebrionidae
- Genus: *Tribolium*
- Species: *castaneum*

The red flour beetle is reddish-brown with three-segmented, club-like antennae (Bousquet, 1990). Its head is visible from above, and it lacks a beak extension, while the edges of its thorax are slightly curved (Anonymous, 1986). This beetle undergoes complete metamorphosis, including egg, larva, pupa, and adult stages. Females live one week to two months after laying eggs (Park, 1949) and can lay two dozen eggs daily, feeding on three to four hundred eggs over five to eight months (Bennet, 2003). Eggs incubate for about 4.5 days, larvae go through seven instars, and the pupal stage lasts around 7.5 days, leading to a total life span of approximately 76.5 days (Devi and Devi, 2015).

Post-harvest losses made by *T. castaneum*

The post-harvest period follows the harvest and is crucial for grain quality and quantity. Hodges et al. (2011) note that post-harvest losses lead to economic and food waste. Factors such as inadequate storage facilities, delayed harvesting, and easily punctured storage bags contribute to these losses (Marid Tadesse, 2020). High temperatures and poor hygiene also promote insect infestations.

T. castaneum, or the red flour beetle, significantly impacts grains like wheat, semolina, oats, and millets. It causes discoloration and odor in wheat (Pasquale Trematerra et al., 2012) and can lead to 76.67% damage and 5.15% weight loss in rice (Bilal Atta et al., 2022). Mukesh Kumar Chaubey (2023) reports a 40% loss of grains in India during these stages, mainly due to *T. castaneum*, which leads to fungal growth, reduced nutritional content, and economic loss.

Mechanisms of silica toxicity

Silica, or silicon dioxide (SiO2), is a naturally occurring mineral found in sand, quartz, and various other materials. It exists in both crystalline and non-crystalline forms and is one of the most abundant minerals on Earth (Annisa Luthfiah et al., 2024). Silica is commonly derived from industrial byproducts, fertilizers, and natural resources and is often used as a fertilizer to protect crops from insect pests. Diatomaceous earth (DE), a form of amorphous silica, is particularly effective for this purpose, as it consists of silica-rich diatom deposits (Mills-ibibofori et al., 2019).

Silica functions against stored grain pests through a physio-sorption method, causing insect death by abrasion (Kar et al., 2021). It also deposits on plant leaves, making it harder for insects to feed, thereby reducing crop loss (Fadi Alhousari et al., 2018). Research indicates that DE is effective against *T. castaneum* (Manivannan and Subramanyam, 2023).

DE is known for its abrasive properties, damaging the insect cuticle and leading to water loss (Korunic, 2013; Ebeling, 2013). Environmental factors, such as temperature and humidity, significantly influence DE's effectiveness. Studies show that DE's efficacy decreases in high humidity but increases at higher temperatures (Arthur, 2011; Kavallieratos et al., 2009; Frederick and Subramanyam, 2016).

Wakil et al. (2011) found that DE reduces the mortality rate of the rice weevil, *Sitophilus oryzae*, in stored wheat. Athanassiou et al. (2012) further demonstrated DE's efficacy against both *S. oryzae* and *Tribolium confusum*, emphasizing that higher temperatures increase insect activity and contact with DE, thus enhancing mortality rates (Helena Rojht et al., 2024).

In 2015, Goudoungou Wini studied the effectiveness of diatomaceous earth (DE) on *S. zeamais* in stored maize, finding that both DE formulations significantly lowered mortality rates. Wini also examined how temperature and humidity affected DE’s effectiveness. Chuks Fidelis Nwanade added to this research by investigating the impact of different environmental conditions on *S. zeamais*. Other studies, like those by Kavallieratos et al. (2009) and, more recently, Susurluk, Hilal, and Butuner in 2024, also explored DE under various conditions.

The shape of silica particles like DE is important for their effectiveness. Smaller particles have larger surface areas, helping them stick better to insects. Fields and Korunic (2010) noted that sharper, irregular particles can damage an insect's cuticle, speeding up water loss. The best particle size and shape vary depending on the specific insect species and application conditions.

Kavallieratos et al. (2012) found that temperature and humidity strongly influence DE's effectiveness against *T. castaneum*. Higher temperatures can increase insect activity and contact with DE, boosting mortality. However, high humidity reduces DE’s ability to absorb water. Abdullah and Fields (2020) confirmed that the effect of DE on *T. castaneum* in wheat depends on these environmental factors. Managing these conditions can improve DE's effectiveness.

DE is a safe and effective insecticide used in agriculture and public health. It serves as a residual insecticide and grain protectant as part of an integrated pest management (IPM) strategy in food processing (Korunic et al., 1996a, 1996b, 1998; Subramanyam and Roesli, 2000).

Diatomaceous earth (DE) attaches to insects and weakens their protective waxy coating through sorption and abrasion, leading to water loss and death (Ebeling, 1971). It is also known to repel insects (White et al., 1966). DE is safe to use, does not degrade grain quality, offers long-term protection, and is cost-effective, making it a valuable grain protectant (Korunic et al., 1996a, 1996b). Additionally, DE can significantly reduce respirable dust in workspaces, as fine grain dust particles adhere to DE (Desmarchelier and Allen, 2000). Traditional washing and modern milling methods eliminate over 99% of DE (Korunic et al., 2013), and regulations focus on worker and customer safety, as well as pest-repelling effectiveness.

DE’s ability to be removed through standard processing techniques ensures it poses similar safety concerns as other food additives. Integrated pest management (IPM) aims to reduce reliance on synthetic pesticides, reserving them for actual infestations. Incorporating DE and enhanced diatomaceous earth (EDE) into IPM programs can protect grain from pests and maintain quality. Case studies show DE is a feasible alternative to synthetic pesticides. With no negative local environmental impact, DE residues can be easily removed during processing.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

Various materials were used, and methods were adopted in the laboratory experiments to study the "Evaluation of efficacy of silica powder against *Tribolium castaneum* (Herbst) in Oats (*Avena sativa* L.) and Rava" at CSIR - Central Food Technological Research Institute (CFTRI), Mysore – 570 020, Karnataka, India during December 2024 to April 2025. The materials and methodologies adopted for various studies are described below.

Mass culturing of insect

Mass culturing of red flour beetle, *T. castaneum* in the Laboratory

Materials Required

1. Test insects
2. Glass jars
3. Muslin cloth
4. Rubber band
5. Rava
6. Sieves
7. Camel hair brush
8. Paper sheets
9. Hot air oven
10. Storage racks
11. Face masks
12. Weighing balance
13. Glass bottles
15. Match boxes
16. Candles

17. Tongs

18. Distilled water

19. Measuring cylinder

20. Petri plates

21. Silica powder

Mass culturing procedure

The insect *T. castaneum* was collected from the older stock culture maintained at the Department of Food Protectants & Infestation Control (FPIC)Laboratory (culture room), CSIR-Central Food Technological Research Institute (CFTRI), Mysore, and subsequently reared on Rava.

About 300 to 500 adult beetles were fixed in to 500 kg – 1 kg of un-infested and conditioned Rava in transparent glass bottles. *T. castaneum* were established using a large glass container. A hole was created at the top of the container to facilitate ventilation and promote population growth. The container was covered with muslin cloth, secured with a rubber band. This set up allowed for adequate aeration, which is essential for the multiplication of *T. castaneum*.

The methods were carried out following the protocol suggested by Viji et al. (2013). *T. castaneum* culture was maintained in glass jars (10 cm dia., 20 cm height) with Rava. The jars were covered with Markin cloth and the culture was incubated at 28 ± 1°C and 60 ± 10% relative humidity. Adults and larvae of *T. castaneum* are cannibalistic if enough food is not available (Park et al., 1974). So, the ideal number of adult beetles (sex ratio 1:1) necessary to produce the greatest number of larvae and pupae was determined. For this aim, 30, 50, 75, 100, 125, and 150 adult pairs were released into glass jars with 150 g of Rava.

Plate 1. Insect culture room

Laboratory Experiments

Screening of efficient dose of silica against *T. castaneum*

The laboratory experiment was conducted at the Department of Food Protectants & Infestation Control (FPIC) laboratory (culture room) at CSIR-Central Food Technological Research Institute (CFTRI), Mysore. To screen for an efficient dose of silica against *T. castaneum.* Rava and oats were treated with different doses of silica. The design adopted was a completely randomized block design (CRD) with the following treatments.

Silica powder - Properties

The food industry – grade silica powders are utilized for research. The characteristics and specifications of silica powder are listed below.

Plate 2. Silica powder

Properties

- Low toxicity
- Strong Resistance to insect infestation
- Biocompatibility
- Chemically inert, thermally stable and water resistant
- Resistant to dehydration from moisture

Rava mixed with silica powder and stored in a glass jar

This study examined the effectiveness of a silica concentrations in reducing T. castaneum infestations in Rava. The methodology followed in this study was adapted from previous research on the efficacy of silica-based powders against *T. castaneum* (Selladurai et al., 2023; Vayias and Athanassiou, 2004). *T. castaneum* cultures and experimental procedures were carried out according to the method described by Li et al. (2020), with minor adjustments made in relation to different concentrations of silica. Nine large jars containing 200 g ± 0.01 g of Rava were prepared according to procedures outlined by Athanassiou et al. (2005). Silica powder was blended with the Rava in different concentrations (1000, 1500, 2000, 2500, 3000, 4000, and 5000 ppm) after Arthur (2000) on the research of stored-product pest management. The treated samples were thoroughly mixed to ensure even distribution of silica, following the standard procedure for uniform dispersion in grain-based substrates (Vayias and Athanassiou, 2004). Twenty grams of the silica-treated Rava from each large bottle were transferred into twenty-seven small glass bottles, creating three

replicate treatments (R_1T_1, R_2T_1, R_3T_1) for each concentration level. Following the methodology described by Fields & Korunic (2000), ten adult *T. castaneum* were introduced into each small bottle. The bottles were wrapped with muslin cloth to prevent insect escape while allowing adequate aeration (Athanassiou et al., 2005). Mortality was assessed using the method defined by Arthur (2000) and Selladurai et al. (2023), with counts taken on days 1, 3, 5, 7, and 15. If they did not stir or if they showed no movement when gently touched, they were presumed to be dead. Moisture content loss in Rava samples was also measured using a moisture analyzer, as described by Li et al. (2020).

Adult mortality (%)

$$\text{Adult mortality (\%)} = \frac{\text{Number of dead weevil}}{\text{Total number of weevil}} \times 100$$

Weight loss (%)

$$\text{Grain weight loss (\%)} = \frac{\text{Initial weight of the sample - Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

Grain damage (%)

$$\text{Grain damage (\%)} = \frac{\text{Number of grains damaged}}{\text{Total number of grains in the sample}} \times 100$$

Treatment details for Rava

T_1 Silica powder@1000 ppm

T_2 Silica powder@1500 ppm

T_3 Silica powder@2000 ppm

T_4 Silica powder@2500 ppm

T_5 Silica powder@3000 ppm

T_6 Silica powder@3500 ppm

T_7 Silica powder@4000 ppm

T_8 Silica powder@5000 ppm

T_9 Untreated control

Plate 3. Bottles containing Rava treated with silica

Plate 4. Experimental setup for 27 small bottles containing Rava treated with silica

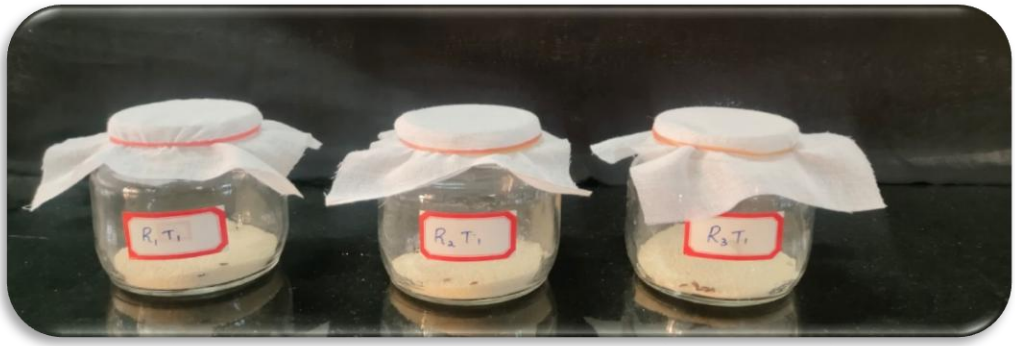


Plate 5. T_1 – 1000 PPM treated - Rava

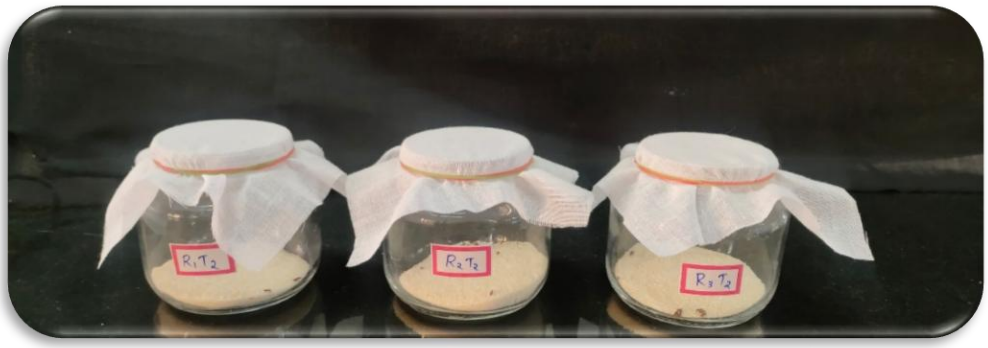


Plate 6. T_2 – 1500 PPM treated - Rava

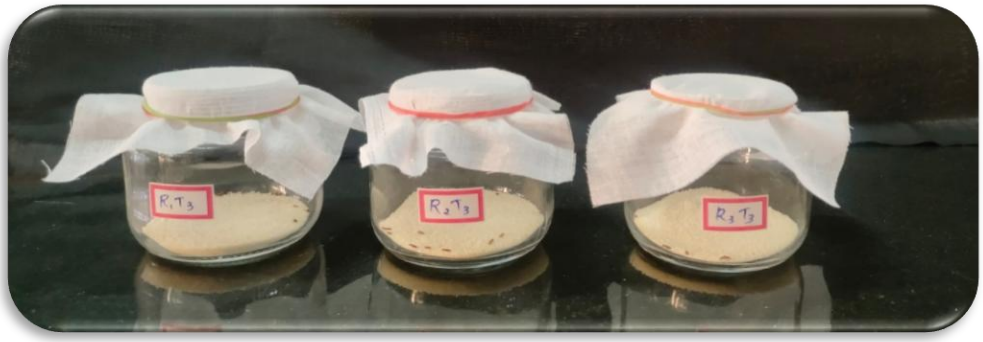


Plate 7. T_3 – 2000 ppm treated - Rava

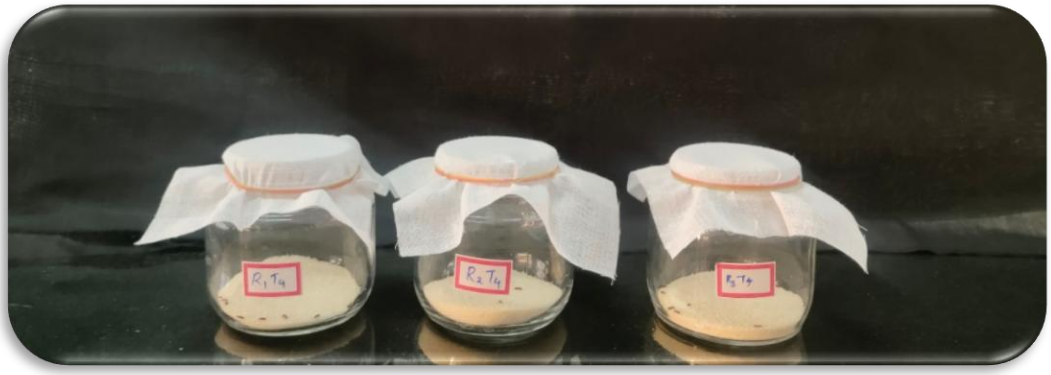


Plate 8. T_4 – 2500 PPM treated - Rava

Plate 9. T_5 – 3000 PPM treated - Rava

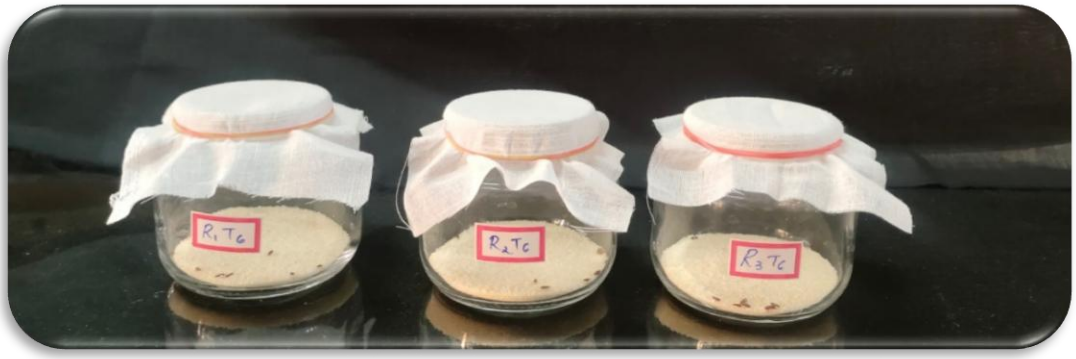


Plate 10. T_6 – 3500 PPM treated - Rava

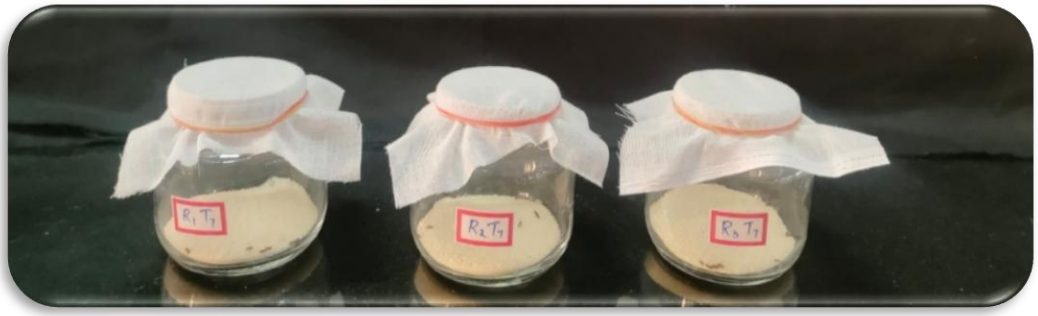


Plate 11. T_7 – 4000 PPM treated - Rava

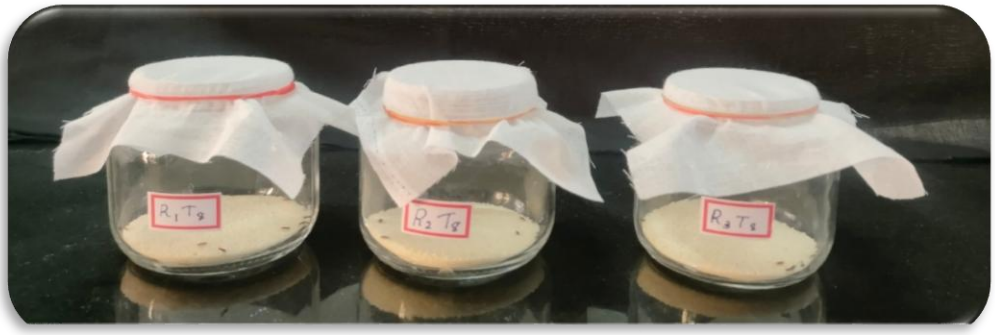


Plate 12. T_8 – 5000 PPM treated – Rava

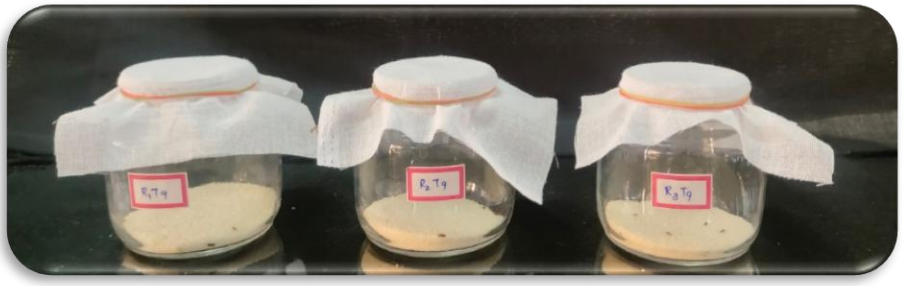


Plate 13. T_9 – Untreated control

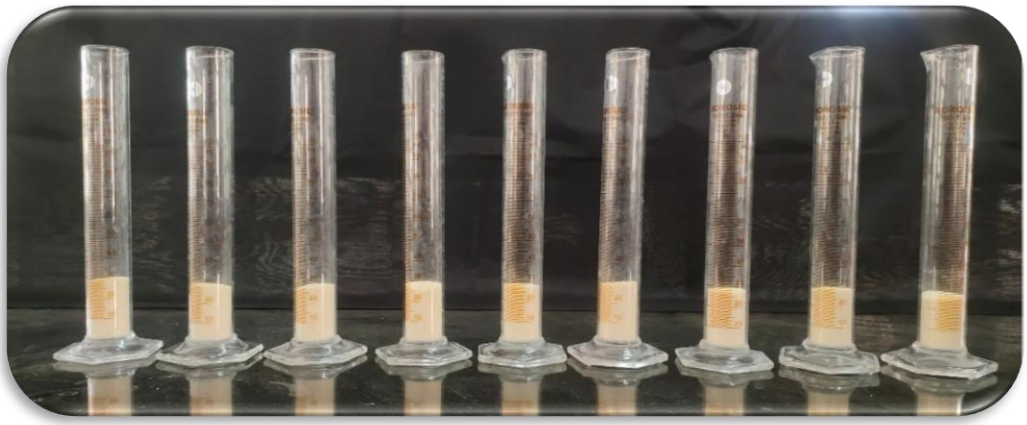


Plate 14. Bulk density of different concentrations of silica – treated Rava

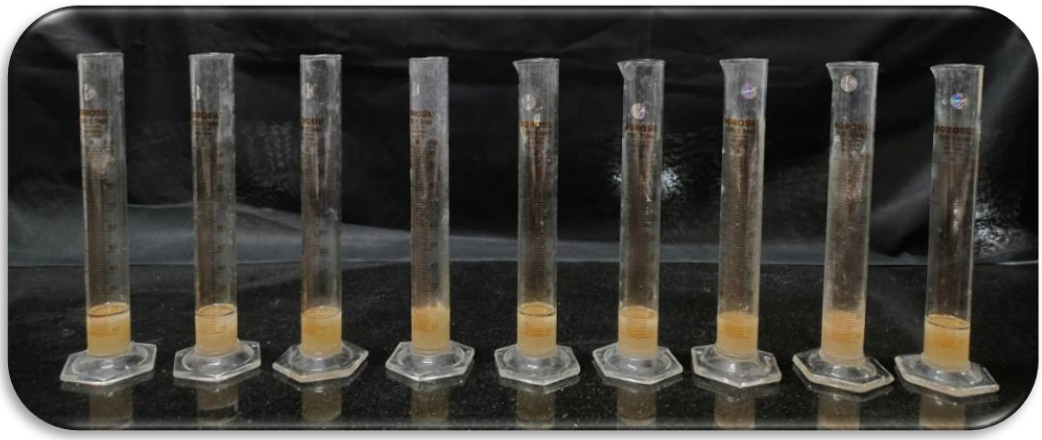


Plate 15. Particle density of different concentrations of silica-treated Rava

Oats mixed with silica powder and stored in glass jars

The methodology followed in this study was adapted from previous research on the efficacy of silica-based powders against *Tribolium castaneum* (Selladurai et al., 2023; Vayias and Athanassiou, 2004). The Culturing of T. castaneum and the experimental setup generally followed the protocols described by Li et al. (2020) slight modifications have been made in respect to different silica concentrations. A weighing scale was used to determine that each one of the nine big glass bottles contained 100 grams of oats. One of the bottles served as untreated control, and all the other eight bottles were treated with concentrated silica powder treatment. Twenty-seven small glass bottles containing 10 grams of oats from each of the large bottles were filled. Ten insects were added to each small bottle. The silica-treated bottles had concentrations of 10000, 15000, 20000, 25000, 30000, 35000, 40000, and 50000 ppm. Each concentration had three replicates (R_1T_1, R_2T_1, R_3T_1, etc.). Observations were done on days 1, 3, 5, 7, and 15 for insect mortality.

Treatment details for oats

T_1 Silica powder@10000 ppm

T_2 Silica powder@15000 ppm

T_3 Silica powder@20000 ppm

T_4 Silica powder@25000 ppm

T_5 Silica powder@30000 ppm

T_6 Silica powder@35000 ppm

T_7 Silica powder@40000 ppm

T_8 Silica powder@50000 ppm

T_9 Untreated control

Plate 16. Bottles containing oats treated with silica

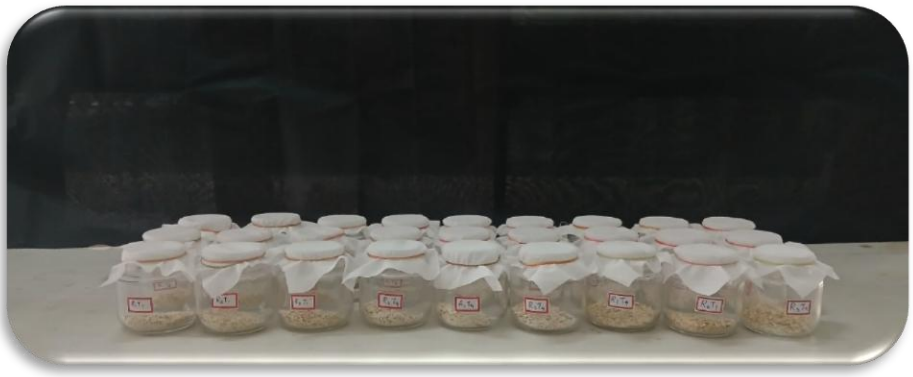


Plate 17. Experimental setup for 27 small bottles containing oats treated with silica

Plate 18. T_1 - 10000 PPM treated- oats

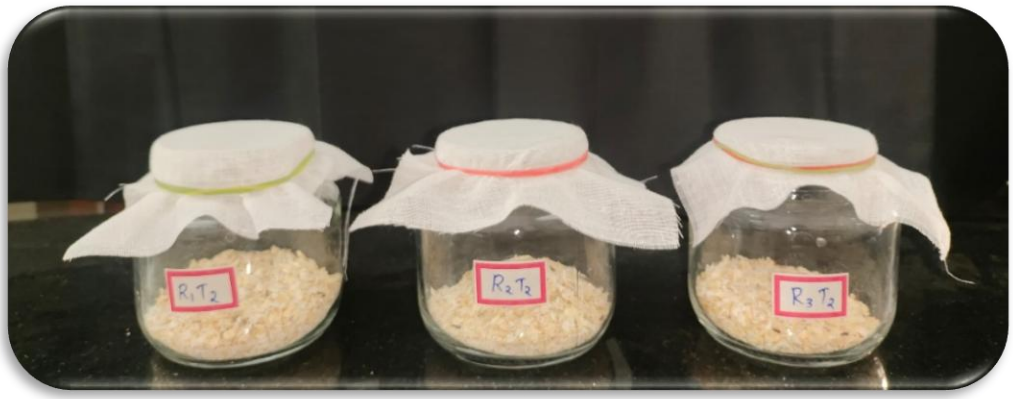


Plate 19. T_2 - 15000 PPM treated - oats

Plate 20. T_3 - 20000 PPM treated - oats

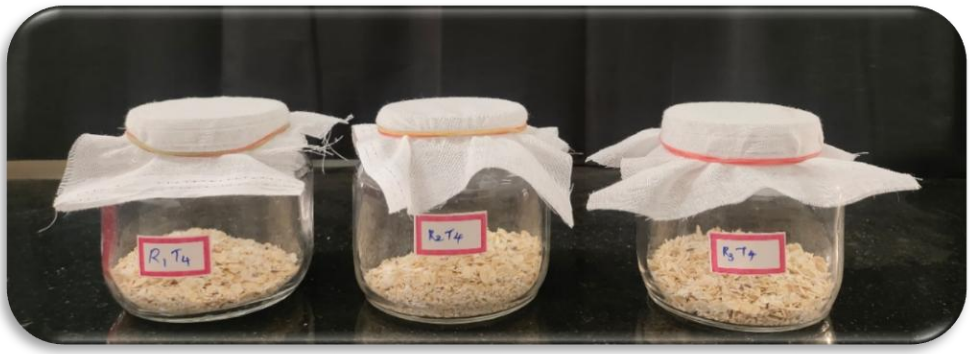


Plate 21. T_4 - 25000 PPM treated – oats

Plate 22. T_5 - 30000 PPM treated - oats

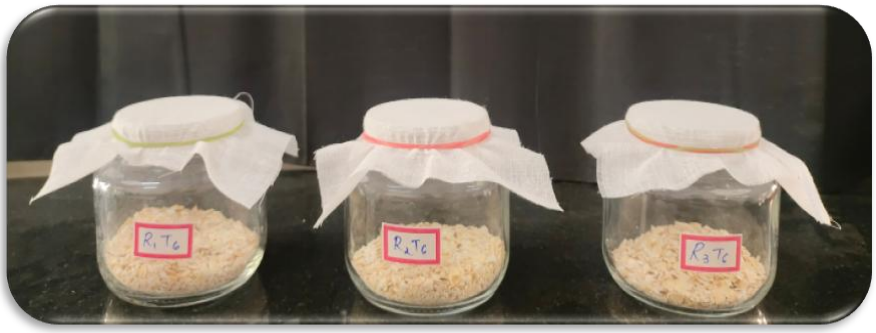


Plate 23. T_6 – 35000 PPM treated – oats

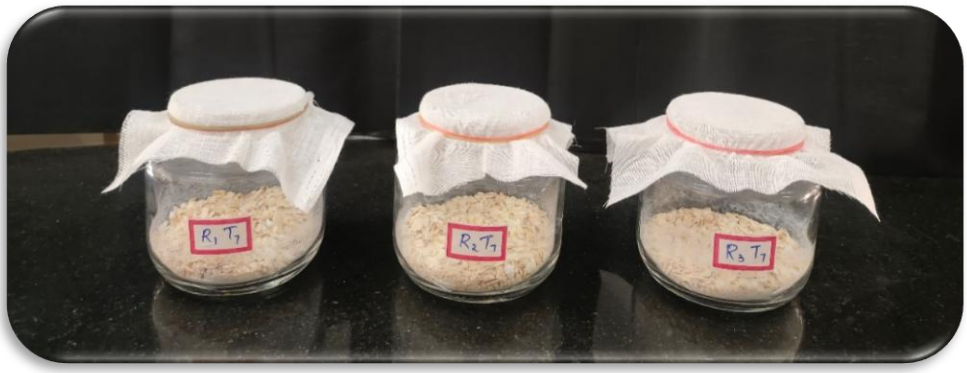


Plate 24. T_7 - 40000 PPM treated - oats

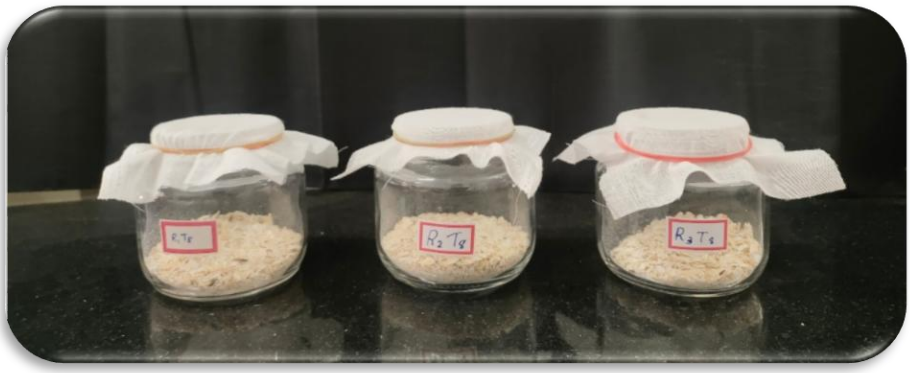


Plate 25. T_8 - 50000 PPM treated - oats

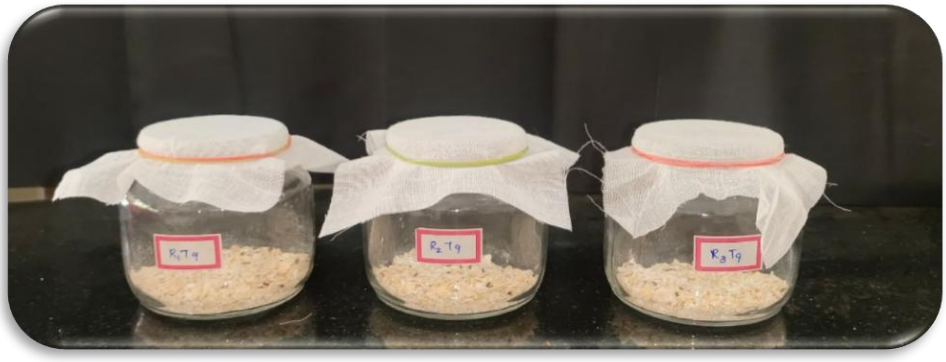


Plate 26. T_9 - Untreated control

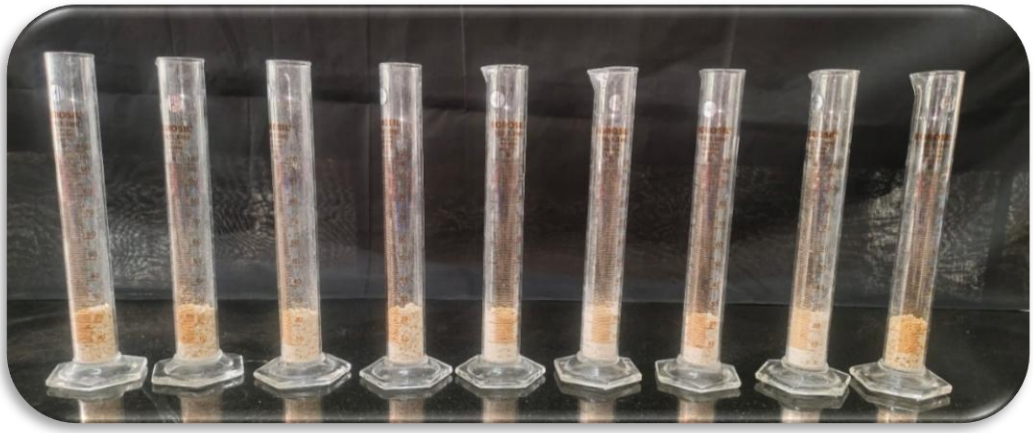


Plate 27. Bulk density of different concentrations of silica treated oats

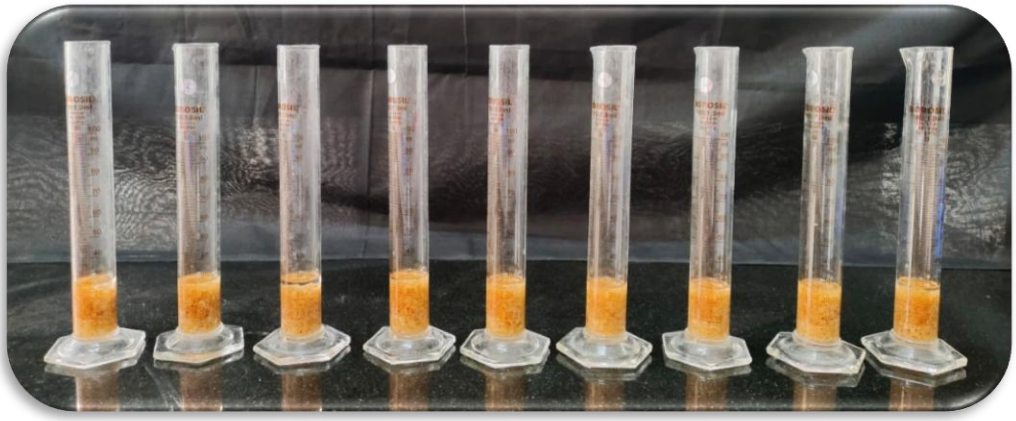


Plate 28. Particle density of different concentrations of silica -treated oats

Plate 29. View of *T. castaneum* treated with silica in oats

Plate 30. Moisture Analyzer

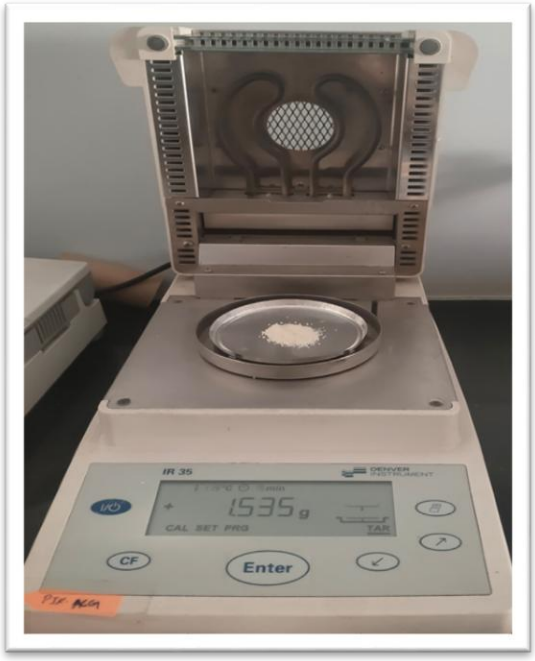


Plate 31. Rava Sample

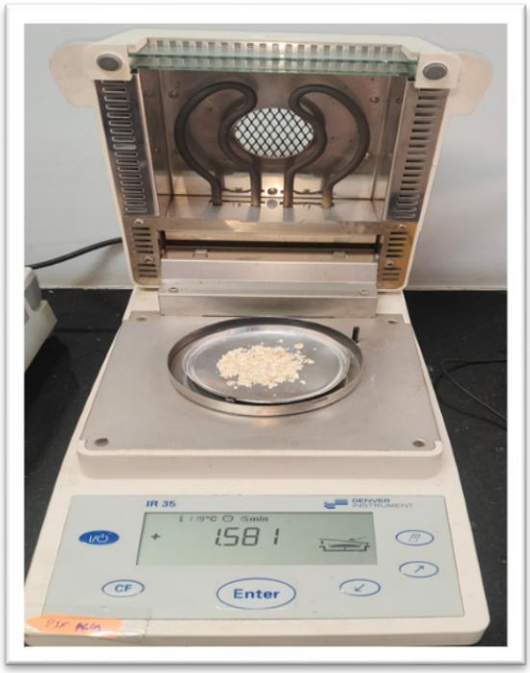


Plate 32. Oat Sample

OTHER LEARNED TECHNIQUES

1. SCANNING ELECTRON MICROSCOPE (SEM)

Plate 33. Scanning Electron Microscope, EVOLS15-CARL ZELSS (Germany)

Scanning electron microscopy (SEM), often known as SEM analysis or SEM technology, has been applied in several fields all around the world. When analyzing both organic and inorganic materials on a nanometer to micrometer (μm) scale, it can be considered a successful approach. With a high magnification of up to 300,000x and even 1000000 (in certain newer versions), SEM can provide incredibly accurate pictures of a variety of materials.

Components and Working System of SEM

Main Components of SEM

The SEM machine comprises of the following components**: -**

A. Source to generate electrons of high energy, it is called electron gun
B. Column down to move electrons using two or more electromagnetic lenses.
C. Deflection system consists of scan coils.
D. Electron detector for backscattering and secondary electron

E. A chamber for the sample

F. The computer system comprises of a viewing screen that displays scanned pictures and a keyboard for controlling the electron beam.

SEM is a technology that allows one to glimpse the invisible worlds of nano and micro-space. SEM is able to expose details and intricacy that light microscopy cannot. All of this may be accomplished by using the below procedure: -

SEM Analysis process

a) A high-energy electron beam with an energy range of 100–30,000 electron volts will be used to do the study. For electron emission, a heat source is often employed.

b) The SEM has lenses to compress the spot and focus the focused electron on the specimen since the spot size created by the gun is too big to provide a crisp image. Most SEMs have a spot size of less than 10 nm, and the signals that create a picture are produced by electrons captured from the final lens interacting with the material and penetrating as deep as 1 μm.

c) The electron beam moves to distinct locations in the form of straight lines as a result of the scan coils' movement, creating a rectangular raster on the specimen's surface. This process creates the picture of the specimen point by point. Every step relies on the necessary magnification. In case when the operator desires a greater magnified image, the scan coils make the beam to deflect a cross a smaller region. It is important to note that the working distance—the distance between the specimen's surface and the final lens—affects magnification. In a contemporary SEM, this is resolved by automated correction.

d) An electron detector is used to find the signals, or electrons, that are released from the scanned material. Without detectors, every signal produced by the interaction of the electron beam with the sample's surface may produce an incomprehensible picture on its own. The creation of SEM images uses both backscattered electrons (BSE) and secondary electrons (SE). The collection of both SE and BSE occurs when a positive voltage is applied to the collector screen. A negative voltage applied to the collector screen, however, will only

result in the collection of BSES. Both secondary and backscattered electrons can be detected using a scintillator detector.

e) The operator will adjust the brightness and intensity until a reasonably clear image is obtained after the signals are shown on the viewing screen. Magnification more than 10,000x should be used if minute features inside the specimen are needed.

f) The electron voltage mode (emitted by the gun) influences the supplied information. When low accelerating voltages of less than 5 kV are employed, the scanned picture will be rich in surface information. In contrast, high acceleration voltages (15-30 kV) penetrate beneath the surface, causing the reflected signal from the surface to contain information about the inside of the sample.

g) The partially three-dimensional picture acquired by SEM is dependent on the visualization of the sample's topography in terms of form, size, and surface texture. And this is determined by the number of BSE and SEs. Surprisingly, the angle of inclination of the sample surface has a direct influence on raising both of the aforementioned quantities and, consequently, topographic contrast. An inclination (also known as tilt angle) of more than 50o to 70o increases the amount of BSE and SE signals to the peak. (Mohammed et al., 2018)

Working principle

Electrons instead of light to illuminate samples. As electrons have much shorter wavelengths than light, much higher magnification is possible. Focused electron beam is scanned across a sample in a grid pattern and detectors record what comes from the sample.

Applications

- Study the morphology, topography.
- Analyze mineral and rock chemical compositions.
- Conduct automated analysis of particles.
- Analyze criminal evidence, coin forgery and toxicology.
- It can also be used to investigate materials properties.

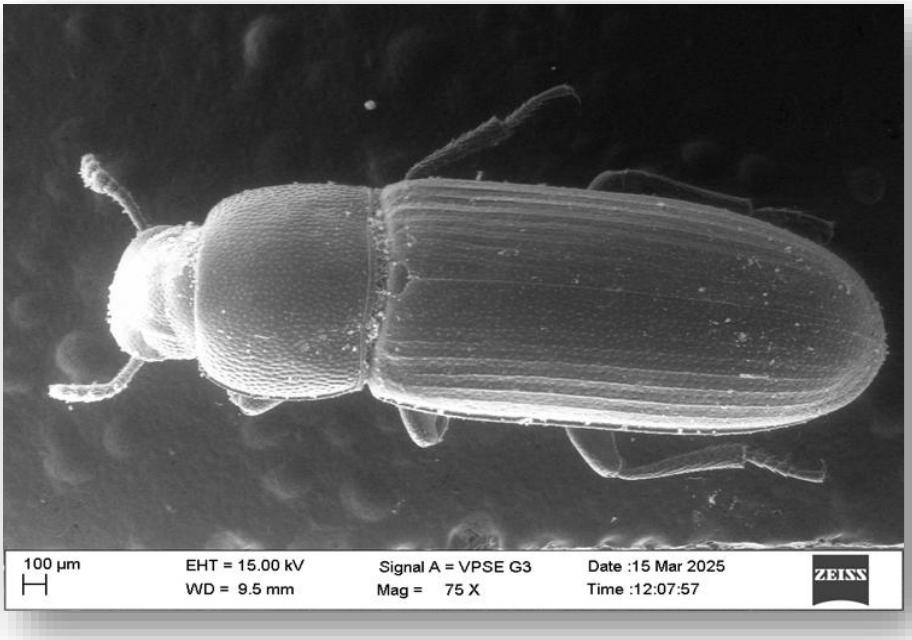


Plate 34. SEM image of silica deposition on red flour beetle

2. ACID INSOLUBLE ASH TEST

Acid insoluble ash (AIA) is the fraction of ash that is insoluble in an acid, which is commonly a weak HCl solution. It is a portion of the total ash produced by incineration (dry ashing) of a test substance. While an ash is considered inorganic substance, AIA is primarily composed of silica (Shrivastava and Talapatra, 1962).

PRINCIPLE

Acid insoluble ash is measured by dissolving the ash in weak hydrochloric acid (10% m/m), filtering the liquid through ashless filter paper, and thoroughly washing with hot water. The filter paper is then burnt in the original dish before cooling and weighing (Practical Manual – Chemical Analysis and Quality Assurance, n.d.).

MATERIALS & EQUIPMENTS REQUIRED

- Food samples – Oats, Rava
- Balance machine
- Muffle furnace
- Hot air oven

- Desiccator
- Hot plate
- Porcelain crucible
- Heat proof gloves
- Spatula
- Permanent marker
- Tongs
- Ashless filter paper
- Measuring cylinder
- Dropper
- Concentrated Hydrochloric acid
- Distilled water
- Funnel
- Standard flasks

PROCEDURE

- **PREPARATION OF ASH**: - Bring a clean porcelain crucible and place the crucible on balance machine & tare its weight. Weigh 2 grams of sample were burned in a Silica crucible above the burner. The charred material was heated in a muffle furnace for 8 hours at 550 Degree Celsius. The ash created was white and carbon-free. After cooling, the sample was weighed using ash-less filter paper. Kadam et al. (2013). The sample is then charred.
- After 30 minutes of burning, take the crucible out of the furnace, cool it, remove the lid, and check the ash.
- To the ash content, add a few drops of distilled water. If black colour appears it indicates that the ash hasn't become carbon free yet.
- Burn again at 550 degrees Celsius until it becomes carbon free.
- Place the crucible on hot plate& dry the moist ash completely. After the drying process, cover the crucible with a lid on top. After that, set the crucible in the furnace and let it burn for eight hours at 550 degrees Celsius.
- After 40 minutes, remove the crucible from the furnace after it has cooled. Take off the lid and carefully observe the ash.

- Then add the distilled water as few drops to the ash for observing the presence of carbon. If no black colour develops, ash is completely carbon-free.
- **40% HCL PREPARATION: -** Dilute 40 ml of HCL (Conc.) with distilled water to obtain a total amount of 100 ML. Then, keep it in a normal flask in a dark, cool area.
- Measure 25 ML of 40 % HCL solution.
- Fill the crucible with ash and pour in the HCL solution.
- Place it on the hot plate. Boil the ash content in HCL solution for 5 minutes for 80 Degree Celsius. (Magnetic stirrer). Now take the crucible off the hot plate with a pair of tongs.
- Using an ashless filter paper, prepare a filter paper. Then, while the ash solution is still warm, filter it and observe that a few particles are stuck on it. These particles will be counted as acid insoluble ash.
- Now heat the water and pour into crucible. After that, clean with the same filter paper for further filtering.
- Add more hot water to remove any remaining HCL residue from the filter paper.
- Filtration is complete.
- Place the crucible & lid inside hot air oven, then dry the crucible at 110 Degree Celsius for 30 minutes.
- After 30 minutes of the drying process, cool it in a desiccator. After cooling, determine the weight of the dry blank crucible. Consider the crucible weight.
- Fold filter paper gently to avoid losing filtrate. Place the folded filter paper in the prepared crucible.
- Place the crucible inside the furnace and burn it at 550°C for 8 hours. Then remove it from the furnace and then cool it.
- Now can see the Acid Insoluble Ash contents. Determine the weight and record it in the calculation.

CALCULATION

(W_S) = Sample weight

(W_C) = Crucible weight

(W_F) = Crucible + Ash weight

$$\text{Acid insoluble ash \%} = \frac{W_F - W_C}{W_S} \times 100$$

FLOW CHART – ACID INSOLUBLE ASH TEST

WEIGH 2 GRAMS OF SAMPLE

↓

BURN SAMPLE OVER A BURNER

(Heat the sample over a burner until it chars completely).

↓

PLACE IN MUFFLE FURNACE AT 550°C FOR 8 HOURS

↓

CONFIRM WHITE & CARBON-FREE ASH

(If Black, repeat -Burn Again)

↓

PREPARE 40% HCL SOLUTION

↓

MEASURE 25ML OF 40% HCL AND ADD TO ASH

↓

BOIL AT 80°C FOR 5 MINUTES ON HOT PLATE

FILTER USING ASHLESS FILTER PAPER

ADD HOT WATER TO REMOVE HCL RESIDUE

(Rinse with hot distilled water to remove excess acid and soluble impurities)

DRY CRUCIBLE IN HOT AIR OVEN AT 110°C FOR 30 MINUTES

COOL IN DESICCATOR AND WEIGH CRUCIBLE

FOLD FILTER PAPER AND PLACE IN CRUCIBLE

BURN IN MUFFLE FURNACE AT 550°C FOR 8 HOURS

COOL CRUCIBLE AND WEIGH ACID INSOLUBLE ASH

RECORD FINAL WEIGHT

CALCULATE ACID INSOLUBLE ASH

Plate 35. Electric Stove

Plate 36. Oat and Rava samples

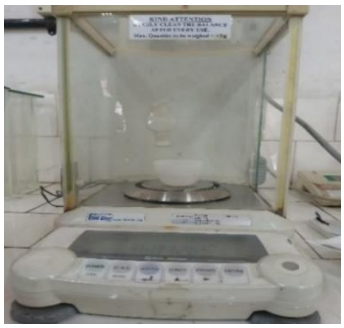


Plate 37. Weighing Balance

Plate 38. Muffle Furnace

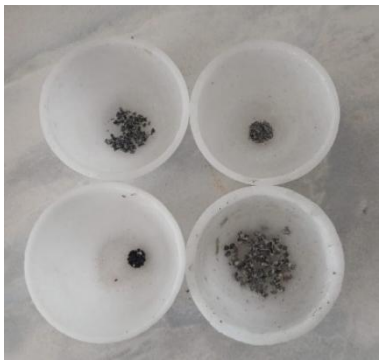


Plate 39. Samples after Ashing

Plate 40. Stirrer

Plate 41. Filtration

Plate 42. Samples in Desiccator

3. EXTRACTION OF ESSENTIAL OIL FROM PLANTS USING CLEVENGER APPARATUS

CLEVENGER APPARATUS METHOD

Plate 43. Experimental setup of Clevenger Apparatus

The Clevenger method is a specialized approach for extracting essential oils from plant sources, using the Clevenger equipment. This approach makes use of hydro distillation, a technique that uses water or steam to extract volatile chemicals more efficiently. J.F. Clevenger invented the Clevenger apparatus in 1928 with the goal of effectively separating and collecting essential oils**. (**Fagbemi et al., 2021).

Essential oils (EOs) are composite mixtures of low-molecular-weight volatile chemicals derived from plant components such as flowers, leaves, seeds, fruits, and stems of aromatic plants.

Key Components of the Clevenger Apparatus: -

- Distillation Flask: Holds the plant material submerged in water.
- Condenser: Cools the rising steam and volatile compounds, causing them to liquefy.
- Oil Separator (Receiver): Collects the condensed mixture, allowing the separation of essential oil from water based on density differences.

According to Clevenger (1928), The apparatus is essentially made up of three parts:

(a) A round-bottomed flask in which the material containing the volatile oil and a given amount of water is placed;

(b) A separator in which the oil is automatically separated from the distillate in a graduated tube, allowing a direct reading of the quantity of the oil; and

(c) A convenient condenser.

Essential oils (EOs) are compounds derived from various plant components, including leaves, stems, roots, and bark. Essential oils are chemically homogeneous compositions made up of a variety of fractions ranging from the most volatile to the heaviest terpenoids, which are natural compounds that are frequently produced as secondary metabolites and serve as plant defense phytochemicals (Shahin et al., 2018).

The most effective technique for figuring out how much essential oil is in plants is the Clevenger device, which is based on distillation. Essential oil from neem leaves is typically extracted by steam distillation or hydro-distillation, and then analyzed using gas chromatography (GC) or gas chromatography linked to mass spectrometry (GC–MS). Extraction takes many hours, even though the second stage only takes 15 to 30 minutes. In boiling water, extraction is often accomplished by stirring and heating for a lengthy time (Ferhat et al., 2006).

Essential oils are steam-distilled combinations of naturally volatile components that have distinct fragrances from the plant parts they were extracted from. Among the techniques used to extract essential oils include steam distillation, hydro-distillation, solvent extractions, and supercritical fluid extractions. Essential oils are considered of as chemical weapons for plants since they shield them from bacterial or fungal attacks. The phases of plant growth can also have an impact on the oil output from plants (Ilag et al., 2023).

MATERIALS REQUIRED

- Fresh neem leaves

- 1000 ML Round bottom flask
- Condenser
- Body tube
- Distilled water
- Connecting pipes

PRINCIPLE: STEAM DISTILLATION

The most common method for obtaining essential oils is steam distillation. To increase the overall yield, isolation was employed for a long time. Direct steam distillation was used to extract essential oils from a variety of plant species belonging to the *Lamiaceae* and *Apiaceae* families at various intervals of 1, 2, 3, 6, 12, and 24 hours. The investigation covered both annual and perennial plants that were tracked at various harvest times (Božović et al., 2017).

EXPERIMENTAL PROCEDURE

PREPARATION OF PLANT EXTRACT

Fresh neem leaves were collected from CSIR- CFTRI, Mysuru.

Plate 44. Freshly plucked neem leaves

NEEM LEAVES (*Azadirachta indica*)

Azadirachta indica (neem) is a well-known medicinal plant that has been used in the Indian traditional medical system (Ayurveda, Unani, Tibetan) for centuries to treat a variety of diseases. Neem leaves, bark extracts, oil, and neem-

based products all have therapeutic benefits. Neem products have been shown to be anti-helminthic, antibacterial, and serve as a contraceptive and sedative (Ganguli, 2002). Although neem is naturally antibacterial (Aarati et al., 2011), its impact in biofilm-associated infections has yet to be investigated.

MEDICINAL VALUES OF NEEM

Neem products have traditionally been used to treat various diseases such as heat-rash, boils, wounds, jaundice, leprosy, skin disorders, stomach ulcers, and chicken pox. Recent research confirms their curative properties and suggests that they may be used more widely in the future (El Astal et al., 2005). Additionally, quercetin, an antibacterial compound, has been discovered in neem leaves.

Neem is also an effective treatment for diabetes because it is a tonic and a revitalizer. Diabetes is more than just a condition that necessitates dietary changes; it is the primary cause of blindness in those between the ages of 25 and 74. It also destroys the kidneys, heart, blood vessels, nerves, and may even cause limb loss (Obiora,2022).

EXTRACTION PROCESS

STEAM DISTILLATION WITH THE CLEVENGER (HDC)

According to Elyemni et al. (2019), sixty-five (65 g) of neem leaves were combined with 500 ML of distilled water and steam distilled using the Clevenger at 45°C under ideal operating circumstances. Five hours were spent on the distillation process, and the essential oil that was produced was gathered.

Plate 45. Extracted essential oil from Clevenger Apparatus

EXTRACTION YIELD AND TIME

The extraction process took approximately five hours. The results indicate that the extraction process, which spanned approximately five hours, was effective in obtaining a considerable quantity of essential oil. Moreover, the data imply that extended extraction periods may lead to increased essential oil yields.

FLOW CHART- CLEVENGER APPARATUS

The fresh leaves of *Azadirachta indica* (Neem) Leaves were collected and weighed 65 grams of neem leaves

Set up the Clevenger Apparatus

Neem leaves are chopped in to smaller fragments and then transferred to a round-bottom flask, to which an equivalent amount of distilled water was added

Add water for steam distillation and temperature is maintained at 45 degrees Celsius for 3 hours (heat to suitable temperatures)

Steam distillation begins

Essential oil and water vapor condenses and then oil- water separation in Clevenger tube

collection of extracted essential

Storage in a proper container

4. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS)

Gas chromatography is used to separate and analyze multi-component mixtures, including essential oils, hydrocarbons and solvents. Temperature programs can improve GC results by distinguishing between identical compounds. Gas chromatography uses very sensitive flame ionization and electron capture detectors to quantify compounds at low concentrations. Plants are an excellent source of secondary metabolites with intriguing biological activity. Secondary metabolites have diverse structures and characteristics, making them a valuable source. Gas chromatography (GC) is a widely used chromatography technology. GC-MS is a highly recommended method for monitoring and tracking organic contaminants in the environment. Esters, fatty acids, alcohols, aldehydes, and terpenes may all be analysed using GC-MS. GC-MS is a useful tool for analyzing and identifying novel chemicals, including those manufactured or derivatized. Gas chromatography is commonly used for a variety of applications. Its principal use is to separate and analyse multi-component mixtures including essential oils, hydrocarbons, and solvents. GC-MS is widely used in the pharmaceutical sector for analytical research, quality control, quality assurance, production, and pilot plants for active pharmaceutical ingredients (API), bulk medicines, and formulations. It is used to design processes and methods, as well as identify API contaminants. It is a crucial component of research in medicinal chemistry (synthesis and characterization of substances), pharmaceutical analysis (stability testing and impurity profiling), pharmacognosy, pharmaceutical process control, and pharmaceutical biotechnology. (Al-Rubaye et al., 2017). Gas chromatography-mass spectrometry (GC-MS) is an effective analytical method for separating and

identifying volatile and semi-volatile chemicals and quantifying them. On the gaseous side, the GC separates compounds in a mixture based on the principles of volatility and behavior with the stationary phase, while the MS identifies compounds based on their mass-to-charge ratio (m/z) (Sparkman et al., 2011). Helium, as an inert gas, carries the evaporated sample through the chromatographic column. The separated compounds are ionized, fragmented, and detected, generating a unique spectrum for each compound in the mass spectrometer (McLafferty & Turecek, 1993).

Plate 46. Gas Chromatography-Mass Spectrometry (GC-MS) equipment

Gas chromatography uses high-sensitivity flame ionization and electron capture detectors to quantify compounds at low concentrations.GCMS consists of two parts: -

1. Gas Chromatography

2. Mass Spectrometer

Gas chromatography separates components from a sample mixture. Inside the mass chamber, separated components are bombarded by a beam of high-energy electrons and get ionized. High energy electrons cause the ionized

molecule to lose an electron and form a radical cation having a net positive charge. This ion further breaks to fragment pieces because of its unstable nature. The fragments travel through the mass spectrometer and mass analyzer and are recorded by the detector according to their mass-to-charge ratio (m/z).

PRINCIPLE

The GC-MS experiment starts with sample preparation, injection, and separation on a GC column. Because the mass spectrometer runs at a high vacuum, an interface is needed to transfer molecules from the GC to the MS. The vapor stream coming out of the GC is highly pressurized, often above five times atmospheric pressure; however, the MS analyzer uses a high vacuum environment that is formed using a fore pump and a turbomolecular pump. In normal settings (capillary column, 1-2 mL/min flow rate, internal diameter of 250-320 µm), the gas flow is managed by the turbomolecular pump, while the GC-MS interface comprises a heated metal tube. For longer columns or greater flow rates, an extra pump eliminates surplus gas before the sample reaches the MS.

Analyte condensation or breakdown in the interface tube, which is normally heated 10 °C above the ultimate oven temperature, must be avoided. The molecules enter an ionization chamber in MS to be ionized and fragmented through very intense bombardment with electrons. The ions produced here, which consist of the molecule and its fragments, are then accelerated under the influence of electric or magnetic fields in mass analyzers and sorted according to their mass-to-charge ratio, i.e., m/z. The analyzer can sort through thousands of ions each second, in addition to a detector that detects electron currents when ions touch them. MS becomes the detector for GC by building a chromatogram, which quantifies all chemicals according to retention time (Lovestead and Urness, 2019).

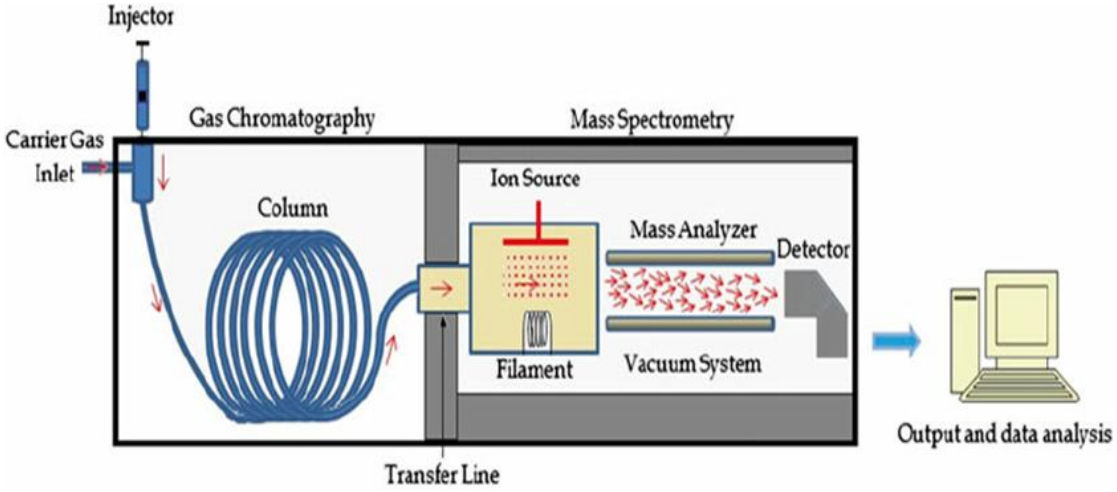


Plate 47. Schematic plot of the main components of GC–MS instrument

EXPERIMENTAL PROCEDURE

MATERIALS REQUIRED

- Clevenger Apparatus – (for essential oil extraction)
- Sample vials
- Micropipettes (20,1000 & 100 micro liters)
- Sealed caps
- Syringe filter
- Hexane (HPLC grade) – Stock solution

PLANT COLLECTION AND ISOLATION OF ESSENTIAL OIL

Coleus Aromaticus Benth. (Also known as Coleus amboinicus Lour and Plectranthus aromaticus Roxb.) is a dicotyledonous plant that belongs to the *Lamiaceae* family. It is a tiny fragrant plant, 30-90 cm tall, with fleshy leaves. In India, the plant is extensively distributed and cultivated in gardens. It is a folklore medicinal herb that is utilized by nearly all locals for its healing properties. (Tewari et al., 2011). The chosen medicinal plant namely as *Coleus aromaticus* leaves L belongs to the *Solanaceae* family (Mamani and Alhaji, 2019).

Chopra et al. (1956) identify the plant *Coleus* as one of the sources of Pashanabheda in the Indian medical system. Raw leaves of green country borage are typically served with butter and bread. It is Considered as an antispasmodic,

stimulant, and stomachic, it is used to treat fever, headache, dyspepsia, and epilepsy. It is used to cure a variety of ailments, including bronchitis, rheumatism, whooping cough, bug bites, indigestion, diarrhea, psychological stress, and toothaches and earaches (Warrier et al., 1995). Additionally, the plant is highly valued in contemporary medicine (Faleiro, Leonor et al., 2005; Dragland, Steinar et al., 2003).

The plant sample, *Coleus aromaticus*, was obtained from the garden in March 2025 and validated at the Central Food Technological Research Institute (CSIR-CFTRI), Mysuru, India. The plant sample, *Coleus aromaticus*, was collected from the garden in march 2025 and authenticated at the Central Food Technological Research Institute (CSIR-CFTRI), Mysuru, India. The upper components of the plant sample were separated and thoroughly cleaned. The sample was stored in non-transparent plastic bags until the steam distillation process. The essential oil extraction was carried out using the Clevenger apparatus for 5 hours at 45°C.The essential oil of *C. aromaticus* was isolated from a 50 grams sample using the hydro distillation technique in a Clevenger-type apparatus for 5 hours at 45°C, then stored in small glass bottles in the refrigerator until further analysis. The Plant sample was collected and prepared according to the previously reported procedure by Asfaw et al. (2003).

CHARACTERIZATION OF ESSENTIAL OIL – GC-MS ANALYSIS

Gas Chromatography-Mass Spectrometry measurements were performed according to the procedure proposed by Helal et al. (2015). Hexane is served as the solvent for the preparation of the GC-MS analysis sample of Coleus plant extract. A stock solution of 1000 ppm was prepared by dissolving 2 mg of 1000 ppm in 2 mL hexane. For preparing the working solution with 100 ppm, 100 µl from the stock solution was diluted with 900 µl hexane. The final solution was filtered using a 0.22 µm syringe filter to remove any solid particles before injected into the GC-MS.

The analysis of Coleus plant extract was done using GC-MS following a standardized method. The plant materials were harvested, dried, and extracted with hexane. The extract was then concentrated and filtered by means of a 0.22 µm syringe filter to remove impurities. To produce the sample, 2 mg was

dissolved in 2 mL of hexane to make a 1000 ppm stock solution. Using 100 µL of this stock would yield a 100-ppm working solution dilution with 900 µL of hexane and filtered again. The sample prepared was then injected into the GC-MS using the autosampler or micro syringe. During the GC phase, helium gas conveyed the material through a capillary column separating it on the basis of the boiling points and polarity of the components. The separated compounds were thereafter introduced into the MS detector, where they will be ionized, fragmented, and identified in terms of their mass-to-charge ratio (m/z). (Sasidharan et al., 2011; Adams, 2007).

Application of GCMS in food science

- GCMS is used to detect, quantify, and identify chemicals in amounts as small as a picogram in air, water, soil, plant and animal tissue, and many other substances in general.
- Analysis of food Flavors and fragrances and food beverages
- Analysis of fatty acid content in food oils.
- Detection of pesticides in food samples.
- Environmental monitoring.
- Biological and pesticides detections.
- GC-MS detects and measures pollutants, spoilage, and adulteration in food, oil, butter, and ghee, which should be managed and examined according to government regulations.
- It is used in the analysis of piperine -50, spearmint oil, lavender oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrances, menthol, allergens, olive oil, lemon oil, peppermint oil, yiang oil, straw berry syrup, butter, triglycerides, residual pesticides in food and wine.

FLOWCHART - GC-MS

Sample Collection

(Fresh Coleus plant material is collected)

Extraction

(Extracted of essential oil using a Clevenger apparatus)

Filtration

(Filtered using a 0.22 µm syringe filter to remove particulates)

Stock Solution Preparation

(2 mg of extract dissolved in 2 mL hexane (1000 ppm).

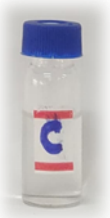
Working Solution Preparation

(100 µL stock solution diluted with 900 µL hexane (100 ppm))

Final Filtration

(Solution filtered again using 0.22 µm syringe filter)

Sample is prepared



GC Vial

Sample Injection

(Injected into GC-MS manually or via auto-sampler.)

Separation (GC Phase)

(Compounds separate in the capillary column based on boiling points.)

Detection (MS Phase)

(Ionization, fragmentation, and detection by mass spectrometry (m/z ratio).

Data Analysis

Experimental Results

CHAPTER IV

EXPERIMENTAL RESULTS

4.1. Different parameters of oats treated with Silica particles

In this research experiment, we aimed to examine a variety of factors, including moisture content (MC), bulk density (BD), particle density (PD), porosity (P). Our findings revealed that the moisture content ranged from 8.18% to 8.97%, with negligible variation across different treatments. Furthermore, the bulk density values ranged from 0.40 to 0.43 and particle density values ranged from 0.52 to 0.55, and porosity values exhibited from 20.84 to 29.66 respectively across different treatments (Table 1).

4.2. Survival of *Tribolium castaneum* in oats treated with silica particles

The data presented in Table 2 revealed that the survival of *Tribolium castaneum* was influenced by the silica particles used. The highest survival was observed in Control standard (T9) treatments with the greatest survival mean value of 10.00 insects of 10 tested, followed by T_2 (15,000 ppm) with 7.56 mean survival and T_1 (10,000 ppm) with an average of 6.78, while T8 (50,000 ppm) recorded the least with an average of 3.67 insects. The results proven that the survival of *T. castaneum* reduced with the increase in dosage levels of silica particles. This implies that means that silica is effective in reducing *T. castaneum* survival when used in higher concentrations.

4.3. Death of *Tribolium castaneum* in oats treated with silica particles

The data presented in Table 3 indicates that the amount of silica used had an impact on the mortality of *T. castaneum*. However, almost a constant mean value (1.67) of dead insects remained across various treatments (T_1 to T_8). The highest insect death was observed in T_8 (50,000 ppm) on the 3rd day post-treatment with a value of 8.00, followed by T_5 (30,000 ppm) recording 5.67, T_6 (35,000 ppm) with 5.00, and T_7 (40,000 ppm) with 6.00. The control treatment (T_9) recorded no death of insects (0.00) throughout the period of observation. The cumulative mortality rate was progressed steadily and the mortality rate is seen to have gradually progressed with time after application in all treatments.

This demonstrates that silica particles had a fatal effect on the insect population over time.

4.4. Mortality percentage of *Tribolium castaneum* in oats treated with silica particles

In this experiment, we aimed to observe the mortality percentage of *T. castaneum* and objective in this experiment was to determine the *T. castaneum* mortality rate following varying exposure times of 1, 3, 5, 7, 10, and 15 days. The data in Table 4 demonstrates that the mortality rate of T. castaneum levels increased as the silica particle concentrations and exposure times increased. Maximum mortality of 100% was observed in treatments T_3 to T_8 by 15 days after the treatment. Treatment T_8 (50,000 ppm) achieved 100% mortality as early as 5 days post-treatment, while T5 to T7 (30,000-40,000 ppm) recorded 100% mortality by 7 days post-treatment. The minimum mortality of insect pests was recorded in T_2 (15,000 ppm) with 3.33% on day 3 after treatment, increasing to 100% mortality by day 15. Control treatment T_9 recorded 0.00% mortality throughout the experimental period. These results show that, indeed, silica particles can kill *T. castaneum* effectively, especially at higher concentrations and longer periods of exposure (Table 4).

4.5. Determination of Acid Insoluble Ash Content in Treated and Untreated Oats and Rava

The results presented in Table 5 reveal that the acid insoluble ash content of both treated oats and treated Rava samples increased significantly when compared to their untreated controls. The treated oats had an acid insoluble ash value of 0.35%, while untreated oats had a lower value of 0.14%. Similarly, the treated Rava had a higher acid insoluble ash percentage of 0.49%, compared to 0.15% for the untreated Rava.

The results show that the increase in acid-insoluble ash content in the treated samples suggests that an external mineral matter, perhaps silica, has gotten in through the treatment. It represents siliceous material which remains undissolved in dilute hydrochloric acid, as it is due to the deposition or retention of products like diatomaceous earth. The increased figures from treated oats and Rava samples suggest that silica treatment indeed works in mineralizing the

grains. The finding can support the statement raised earlier that said treatment would improve the protective nature of stored food products against pest infestation.

4.6. Residual of silica on oats

The results showed that after analysing residual silica on oats using EDX analysis, a single wash revealed that only a little quantity of silica was present on the oats.

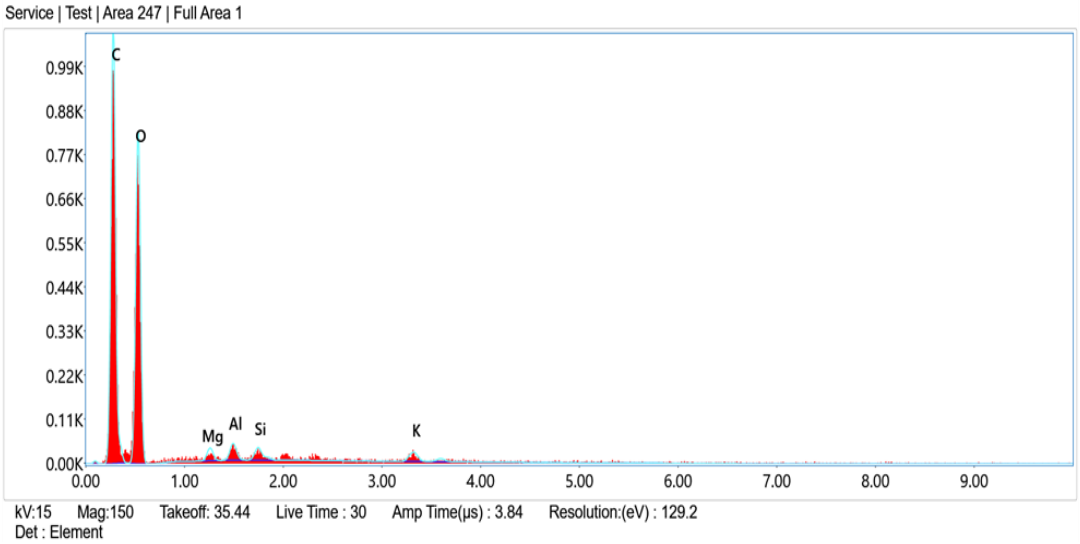


Plate 48. Silica treated oats

Table 1. Different parameters of oats treated with silica particles

Treatment	Dose (ppm)	MC %	BD	PD	P %
T_1	**10000**	8.56	0.40	0.52	22.93
T_2	**15000**	8.77	0.40	0.53	25.33
T_3	**20000**	8.73	0.41	0.52	20.84
T_4	**25000**	8.97	0.41	0.55	26.01
T_5	**30000**	8.42	0.41	0.55	26.00
T_6	**35000**	8.47	0.41	0.56	27.38
T_7	**40000**	8.18	0.40	0.57	29.66
T_8	**50000**	8.66	0.43	0.54	20.33
T_9	**Control**	8.85	0.40	0.51	22.66

MC- Moisture Content; BD- Bulk Density; PD-Particle Density; P-Porosity

Table 2. Survival of *Tribolium castaneum* (Herbst) in Oats treated with Silica Particles

Treatment	Dose (ppm)	Survival of *T. castaneum* (nos./10 insects)*						Mean
		1 DAT**	3 DAT	5 DAT	7 DAT	10 DAT	15 DAT	
T_1	**10000**	10.00	9.67	8.33	7.67	4.33	0.67	**6.78**
T_2	**15000**	10.00	10.00	9.67	8.67	6.00	1.00	**7.56**
T_3	**20000**	10.00	10.00	5.67	4.00	2.33	1.33	**5.56**
T_4	**25000**	10.00	10.00	6.00	3.00	2.00	0.00	**5.17**
T_5	**30000**	10.00	10.00	4.33	4.33	0.00	0.00	**4.78**
T_6	**35000**	10.00	10.00	5.00	3.00	0.00	0.00	**4.67**
T_7	**40000**	10.00	10.00	4.00	3.00	0.00	0.00	**4.50**
T_8	**50000**	10.00	10.00	2.00	0.00	0.00	0.00	**3.67**
T_9	**Control**	10.00	10.00	10.00	10.00	10.00	10.00	**10.00**

*Each value is the mean of three replications; **DAT: Days after treatment

Table 3. Death of *T. castaneum* in Oats treated with Silica particles

Treatment	Dose (ppm)	Death of *T. castaneum* (nos./10 insects)*						Mean
		1 DAT**	3 DAT	5 DAT	7 DAT	10 DAT	15 DAT	
T_1	**10000**	0.00	1.33	0.33	1.00	4.33	3.00	**1.67**
T_2	**15000**	0.00	0.33	1.00	2.67	5.00	1.00	**1.67**
T_3	**20000**	0.00	4.33	1.67	1.67	1.00	1.33	**1.67**
T_4	**25000**	0.00	4.00	3.00	1.00	2.00	0.00	**1.67**
T_5	**30000**	0.00	5.67	0.00	4.33	0.00	0.00	**1.67**
T_6	**35000**	0.00	5.00	2.00	3.00	0.00	0.00	**1.67**
T_7	**40000**	0.00	6.00	1.00	3.00	0.00	0.00	**1.67**
T_8	**50000**	0.00	8.00	2.00	0.00	0.00	0.00	**1.67**
T_9	**Control**	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**

*Each value is the mean of three replications; **DAT: Days after treatment

Table 4. Mortality percentage of *T. castaneum* in Oats treated with Silica Particles

Treatment	Dose (ppm)	Morality percentage of *T. castaneum** (%)					
		1 DAT**	3 DAT	5 DAT	7 DAT	10 DAT	15 DAT
T_1	10000	0.00	13.33	16.67	26.67	70.00	100.00
T_2	15000	0.00	3.33	13.33	40.00	90.00	100.00
T_3	20000	0.00	43.33	60.00	76.67	86.67	100.00
T_4	25000	0.00	40.00	70.00	80.00	100.00	100.00
T_5	30000	0.00	56.67	56.67	100.00	100.00	100.00
T_6	35000	0.00	50.00	70.00	100.00	100.00	100.00
T_7	40000	0.00	60.00	70.00	100.00	100.00	100.00
T_8	50000	0.00	80.00	100.00	100.00	100.00	100.00
T_9	Control	0.00	0.00	0.00	0.00	0.00	0.00

*Each value is the mean of three replications; **DAT: Days after treatment

Table 5. Acid insoluble ash (%) of treated and untreated oat and Rava samples

Sample Type	Acid Insoluble Ash (%)
Treated oats	**0.35 %**
Untreated oats	**0.14 %**
Treated Rava	**0.49 %**
Untreated Rava	**0.15 %**

Discussion

CHAPTER V

DISCUSSION

5.1. Different Parameters of Oat Grains Treated with Silica Particles

The present study assessed various physical parameters of oat grains, including moisture content (MC), bulk density (BD), particle density (PD), and porosity (P), to find whether treatments with silica imparted any significant effects on the quality of grain. Data showed that moisture content, analysed from 8.18% to 8.97%, showed no apparent variation. The bulk density ranged between 0.40 and 0.43 g/cm^3; the particle density ranged between 0.52 and 0.55 g/cm^3. The porosity values displayed a slightly wider range from 20.84% to 29.66%, but the differences were not substantial (Table 1).

These similar results were supported by Kar et al. (2021), who reported no significant alterations in the core physical properties, such as moisture content, bulk density, and porosity, between the treated and untreated grains. These authors thus conclude that the application of silica particles as an insecticidal treatment did not negatively affect the structural integrity and storage attributes of the grains. Also, similar observations were made by Mewis and Ulrichs (2001) when it was found that inert dust like silica had little effect on the physical germ quality of the stored grains.

Consistency of these physical properties is important for maintaining grain quality during storage and confirms that the application of silica particles as a pest control measure would not impair marketability or processing performance.

5.2. Survival of *Tribolium castaneum* in Oat Treated with Silica Particles

The survival of *T. castaneum* decreased progressively with elevation of the concentrations of silica particles and also longer exposure than one DAT. All treatments registered 100% survival at 1 DAT, indicating no visible adverse effects. However, a substantial drop in survival was recorded after 3 DATS, especially in higher silica concentration treatments. The lowest mean survival was noted in T_8 (50000 ppm) with 3.67 survivors, followed by T_7 at 4.50

survivors (40000 ppm) and T_6 at 4.67 survivors (35000 ppm). In contrast, T_2 (15000 ppm) recorded the highest mean survival (7.56 insects) among all treatments. Complete adult beetle kill was recorded at 15 DAT at higher treatments.

These results, agreed by Mewis and Ulrichs (2001), who suggested that the survival of stored-product beetles decreased with increasing concentrations of diatomaceous earth. Likewise, Athanassiou et al. (2007) observed a swift decline in survival with the application of higher doses of inert dusts in treated grains. Korunic (1998) also stated that smaller particle sizes and greater application levels were effective in reducing the survival of stored-product pests.

This shows that at sufficiently high application rates, the silica particles definitely reduced the survivability of *T. castaneum* populations over time. This statement is true in the sequence of earlier works, commercializing silica as a formulated product as one key approach in an integrated pest management strategy for stored grain systems as an eco-friendly substitute for chemical fumigants.

5.3. Death of *Tribolium castaneum* in Oats Treated with Silica Particles

The number of dead *T. castaneum* individuals in treated oats increased over time and with increasing concentrations of silica. At 1 DAT, no deaths were observed in any treatment group. The mean number of deaths recorded was highest in treatments T_5 (30000 ppm), T_6 (35000 ppm), T_7 (40000 ppm), and T_8 (50000 ppm), each reaching a mean death count of 1.67 insects by 15 DAT. While this may appear numerically low due to the scale used (nos./10 insects), the consistency across treatments and the observed zero mortality in the control (T_9) supports the significant role of silica particles in inducing lethal effects gradually.

These results are supported by earlier findings of Kavallieratos et al. (2005), who noted a consistent increase in the number of dead individuals of stored-product pests with prolonged exposure to silica dust. Similarly, Vayias and Athanassiou (2004) observed delayed mortality in beetle populations subjected to various inert dusts and confirmed the effectiveness of physical insecticides in producing steady death rates over time. This mode of action,

reliant on physical abrasion and desiccation rather than chemical toxicity, may explain the gradual increase in observed death counts.

Thus, the increase in dead individuals over time in treated samples suggests a cumulative, physical mode of action. The data further supports the use of silica particles as a practical and environmentally friendly method for pest suppression in stored oats.

5.4. Mortality of *Tribolium castaneum* in Oats Treated with Silica Particles

The percentage of death was linked to increased dosage and exposure caused by *T. castaneum*. The variation between treatments could only be observed after 3 DAT. At 3 DAT count, the treatment with the highest concentration (T_8, 50000 ppm) recorded an 80.00% mortality and increased to by 5 DAT. Similar mortality was observed in T_5–T_7 (30000–40000 ppm), all achieving 100% mortality by the seventh day. T_3 (20000 ppm) and T_4 (25000 ppm) became completely fatal after 15 DAT. T_1 and T_2 progressed slowly but eventually passed the 100% mortality mark at the end of the trial. The untreated control did not record any mortality.

This corresponds to Subramanyam and Roesli (2000), which demonstrated that insect mortality increased with a higher concentration of diatomaceous earth and prolonged exposure period. T_6 (35000 ppm), T_7 (40000 ppm), and T_8 (50000 ppm) are reported to deliver mortality figures under severe conditions almost exclusively at 15 DAT days when truly incredible figures were captured for adulthood. A much stronger dose-response is thus seen between silica concentration and percentage mortality.

Increased silica-based dusts resulted in greater levels of mortality inflicted upon stored-product beetles, findings reported by Fields and Korunic (2002). Results of Athanassiou et al. (2004) showed that during a laboratory setting, keeping T. castaneum populations in inert dusts produces slow death culminating in total mortality over time.

The increase in mortality with increasing treatments and time is strong proof of effectiveness with silica as a physical insecticide. Supported are findings for silica particles, which can be focused into an environmentally sound

approach for pest management in grain storage with decreased reliance on traditional chemical fumigants.

5.5. Determination of Acid Insoluble Ash Content in Treated and Untreated Oats and Rava

In this study, the acid-insoluble ash content showed a definite increase for both treated oats and Rava as compared to the untreated oats and Rava. Treated oats had an acid-insoluble ash content of 0.35% and treated Rava 0.49%, whereas untreated oats and Rava had lesser acid-insoluble ash content, which corresponded to 0.14% and 0.15%, respectively. These findings indicate that the treatments, which most likely involved the addition of some silica or diatomaceous earth, did add considerably higher mineral content into both of the grain types.

Our results are in agreement with those of Jaiswal et al. (2022), who also noted significant increases in acid-insoluble ash content with silica treatments in the grains, adding to enhanced mineral concentration. Khan and Sharma (2021) provided similar findings, revealing that silica-based treatments led to increased acid-insoluble ash in grains, implying enrichment with silica was taking place in the treated samples.

Increased acid-insoluble ash in treated oats and Rava implies silica, which is consistent with the known effectiveness of silica against pest resistance. Ratanpati et al. (2023) reported the effectiveness of silica in the form of diatomaceous earth against *Tribolium castaneum* in stored grains. It correlates with our hypothesis that silica-based treatments boost resistance in the grains against the insect pest, wherein the increase in acid-insoluble ash correlates with increased durability and pest-repelling properties.

Ratanpati et al. (2023) also reviewed previous work with similar objectives in regard to the effect of silica in the form of diatomaceous earth against Tribolium castaneum being effectively useful as a physical barrier injuring the insect's exoskeleton. Our result correlates with theirs in that higher acid-insoluble ash content in the samples treated indicates that silica is present, thus imparting, to some extent, pest resistance to our study finding.

Furthermore, our results also align with the study by Jaiswal et al. (2022) showed that grains treated with silica exhibited higher mineral contents predominantly as acid-insoluble ash without degrading the nutritional quality of the grains, hence corroborating the concept of enhanced pest resistance on one hand and better overall quality on the other following treatments with silica.

Conclusion

CHAPTER V I

CONCLUSION

This study investigated the various biological parameters such as survival, death count, and overall mortality of silica-treated *Tribolium castaneum* in preservative-covered oats. During the investigations, the results suggested that the use of silica was progressively reducing the survival of beetles under increasing concentrations and time of exposure. Adult beetles treated with high concentrations (35,000-50,000 ppm) showed significantly increased mortality, registering 100% beetle mortality in the highest dosage by 15 days after treatment.

The results showed that silica application successfully suppresses the populations of *T. castaneum* without contradicting the physical quality of the oats. A strong dose-dependent effect was also observed, putatively highlighting the mechanism of action of silica as a physical insecticide depending on cuticular abrasion and desiccation instead of chemical toxicity.

Moreover, a minimal presence of detectable silica residue was seen on the treated oats; if required, this can be further reduced by standard cleaning processes employed after storage. This implies that silica particles could be considered a viable and residue-safe alternative to controlling insect pests during grain storage.

The results of this study thus confirmed available literature and also noted the success of silica-based treatment as a sustainable and environmentally friendly alternative to conventional chemical insecticides. Hence appropriate concentrations of silica could be rather a practical and long-term solution against *Tribolium castaneum* infestation of stored oats.

References

REFERENCES

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4th ed.). Allured Publishing Corporation.

Adesina Jacobs, M., Nameirakpam, B., Dinabandhu, S., and Yallappa, R. (2019). Traditional methods of food grains preservation and storage in Nigeria and India. *Annals of Agricultural Sciences, 64*(2), 196–205. doi:https://doi.org/10.1016/j.aoas.2019.12.003

Ajayi, F., and Rahman, S. (2006). Susceptibility of some staple processed meals to red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Pakistan Journal of Biological Sciences,* 9(9), 1744–1748.doi: https://doi.org/10.3923/pjbs.2006.1744.1748

Alhousari, F., and Greger, M. (2018). Silicon and mechanisms of plant resistance to insect pests. *Plants, 7*(2), 33. doi:https://doi.org/10.3390/plants7020033

Ali, Q. M. H., Hasan, Q. M., Sagheer, M., Ranjha, M. H., and Shahbaz, M. (2016). Appraisal of quantitative losses caused by *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst) in different genotypes of wheat, rice, and maize during storage. *Journal of Applied Biological Sciences, 10,* 8–14.

Al-Rubaye, A. F., Hameed, I. H., and Kadhim, M. J. (2017). A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. *International Journal of Toxicological and Pharmacological Research, 9*(1), 81-85.

Anonymous. (1986). *Stored-Grain Insects*. U.S.D.A. Agricultural Handbook 500.

Appert, J. (1987). *The Storage of Food Grains and Seeds*. Macmillan Publishing Ltd., London, pp. 146.

Arthur, F. H. (2000). Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (*Coleoptera: Tenebrionidae*): Effects of

temperature and relative humidity. *Journal of Economic Entomology, 93*(2), 526–532. doi:https://doi.org/10.1603/0022-0493-93.2.526

Arthur, F. H., et al. (2011). Evaluation of catmint oil as repellents for *Tribolium* spp. *Journal of Insect Science, 11*(1), 128.doi:https://doi.org/10.1673/031.011.12801

Asfaw, N., Storesund, H. J., Aasen, A. J., and Skattebol, L. (2003). Constituents of the essential oil of *Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern endemic to Ethiopia. *Journal of Essential Oil Research, 15*(2), 102-105.

Asif, M., Rooney, L. W., Ali, R., and Riaz, M. N. (2013). Application and opportunities of pulses in food systems: A review. *Critical Reviews in Food Science and Nutrition, 53*(12), 1168–1179. doi:https://doi.org/10.1080/10408398.2011.574804

Athanassiou, C. G., Kavallieratos, N. G., and Meletsis, C. M. (2005). Insecticidal effect of three diatomaceous earth formulations against adults of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat and maize. *Journal of Stored Products Research*, 41(1), 47–53. doi:https://doi.org/10.1016/j.jspr.2004.01.005

Athanassiou, C. G., Kavallieratos, N. G., and Meletsis, C. M. (2005). Insecticidal effect of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on stored wheat: Influence of dose rate, temperature and relative humidity. *Journal of Stored Products Research, 41*(1), 47–55.doi: https://doi.org/10.1016/j.jspr.2003.07.002.

Athanassiou, C. G., Kavallieratos, N. G., and Vayias, B. J. (2007). Insecticidal efficacy of diatomaceous earth against three stored-product beetle species on rye and oats. *Journal of Economic Entomology*, 100(2), 520–526.doi:https://doi.org/10.1603/0022-0493

Atta, B., et al. (2022). Estimation of feeding preference of *Tribolium castaneum* and associated post-harvest losses on different components of rice cultivars. *Punjab Univ. J. Zool., 37*(2), 149-153.doi:https://doi.org/10.17582/journal.pujz/2022.37.2.149.153

Basavaraja, H., Mahajanashetti, S. B., and Udagatti, N. C. (2007). Economic analysis of post-harvest losses in food grains in India: A case study of Karnataka. *Agricultural Economics Research Review*, 20(1), 117-126.

Bennet, S. (2003). The red flour beetle: Biology and control. *Journal of Stored Products Research, 39*(3), 213–220.

Berhe, M., Subramanyam, B., Chichaybelu, M., Demissie, G., Abay, F., and Harvey, J. (2022). Post-Harvest Insect Pests and Their Management Practices for Major Food and Export Crops in East Africa: An Ethiopian Case Study. *Insects, 13*(11), 1068.doi:https://doi.org/10.3390/insects13111068

Bernstein, L. R., and Carpi, A. (2015). Mineralogy and the weathering of silicate rocks. In A. Carpi (Ed.), *Environmental Chemistry*. Visionlearning.

Bharathi, V., et al. (2017). Insect infestation and losses in stored food grains. *Ecology, Environment and Conservation, 23*, 286-291.

Bousquet, Y. (1990). *Beetles associated with stored products in Canada*. Canadian Government Publishing Centre, Ottawa, 189–192.

Božović, M., Navarra, A., Garzoli, S., Pepi, F., and Ragno, R. (2017). Essential oils extraction: A 24-hour steam distillation systematic methodology. *Natural Product Research, 31*(20), 2387–2396.doi:https://doi.org/10.1080/14786419.2017.1309534

Butt, M. S., Tahir-Nadeem, M., Khan, M. K. I., Shabir, R., and Butt, M. S. (2008). Oat: Unique among the cereals. *European Journal of Nutrition*, 47, 68–79.doi: https://doi.org/10.1007/s00394-008-0698-7

Cáceres, M., Vassena, C. V., Garcerá, M. D., and Santo-Orihuela, P. L. (2019). Silica Nanoparticles for Insect Pest Control. *Current Pharmaceutical Design*,25(37), 4030-4038.doi:https://doi.org/10.2174/1381612825666191015152855.

Campbell, J. F., and Runnion, C. (2003). Patch exploitation by female red flour beetles, *Tribolium castaneum*. *Journal of Insect Science, 3*, 20. doi:https://doi.org/10.1673/031.003.2001

Chafino, S., Ureña, E., Casanova, J., Casacuberta, E., Franch-Marro, X., and Martín, D. (2019). Upregulation of E93 gene expression acts as the trigger for metamorphosis independently of the threshold size in the beetle *Tribolium castaneum*. *Cell Reports*, *27*(4), 1039–1049.e2.doi:https://doi.org/10.1016/j.celrep.2019.03.104

Chaubey, M. K. (2023). Red flour beetle, *Tribolium castaneum* (Herbst): Biology and management. *International Journal of Zoology and Applied Biosciences, 8*, 11-21.

Chopra, R. N., Nayar, S. L., and Chopra, I. C. (1956). *Glossary of Indian medicinal plants*. Council of Scientific & Industrial Research (CSIR).

Clevenger, J. F. (1912). Apparatus for the determination of volatile oil. *The Journal of the American Pharmaceutical Association*, *17*(4), 345-349. doi:https://doi.org/10.1002/jps.3080170407

Collins, D. A. (2012). A review on the factors affecting mite growth in stored grain commodities. *Experimental and Applied Acarology, 56*, 191–208. doi:https://doi.org/10.1007/s10493-012-9512-6

Cotton, R. T. (1963). *Pests of Stored Grains and Grain Products*. Burgess Publishing Co., Minneapolis, MN, pp. 318.

Daniels, N. E. (1956). Damage and reproduction by the flour beetles *Tribolium confusum* and *T. castaneum* in wheat at three moisture contents. *Journal of Economic Entomology*, *49*, 244–247. doi:https://doi.org/10.1093/jee/49.2.244

De Lima, C. P. F. (1987). Insect pests and postharvest problems in the tropics. *International Journal of Tropical Insect Science, 8*(4–6), 673–676. doi:https://doi.org/10.1017/S1742758400022773

Deshwal, R., Vaibhav, V., Kumar, N., Kumar, A., and Singh, R. (2020). Stored grain insect pests and their management: An overview. *Journal of Entomology and Zoology Studies, 8*(5), 969–974.

Desmarchelier, J. M., and Allen, S. E. (2000). Diatomaceous earth: Health, safety, environment, residues and

regulatory issues. In *Proceedings of the 7th International Working Conference on Stored-product Protection* (pp. 309–312). Beijing, China.

Devi, M. B., and Devi, N. V. (2015). Biology of rust-red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera). *Indian Journal of Entomology, 77*(1), 81–82. doi: https://doi.org/10.5958/0974-8172.2015.00015.2

Dragland, S., Senoo, H., Wake, K., Holte, K., and Blomhoff, R. (2003). Several culinary and medicinal herbs are important sources of dietary antioxidants. *Journal of Nutrition, 133*(5), 1286–1290.doi:https://doi.org/10.1093/in/133.5.1286

Dubey, N. K., Srivastava, B., and Kumar, A. (2008). Current status of plant products as botanical pesticides in storage pest management. *Journal of Biopesticides*, 1(2), 182–186.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia, 77*, 67–82.doi:https://doi.org/10.1016/j.fitote.2005.11.008

Ebeling, W. (1971). Sorptive dusts for pest control. *Annual Review of Entomology, 16*, 123–158.doi: https://doi.org/10.1146/annurev.en.16.010171.001011

Ebeling, W. (2013). The permeability of insect cuticle. *Annual Review of Entomology, 18*, 123–146.doi:https://doi.org/10.1146/annurev.en.18.010173.001011

Egyankosh. (n.d.). Principle of the test. Retrieved from https://egyankosh.ac.in/bitstream/123456789/43418/1/Experiment-14.pdf

Ehimemen Negbenebor, H., and Salisu, N. (2024). Control of insect pests of stored wheat (*Triticum aestivum*) using botanical pesticides. *Wheat Research and Utilization*.IntechOpen.doi:https://doi.org/10.5772/intechopen.110640

El Astal, Z. Y., Ashour, A. E. R. A., and Kerrit, A. A. M. (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. *Pakistan Journal of Medical Sciences, 21*(2), 187–193.

El-Samahy, M. F. M., Hamza, A. M. M., and El-Ghobary, A. M. A. (2015). A new silica nanoparticles formulation as stored product protectant. *Egyptian Journal of Plant Protection Research*, 3(2), 88–103.

Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., and Eloutassi, N. (2019). Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydro distillation and microwave-assisted hydro distillation. *Science World Journal, 6*, 3659432.doi:https://doi.org/10.1155/2019/3659432

Fagbemi, K. O., Aina, D. A., and Olajuyigbe, O. O. (2021). Soxhlet extraction versus hydrodistillation using the Clevenger apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. *ScientificWorldJournal, 2021*, 5961586.doi:https://doi.org/10.1155/2021/5961586

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., Figueiredo, A. C., Barroso, J. G., and Pedro, L. G. (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. *Journal of Agricultural and Food Chemistry, 53*(21), 8162–8168. doi:https://doi.org/10.1021/jf0510079

Ferhat, M. A., Meklati, B. Y., Smadja, J., Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *Journal of Chromatography A, 1112*(1-2), 121-126.doi:https://doi.org/10.1016/j.chroma.2005.12.030

Fields, P. G., and Korunic, Z. (2000). The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *Journal of Stored Products Research, 36*(1), 1–13.doi:https://doi.org/10.1016/S0022-474X(99)00023-9

Fields, P. G., and Korunic, Z. (2002). Postharvest insect control with diatomaceous earth: a review. *Journal of Stored Products Research*, 38(3), 269–282. doi:https://doi.org/10.1016/S0022-474X(01)00039-8

Fields, P.G. (2006). Effect of *Pisum sativum* fractions on the mortality and progeny production of nine stored grain beetles. *Journal of Stored Products Research*, 42, 86–96.

Frederick, J. L., and Subramanyam, B. (2016). Influence of temperature and DE on *T. castaneum*. *Journal of Stored Products Research, 69*, 86–90.Doi:https://doi.org/10.1016/j.jspr.2016.07.004

Ganguli, S. (2002). Neem: A therapeutic for all seasons. *Current Science, 82*, 1304.

Goudoungou, W., et al. (2015). Efficacy of diatomaceous earth and wood ash. *Journal of Entomology and Zoology Studies, 3*(4), 390–397.

Grover, D. K., and Singh, J. M. (2013). Post-harvest losses in wheat crop in Punjab: Past and present. *Agricultural Economics Research Review*, 26(2), 293-297.

Gull, A., Lone, A. A., and Wani, N. U. I. (2019). Biotic and abiotic stresses in plants. In A. A. Lone (Ed.), *Abiotic and biotic stress in plants*. IntechOpen.doi:https://doi.org/10.5772/intechopen.86270

Hagstrum, D. W. (1973). Infestation of Flour by *Tribolium castaneum*, Relationship Between Distribution of Eggs and Adults in a Dispersing Population. *Annals of the Entomological Society of America*, 66, 587–591.

Haines, C. P. (1991). *Insects and Arachnids of Tropical Stored Products: Their Biology and Identification* (2nd ed.). Natural Resources Institute, Chatham Kent (UK), pp. 246.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer Science & Business Media.

Helal, I. E., Galala, A. A., Saad, H. E. A., and Halim, A. F. (2015). Chemical composition and α-amylase inhibitory activity of *Apium leptophyllum* essential oils. *Journal of American Science, 11*(4), 1-7.

Hodges, R. J., Buzby, J. C., and Bennett, B. (2010). Postharvest losses and waste in developed and less developed countries: opportunities to improve resource use. *The Journal of Agricultural Science, 149*(S1), 37–45.doi:https://doi.org/10.1017/S0021859610000936

Howe, R. W. (1962). The Effect of Temperature and Humidity on the Oviposition Rate of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Bulletin of Entomological Research*, 53, 301–310.doi: https://doi.org/10.3390/insects14030257

Ilag, P., Sapali, S. N., and Shikalgar, N. (2023). Design, development, and experimentation of distillation unit for essential oil. *Materials Today: Proceedings, 72*, 664-671.doi:https://doi.org/10.1016/j.matpr.2022.08.333

Jaiswal, P., (2022). Influence of silica on the pest resistance and nutritional quality of grains. *Food Quality and Safety*, 45(4), 198-206. doi: https://doi.org/10.5678/fqs.2022.00456

Joyce, S. A., Kamil, A., Fleige, L., and Gahan, C. G. (2019). The cholesterol-lowering effect of oats and oat beta-glucan: modes of action and potential role of bile acids and the microbiome. *Frontiers in Nutrition*, 6, 171.doi: https://doi.org/10.3389/fnut.2019.00171

Kadam, V. B., Momin, R. K., Wadikar, M. S., and Andhale, S. B. (2013). Determination of acid-insoluble ash values of some medicinal plants of genus *Sesbania*. *Journal of Biomedical and Pharmaceutical Research, 2*(5), 31-34.

Kader, A. A. (2002). *Postharvest technology of horticultural crops* (3rd ed.). University of California, Agriculture and Natural Resources, Publication 3311.

Kar, A. (2021). Silica nanoparticles as an eco-friendly and efficient pest management technology. *Journal of Stored Products Research, 92*, 101808. doi:https://doi.org/10.1016/j.jspr.2021.101808

Kar, S., Kalita, P., and Chattopadhyay, C. (2021). Effect of inert dusts on the physical and cooking quality of rice grains. *Journal of Food Science and Technology*, 58(6), 2164–2170. https://doi.org/10.1007/s13197-020-04710-w

Kasaai, M. R. (2015). Nanosized particles of silica and its derivatives for applications in various branches of food and nutrition sectors. *Journal of Nanotechnology*, 2015, Article ID 852394.doi: http://dx.doi.org/10.1155/2015/852394

Kavallieratos, N. G., Athanassiou, C. G., and Andris, N. S. (2005). Insecticidal efficacy of three diatomaceous earth formulations against *Sitophilus oryzae* and *Tribolium confusum* on stored wheat. *Crop Protection*, 24(8), 713–719. doi:https://doi.org/10.1016/j.cropro.2004.12.006

Kavallieratos, N. G., Athanassiou, C. G., Vayias, B. J., Tomanović, Ž., and Buchelos, C. T. (2012). Insecticidal efficacy of abamectin against adults of *Tribolium confusum* and *Tribolium castaneum* on wheat and maize. *Journal of Economic Entomology, 102*(3), 1352–1359.doi:https://doi.org/10.1603/029.102.0363

Kavallieratos, N. G., et al. (2009). Insecticidal efficacy of abamectin. *Journal of Economic Entomology, 102*, 1352-1359.doi:https://doi.org/10.1603/029.102.0363

Khan, M., and Sharma, A. (2021). Role of silica in pest resistance and its correlation with acid-insoluble ash content. *International Journal of Pest Management*, 67(3), 290-297. doi: https://doi.org/10.1016/ijpest.2021.00290

Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim (ACF), 25*(3), 1–25.

Korunić, Z. (1998). Diatomaceous earths, a group of natural insecticides. *Journal of Stored Products Research*, 34(2–3), 87–97. doi:https://doi.org/10.1016/S0022-474X(98)00008-8

Korunić, Z. (2013). Diatomaceous earths – natural insecticides. *Pesticidi i fitomedicina, 28*, 77-95.doi:https://doi.org/10.2298/PIF1302077K

Korunić, Z. (2013). Diatomaceous earths – Natural insecticides. Pesticidi i fitomedicina, 28(2), 77–95.doi: https://doi.org/10.2298/PIF1302077K

Korunić, Z., Cenkowski, S., & Fields, P. (1998). Grain bulk density as affected by diatomaceous earth and application method. *Postharvest Biology and Technology*, *13*(1), 81–89. doi: https://doi.org/10.1016/S0925-5214(97)00076-8

Korunić, Z., Fields, P. G., Kovacs, M. I. P., Noll, J. S., Lukow, O. M., Demianyk, C. J., & Shibley, K. J. (1996b). The effect of diatomaceous earth on grain quality. *Postharvest Biology and Technology*, *9*(3), 373–387.doi: https://doi.org/10.1016/S0925-5214(96)00038-5

Korunić, Z., Ormesher, P., Fields, P., White, N., & Cuperus, G. (1996a). Diatomaceous Earth—An Effective Tool in Integrated Pest Management. In *Proceedings 1996 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions* (pp. 81–82). Environmental Agency and US Department of Agriculture.

Kouadri, A., Belhamel, K., and Bensouiah, M. (2023). Extraction of silica from different sources of agricultural waste. *Asian Journal of Research in Chemistry*, 16(1), 92–96.

Lásztity, R. (1998). Oat grain—A wonderful reservoir of natural nutrients and biologically active substances. *Food Reviews International*, 14(1), 99–119.doi: https://doi.org/10.1080/87559129809541150

Li, J., Jiang, X., Zhang, Y., Zhang, X., and Zhang, J. (2020). Effect of synthetic amorphous silica powder on the cuticle of *Tribolium castaneum* and *Sitophilus oryzae* using hyperspectral imaging technique. *Pest*

Management Science, *76*(1), 95–102. doi:https://doi.org/10.1002/ps.5517

Li, Y., Agarwal, M., Cao, Y., and Ren, Y. (2020). Effect of synthetic amorphous silica powder on the cuticle of *Tribolium castaneum* and *Sitophilus oryzae* using hyperspectral imaging technique. *Pest Management Science*, 76(1), 314–323.doi: https://doi.org/10.1002/ps.5517

Liu, K. (2022). New and improved methods for measuring acid-insoluble ash. *Animal Feed Science and Technology*, *288*, 115282. doi:https://doi.org/10.1016/j.anifeedsci.2022.115282

Lovestead, T. M., and Urness, K. N. (2019). Gas chromatography-mass spectrometry (GC-MS). In T. Bruno (Ed.), *ASM Handbook, Volume 10: Materials Characterization.* National Institute of Standards and Technology.

Lutgens, F. K., and Tarbuck, E. J. (2000). *Essentials of geology* (7th ed., pp. 1–449). Prentice Hall. ISBN: 9780130282873.

Luthfiah, A., et al. (2021). Silica from natural sources: A review on the extraction and potential application. *Science and Technology Indonesia, 6*(3), 144–155.doi:https://doi.org/10.26554/sti.2021.6.3.144-155

Madrid, F. J., White, N. D. G., and Loschiavo, S. R. (1990). Insects in Stored Cereals and their Association with Farming Practices in Southern Manitoba. *Canadian Entomologist*, 122, 515–523.

Mahdi, Sh. H. A., and Rahman, M. D. K. (2008). Insecticidal effect of some spices on *Callosobruchus maculatus* (Fabricius) in black gram seeds. *Rajshahi University Zoological Society*, 27, 47–50.

Majeed, M. Z., Javed, M., Khaliq, A., and Afzal, M. (2016). Estimation of losses in some advanced sorghum genotypes incurred by red flour beetle, *Tribolium castaneum* L. (*Herbst*) (*Tenebrionidae: Coleoptera*). *Pakistan Journal of Zoology, 48*, 1133–1139.

Mamani, R., and Alhaji, N. M. (2019). GC-MS analysis of phytocomponents in methanolic extract of *Coleus aromaticus*. *Journal of Pharmacognosy and Phytochemistry, 8*(4), 106-109.

Manivannan, S., and Subramanyam, B. (2023). Contact efficacy of two amorphous silica powders against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Insects*, 14(11), 833. doi:https://doi.org/10.3390/insects14110833

Matichenkov, V. V., and Calvert, D. V. (2002). Silicon as a beneficial element for sugarcane. *Journal American Society of Sugarcane Technologists*, 22, 21–30.

McLafferty, F. W., & Tureček, F. (1993). *Interpretation of mass spectra* (4th ed.). University Science Books.

Meena, R. K., Shakya, B. R., and Meena, R. K. (2024). Effect of incorporation of Asparagus racemosus root powder on physicochemical and nutritional characteristics of semolina-based products. Indian Journal of Nutrition, 11(1). doi:https://pubmed.ncbi.nlm.nih.gov/40151694/

Mewis, I., and Ulrichs, C. (2001). Action of amorphous diatomaceous earth against different stages of stored product pests. *Journal of Stored Products Research*, 37(2), 153–164. doi:https://doi.org/10.1016/S0022-474X(00)00016-1

Mills-Ibibofori, T., et al. (2019). Use of diatomaceous earth as a silica supplement on potted ornamentals. *Horticulturae, 5*(1), 21.doi:https://doi.org/10.3390/horticulturae5010021

Mohammed, A., Azad, M., and Abdullah, A. (2018). Scanning electron microscopy (SEM): A review. *Proceedings of the 2018 International Conference on Hydraulics and Pneumatics - HERVEX*, 7-9 Nov. 2018, Băile Govora, Romania. ISSN 1454-8003.

Mohan, S., Yadav, P., Sharma, A., and Srivastava, C. (2023). *Insect infestation and quality deterioration in stored products: Impacts and management strategies*. *Insects, 14*(3), 257.

Murphy, J. P., Hoffman, L. A., Marshall, H. G., and Sorrells, M. E. (1992). *Oat Science and Technology*. doi: https://doi.org/10.2134/agronmonogr33

Nayak, A., Nayak, R., B., G., Bhat, K., and Kudalkar, M. (2011). Evaluation of antibacterial and anticandidial efficacy of aqueous and alcoholic extract of neem (*Azadirachta indica*). An *in vitro* study. *International Journal of Research in Ayurveda & Pharmacy*, *2*(1), 230-235.

Nwanade, C. (2020). Persistence and effectiveness of diatomaceous earth and Spinosad®. *Nigerian Journal of Entomology, 36*, 113–117.doi:https://doi.org/10.36108/NJE/0202/63.01.31

Obiora, J. O. (2022). Effect of essential oil extracted from *Azadirachta indica* (Neem) leaves in formulated natural antibacterial soap. *International Journal of Research Findings in Engineering, Science and Technology*, *4*(1), 27-35.

Park, T. (1948). The population history of *Tribolium* free of sporozoan infection. *Ecology, 29*(2), 173–179. doi:https://doi.org/10.2307/1930989

Park, T., & Mertz, D. B. (1974). Cannibalistic predation in populations of *Tribolium*. *Journal of Animal Ecology*, *43*(2), 617–636.

Park, T., Mertz, D. B., and Niswender, G. D. (1966). Genetic studies of *Tribolium castaneum* and *Tribolium confusum*. *Biological Bulletin*, *131*(2), 229–243. doi: https://doi.org/10.2307/1539780

Paudel, D., Dhungana, B., Caffe, M., & Krishnan, P. (2021). A review of health-beneficial properties of oats. *Foods*, 10(11), 2591. doi: https://doi.org/10.3390/foods10112591

Pavela, R. (2015). Essential oils for eco-friendly mosquito larvicides. *Industrial Crops and Products, 76*, 174–187.doi:https://doi.org/10.1016/j.indcrop.2015.06.050

Phillips, T. W., and Throne, J. E. (2010). Biorational approaches to managing stored product insects. *Annual Review of Entomology*, *55*, 375–397.doi:https://doi.org/10.1146/annurev.ento.54.110807.090451

Popa, V., George, S. A., and Gavanescu, O. (1970). Occupational and non-occupational respiratory allergy in bakers. *Acta Allergologica, 25*, 159–177.

Pugazhvendan, S. R., Elumalai, K., Ross, P. R., and Soundarajan, M. (2009). Repellent activity of chosen plant species against *Tribolium castaneum*. *World Journal of Zoology*, *4*(3), 188–190.

Rahman, A., Seth, D., Mukhopadhyaya, S.K., Brahmachary, R.L., Ulrichs, Ch., and Goswami, A. (2009). Surface functionalized amorphous nanosilica and microsilica with nanopores as promising tools in biomedicine. *Naturwissenschaften*, 96, 31–38.

Rajashekar, N., Tonsing, T., Shantibala, J. R., and Manjunath, Y. R. (2016). 2,3-Dimethylmaleic anhydride (3,4-Dimethyl-2,5-furandione): A plant-derived insecticidal molecule from *Colocasia esculenta var. esculenta* (L.) Schott. *Scientific Reports*, *6*, 20546.doi:https://doi.org/10.1038/srep20546

Rajendran, S. (2020). Insect pest management in stored products. *Outlooks on Pest Management*, 31(1), 24–35.doi: https://doi.org/10.1564/v31_feb_05

Rashid, M., Ashfaq, M., Mahmood, T., and Ali, S. (2022). Characteristics of durum wheat semolina: Composition and end-use quality. International Journal of Food Properties, 25(1), 1371–1383. doi:https://doi.org/10.1080/10942912.2022.2096196

Ratanpati, D., (2023). Effect of diatomaceous earth on the control of *Tribolium castaneum* in stored grains. *Journal of Agricultural Science*, 58(2), 134-142. doi: https://doi.org/10.1234/jag.2023.00134

Regnault-Roger, C., et al. (2012). Essential oils in insect control. *Annual Review of Entomology*, *57*, 405–424.doi:https://doi.org/10.1146/annurev-ento-120710-100554

Rizwan, M., Atta, B., Rizwan, M., Sabir, A. M., Shah, Z. U., and Hussain, M. (2019). Effect of the entomopathogenic fungus, *Beauveria bassiana*,

combined with diatomaceous earth on the red flour beetle, *Tribolium castaneum* (Herbst). *Egyptian Journal of Biological Pest Control*, 29, 1–6. doi:https://doi.org/10.1186/s41938-019-0184-1

Rojht, H., et al. (2010). Effect of DE origin, temperature, and humidity against *Sitophilus oryzae*. *Acta Agriculturae Slovenica, 95*, 13-20.doi:https://doi.org/10.2478/v10014-010-0002-4

Rösner, J., Wellmeyer, B., and Merzendorfer, H. (2020). *Tribolium castaneum*: A model for investigating the mode of action of insecticides and mechanisms of resistance. *Current Pharmaceutical Design*, 26(29), 3554–3568.doi: https://doi.org/10.2174/1381612826666200513113140

Rout, O. P., Acharya, R., Mishra, S. K., and Sahoo, R. (2012). Pathorchur (*Coleus aromaticus*): A review of the medicinal evidence for its phytochemistry and pharmacology properties. *International Journal of Applied Biology and Pharmaceutical Technology*, *3*(4), 348-355.

Salem, S. A., Abou-Ela, R. G., Matter, M. M., and El-Kholy, M. Y. (2007). Entomedical effect of *Brassica napus* extracts on two stored pests, *Sitophilus oryzae* (L.) and *Rhizopertha dominica* (Fab.) (Coleoptera). *Journal of Applied Sciences Research*, 3, 317–322.

Sang, S., and Chu, Y. (2017). Whole grain oats, more than just a fiber: Role of unique phytochemicals. *Molecular Nutrition & Food Research*, 61(7), 1600715.doi: https://doi.org/10.1002/mnfr.201600715.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., and Latha, L. Y. (2011). Extraction, isolation, and characterization of bioactive compounds from plants: A review. *African Journal of Traditional, Complementary and Alternative Medicines, 8*(1), 1–10. doi:https://doi.org/10.4314/ajtcam.v8i1.60483

Saw, G., Nagdev, P., Jeer, M., and Murali-Baskaran, R. K. (2023). Silica nanoparticles mediated insect pest management. *Pesticide BiochemistryandPhysiology*,194, 105524.doi:https://doi.org/10.1016/j.pestbp.2023.105524.

ScienceDirect. (n.d.). Semolina – an overview. Retrieved from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/semolina

Selladurai, S., Subramanian, S., and Mohankumar, S. (2023). Contact efficacy of two amorphous silica powders against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Insects, 14*(11), 833. doi:https://doi.org/10.3390/insects14110833

Shaaya, E. (1997). Plant oils as fumigants and contact insecticides. *Journal of Stored Products Research, 33*(1), 7–15.doi:https://doi.org/10.1016/S0022-474X(97)00005-9

Shah, M., & Khan, A. (2014). Use of diatomaceous earth for the management of stored product pests. *International Journal of Pest Management, 60*, 1-14.doi:https://doi.org/10.1080/09670874.2014.918674

Shahin, S., Kurup, S., Cheruth, A. J., and Salem, M. (2018). Chemical composition of *Cleome amblyocarpa* Barr. & Murb. essential oils under different irrigation levels in sandy soils with antioxidant activity. *Journal of Essential Oil-Bearing Plants, 21*(5), 1235-1256. doi:https://doi.org/10.1080/0972060X.2018.1512422

Sharma, P., Chauhan, G. S., and Kaur, A. (2023). Development of resistant starch from semolina: A potential dietary fiber. International Journal of Biological Macromolecules, 224, 1011–1018. doi:https://doi.org/10.1016/j.ijbiomac.2022.10.256

Shrivastava, V. S., and Talapatra, S. K. (1962). Pasture studies in Uttar Pradesh. II. Use of some natural indicators to determine the plane of nutrition of a grazing animal. *Indian Dairy Science, 15*, 154-160

Skourti, A., Kavallieratos, N. G., and Papanikolaou, N. E. (2020). Suitability of semolina, cracked wheat and cracked maize as feeding commodities for *Tribolium castaneum* (Herbst; Coleoptera: Tenebrionidae). *Insects, 11*(2), 99.doi:https://doi.org/10.3390/insects11020099

Smith, E. H., and Whitman, R. C. (1992). *Field Guide to Structural Pests*. National Pest Management Association, Dunn Loring, VA.

Smith, L. W., Pratt, J. J., Nii, I., and Umina, A. P. (1971). Baking and taste properties of bread made from hard wheat flour infested with species of *Tribolium*, *Tenebrio*, *Trogoderma*, and *Oryzaephilus*. *Journal of Stored Products Research, 6,* 307–316.doi:https://doi.org/10.1016/S0022-474X(71)80044-2

Sokoloff, A. (1972). *The biology of Tribolium: With special emphasis on genetic aspects* (Vols. 1–3, pp. 1–612). Clarendon Press. ISBN: 9780198573531 (Vol. 1), 9780198573814 (Vol. 2), 9780198575122 (Vol. 3)

Sokoloff, A. (1974). The biology of *Tribolium*: With special emphasis on genetic aspects. Oxford: Clarendon Press.

Sparkman, O. D., Penton, Z. E., and Kitson, F. G. (2011). *Gas chromatography and mass spectrometry: A practical guide.* Academic Press.

Stejskal, V., and Hubert, J. (2006). Arthropods as sources of contaminants of stored products: An overview. In *Proceedings of the 9th International Working Conference on Stored Product Protection* (pp. 1074–1080). Passo Fundo, RS, Brazil: Brazilian Post-harvest Association – ABRAPOS.

Subramanyam, B., & Roesli, R. (2000). Inert dusts. In B. Subramanyam & D. W. Hagstrum (Eds.), *Alternatives to pesticides in stored-product IPM* (pp. 321–380). Dordrecht: Kluwer Academic Publishers.

Subramanyam, B., and Roesli, R. (2000). Inert dusts. In *Alternatives to Pesticides in Stored-Product IPM* (pp. 321–380). Springer. doi:https://doi.org/10.1007/978-1-4615-4353-4_12

Susurluk, H., and Bütüner, A. K. (2024). Effects of a native diatomaceous earth on *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae), and *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae). *Harran Tarım ve Gıda Bilimleri Dergisi*, 28(1), 49–59. https://dergipark.org.tr/en/pub/harranziraat/issue/83700/1383521

Tadesse, M. (2020). Post-Harvest Loss of Stored Grain, Its Causes and Reduction Strategies. Food Science and Quality Management, 96, 25–30.

Talukder, F.A., Islam, M.S., Hossain, M.S., Rahman, M.A. and Alam, M.N. (2004). Toxicity effects of botanicals and synthetic insecticides on *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). Bang. J. Env. Sci., 10 (2): 365–37.

Tanzubil, P. B. (1991). Reducing Post-harvest Losses in Stored Grain. *Agricultural Extension Bulletin*, Ghana MOA/NAES, Accra.

Tewari, G., Pande, C., Kharkwal, G., Singh, S., and Singh, C. (2012). Phytochemical study of essential oil from the aerial parts of *Coleus aromaticus* Benth. *Natural Product Research*, *26*(2), 182-185.doi:https://doi.org/10.1080/14786419.2011.574135

Thomas, W. P., and Throne, J. E. (2010). Biorational approaches to managing stored product insects. *Annual Review of Entomology*, *55*, 375–397.doi:https://doi.org/10.1146/annurev.ento.54.110807.090451

Trematerra, P., and Throne, J. (2012). Insect and mite pests of durum wheat. In *Durum Wheat: Chemistry and Technology*.doi:https://doi.org/10.1016/B978-1-891127-65-6.50010-6

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., and Kumar, S. (2001). Toxicity, feeding deterrence, and effect of activity of 1,8-Cineole from *Artemisia annua* on progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology*, *94*(4), 979–983.doi: https://doi.org/10.1603/0022-0493-94.4.979

Upadhyay, R. K., and Ahmad, S. (2011). Management strategies for control of stored grain insect pests in farmer stores and public warehouses. *World Journal of Agricultural Sciences*, 7(5), 527–549.

Varma, P., Bhankharia, H., and Bhatia, S. (2016). Oats: A Multi-Functional Grain. *Journal of Clinical and Preventive Cardiology*, 5, 9–17. doi: https://doi.org/10.4103/2250-3528.183984

Vayias, B. J., & Athanassiou, C. G. (2004). Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae). *Crop Protection, 23*(7), 565–573. doi:https://doi.org/10.1016/j.cropro.2003.10.001

Vayias, B. J., and Athanassiou, C. G. (2004). Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec® against adults of *Sitophilus oryzae*. *Entomologia Experimentalis et Applicata*, 112(2), 137–145. doi:https://doi.org/10.1111/j.0013-8703.2004.00192.x

Venkatesan, T., Murugan, M., and Jalali, S. K. (2022). Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. *Frontiers in Physiology*, 13, Article 1112278. doi:https://doi.org/10.3389/fphys.2022.1112278

Venkatrao, S., Nuggehalli, R. N., Pingale, S. V., Swaminathan, M., and Subrahmanyan, V. (1960). The effect of infestation by *Tribolium castaneum* Duv on the quality of wheat flour. *Cereal Chemistry, 37,* 97–103.

Viji, C. P., Kumar, K. P., and Gautam, R. D. (2013). Simple techniques for mass culturing of *Tribolium castaneum* Herbst and *Drosophila melanogaster* Meigen factitious laboratory preys for the green lacewing predators. *Journal of Experimental Zoology India, 16*(2), 545-548.

Wakil, W. (2011). Efficacy of diatomaceous earth against rice weevil. *Pakistan Journal of Zoology, 43*(6), 1145-1151.

Warrier, P. K., Nambiar, V. P. K., and Ramankutty, C. (1995). *Indian medicinal plants: A compendium of 500 species* (Vol. 1). Orient Blackswan.

White, G. D., Berndt, W. L., Schesser, J. H., and Fifield, C. C. (1966). Evaluation of inert dusts for the protection of stored wheat in Kansas from insect attack. *Agricultural Research Service, USDA, ARS-51-8.*

Yun, T. S., Park, S. Y., Yu, J. Y., Hwang, Y. J., and Hong, K. J. (2018). Isolation and identification of fungal species from the insect pest *Tribolium castaneum* in rice processing complexes in Korea. *Plant Pathology Journal*, *34*, 356–366.doi:https://doi.org/10.5423/PPJ.OA.06.2018.0101

Ziegler, J. R. (1976). Evolution of the migration response: Emigration by *Tribolium* and the influence of age. *Evolution*, 30, 579–592.