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Them

Formulation based on essential oils of three plant spies for the control of *Tribolium castaneum* in stored foodstuffs

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إهــداء

بسـم الله الرحمان الرحيـم

الحمد لله الذي بنعمته تتـم الصالحات الحمد لله الذي ما تم جهد الا بفضله

الشكـر موجه الى تلك التي جعلت هاته الاطروحة ممكنة والتي رافقتنا في رحلتنا وعلى العطاء وجودة اشرافكـم المبنية

على الدقة والثقة والمصداقية استاذتنا الغالية

" الدكتورة بوعفيان نريمان مبروكة "

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الى كل من ساهم في انجاح هذا العمل المتواضع من قريب او من بعيد

إهــداء

الحمد لله حبا وشكرا وامتنانا على البدء والختام

(واخر دعواهم ان الحمد لله رب العالمين)

لم تكن الرحلة قصيرة ولا الطريق محفوفا بالتسهيلات لكنني فعلتها فالحمد لله الذي يسر البدايات وبلغنا النهايات . بفضله وكرمه

اهدي هذا النجاح لنفسي بعد مسيرة اعوام من التعب والصعوبات، ها انا اليوم أقف على عتبة تخرجي اقطف ثمار تعبي وارفع قبعتي بكل فخر

وبكل حب وامتنان اهدي ثمرة نجاحي وتخرجي الى معلمي الاول في الحياة الى النور الذي انار دربي والذي بذل جهد السنين من اجل ان اعتلي سلالم النجاح الى من احمل اسمه بكل فخر لطالما عاهدته بهذا النجاح. ها انا اتممت وعدي واهديته اليك

"بابا علي"

الى من علمتني الاخلاق قبل الحروف الى الجسر الصاعد بي الى الجنة الداعمة الاولى لي في الحياة حبيبة القلب والروح اليد الخفية التي ازالت عن طريقي الاشواك واحفتني بدعواتها

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اخوتي الغوالي **" بدر الدين ,سفيان "**

اخواتي الغاليات **"حنان زهيرة كوثر دلال مريم ومارية "**

الى كل ابناء وبنات الاخوة والاخوات الاعزاء

الى اختي التي انجبتها لي الايام انيسة روحي ورفيقة دربي وصديقة حياتي غاليتي

"لينـه"

واخيرا من قال انا لها "نالها" وانا لها ان ابت رغما عنها اتيت بها , فالحمد لله الذي ما تيقنت به خيرا واملا الا واغرقني سرورا وفرحا ينسيني مشقتي .

سيرين

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(وآخر دعواهم أن الحمد لله رب العالمين)

لنفسي، التي خاضت هذا الدرب بشجاعة وإصرار، التي آمنت بقدراتها وسعت لتحقيق احلامها . هذا التخرج بداية لرحلة جديدة من التعلم والتميز، وإيمان بأن كل حلم يمكن أن يصبح حقيقة

إلى سندي وعزوتي قدوتي ومثلي الأعلى، يا من غرست في الطموح وحب العلم يا من علّمني معنى الجد والكفاح. الذي لم يبخل يومًا بجهده دعمه وتضحياته أهدي إليك هذا الإنجاز الذي هو جزء صغير من عطائك الكبير (والدي العزيز حفظه الله)

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الى التي تعجز كل الكلمات عن وصفها التي كانت النور في عتمتي التي كان دعاؤها سر نجاحي الى الانسانة العظيمة التي لطالما تمنت ان تقر عينها في يوم كهذا

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إلى من شاركوني لحظات الفرح والتعب وإلى من كانوا لي خير عون وسند إلى إخوتي يا من زرعتم في الأمل وشجعتموني على المضي قدمًا أهدي إليكم هذا الإنجاز الذي طالما حلمنا به معًا، فهو لكم بقدر ما هو لي أدعو الله أن يجمعنا دائمًا على الخير والنجاح

اخوتي الغاليين **"وائل خليفه ، عائد العزوزي"**

اخواتي الغاليات **" فريال، تقوى ، ابتهال "**

ابناء الاخت الغوالي **" رتيل و رفيف رابوب "**

إلى رفيقة الدرب وشريكة النجاح وأعز الأصدقاء من شاركتني اللحظات الحلوة والمُرة وكانت سندًا لي في كل خطوة

غاليتي **"سيرين"**

(إِنِّي جَزَيْتُهُمُ الْيَوْمَ بِمَا صَبَرُوا أَنَّهُمْ هُمُ الْفَائِزُونَ)

الحمد لله الذي بنعمته تتم الصالحات أخيراً تحقق حلم التخرج. لطالما انتظرت هذا اليوم كي ارى الفخر والسعادة في عين أمي وأبي

لينه

RÉSUMÉ

Le principal objectif de ce travail est de développer une formulation naturelle à l'efficacité insecticide contre les ravageurs des denrées stockées, en ciblant spécifiquement le tribolium de la farine, *Tribolium castaneum*.

Dans ce contexte, la toxicité d'extraits de plantes de graines de Neem (*Azadirachta indica*), de graines d'Harmel (*Peganum harmala*) et de gomme d'Asafoetida (*Ferula assa-foetida*) a été testée séparément sur des adultes de tribolium de la farine (*Tribolium castaneum*) à différentes concentrations croissantes, et un mélange de ces extraits de plantes a également été évalué.

Une formulation d'huiles essentielles a été appliquée par contact et par fumigation à des doses de (0,5 µl/ml, 1,5 µl/ml, 3 µl /ml et 6 µl/ml) . Les taux de mortalité ont été observés dès la première heure du début du traitement et après 24 heures, avec un taux de mortalité de 100 % enregistré.

De même, une formulation alcoolique d'extraits de plantes de graines de Neem (*Azadirachta indica*) et de graines d'Harmel (*Peganum harmala*) a été testée par contact et par fumigation à des doses de (0,5 µl/ml, 1 µl/ml, 2,5 µl/ml et 5 µl/ml) , et les taux de mortalité ont été observés à 1 heure et 24 heures. Un taux de mortalité de 100 % a été enregistré dans les premières 24 heures.

Selon nos résultats, le taux de mortalité enregistré variait en fonction de la dose, de la durée d'exposition et de l'espèce végétale.

L'efficacité de la formulation contenant un mélange d'extraits de plantes de Neem, d'Asafoetida et d'Harmel s'est avérée plus puissante et à action plus rapide que la toxicité de chaque extrait individuellement.

Mots-clés :

Formulation naturelle, Extraits de plantes, Extrait alcoolique, Huiles essentielles, *Tribolium castaneum*, *Peganum harmala*, *Azadirachta indica*, *Ferula assa-foetida*.

Abstract:

The primary objective of this work is to develop a natural formulation with insecticidal effectiveness against storage material pests, specifically targeting the flour beetle *Tribolium castaneum*.

In this context, the toxicity of plant extracts from Neem (*Azadirachta indica*) seeds, Harmal (*Peganum harmala*) seeds, and Asafoetida (*Ferula assa-foetida*) gum was tested separately against adult flour beetles *Tribolium castaneum* at different increasing concentrations, and a mixture of these plant extracts was also evaluated.

An essential oil formulation was applied using contact and fumigant methods at doses of (0.5 µl/ml, 1.5 µl/ml, 3 µl/ml, and 6 µl/ml). Mortality rates were observed in the first hour of treatment initiation and after 24 hours, with a 100% mortality rate recorded.

Similarly, an alcoholic formulation of plant extracts from Neem(*Azadirachta indica*) seeds and Harmal*(Peganum harmala*) seeds was tested using contact and fumigant methods at doses of (0.5 µl/ml, 1 µl/ml, 2.5 µl/ml, and 5 µl/ml), and mortality rates were observed at 1 hour and 24 hours. A 100% mortality rate was recorded within the first 24 hours.

According to our findings, the recorded mortality rate varied depending on the dose, exposure duration, and plant species.

The efficacy of the formulation containing a mixture of Neem, Asafoetida, and Harmal plant extracts together was more potent and faster-acting compared to the toxicity of each extract individually.

Keywords:

Natural formulation, Plant extracts, Alcoholic extract, Essential oils, *Tribolium castaneum*, *Peganum harmala*, *Azadirachta indica*, *Ferula assa-foetida*.

الملخص

يهدف هذا العمل بشكل أساسي إلى تطوير تركيبة طبيعية فعالة لمكافحة آفات مخازن المواد الغذائية، مع التركيز على تأثيرها المبيد لحشرة خنفساء الدقيق Tribolium castaneum.

وقد تم في هذا السياق تقييم السمية المنفصلة لمستخلصات نباتية من بذور النيم (*Azadirachta indica*)، وبذور الحرمل (*Peganum harmala*)، وصمغ الحلتيت (Ferula assa-foetida) ضد الخنافس البالغة ل *Tribolium castaneum* بتراكيز متزايدة، بالإضافة إلى اختبار خليط من هذه المستخلصات.

كما تم تطبيق تركيبة من الزيوت العطرية بطريقتي التلامس والتبخير بتراكيز (0.5 µl, 1.5 µl, 3 µl, 6 µl) / مل ,ولوحظت معدلات النفوق بعد ساعة واحدة و 24 ساعة من المعاملة، حيث سجلت نسبة نفوق بلغت 100%.

وبالمثل، تم اختبار تركيبة كحولية لمستخلصات بذور النيم*Azadirachta indica* وبذور الحرمل *Peganum harmal* بطريقتي التلامس والتبخير بتراكيز (0.5 µl, 1 µl, 2.5 µl, 5 µl) / مل ,ولوحظت معدلات النفوق بعد ساعة و 24 ساعة، حيث سجلت نسبة وفيات 100% خلال الـ 24 ساعة الأولى.

وفقا للنتائج، تبين أن معدل الوفيات يعتمد على الجرعة، ومدة التعرض، ونوع النبات المستخدم. وقد أظهرت تركيبة خليط مستخلصات *Azadirachta indica* و*Ferula assa-foetidaa* و *Peganum harmala* فعالية مبيدة أسرع وأعلى مقارنة بسمية كل مستخلص على حدة.

الكلمات المفتاحية:

تركيبة طبيعية، مستخلصات نباتية، مستخلص كحولي، زيوت عطرية، خنفساء الدقيق ، الحرمل ، النيم ، الحلتيت.

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Introduction

Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, serves as a prominent model organism and a ubiquitous pest of stored grain ecosystems, particularly infesting milled cereal products, stored flour, and fungus-contaminated grains. This species is recognized as a major contributor to post-harvest losses in flour mills and storage facilities for dried foods and cereal products, ranking among the most economically significant pests of stored grains and processing environments(SJ Brown, TD Shippy et al ;2009).

Consequently, heightened consumer demand for pest-free flour and grains underscores the critical importance of effective *T. castaneum* management strategies within grain storage facilities(U Shamjana and T Grace; 2021).

A variety of chemical insecticides are used to control *Tribolium castaneum* (red flour beetle). This group includes organophosphates such as malathion and pirimiphos-methyl, as well as pyrethroids like deltamethrin and cyfluthrin. Fumigants such as phosphine and methyl bromide are also used in airtight storage, and carbon dioxide is another option for fumigation in controlled atmosphere environments. Recent studies have explored the effectiveness of newer insecticides like spinosad and insect growth regulators such as (S)-methoprene. However, it is important to note that the red flour beetle has shown resistance to some of these chemicals(JF Campbell and CG Athanassiou; 2022).

Insecticides are widely employed in agriculture for pest and weed control, with their efficacy predicated on their chemical composition. However, the prolonged and extensive application of these substances has raised significant concerns regarding their environmental persistence, food safety, and public health risks. These pesticides have been shown to contaminate various environmental matrices, including soil, surface water, and groundwater, posing potential threats to ecosystems and human health (B Zhou and X Li ; 2021).

Human exposure to insecticides occurs through direct and indirect pathways. Direct contact happens during application on crops, affecting the skin, eyes, mouth, and respiratory system, causing acute reactions such as headaches, irritation, vomiting, sneezing, and skin rashes. The severity of these effects depends on the duration and concentration of exposure. (VM Pathak, VK Verma,et al; 2022).

To avoid the negative points inherent to the use of synthetic insecticides, it is necessary to find more efficient alternatives with less environmental impact that can control the pest populations. One of those alternatives is the use of naturally derived insecticides, such as plant extracts, that are more environmentally friendly and sustainable than the commercial synthetic insecticides widely used in agriculture (MC Barreto and AML Seca; 2021).

The efficacy of plant essential oils and their constituent compounds for the management of *Tribolium* spp. (*T. castaneum* and *T. confusum*) have demonstrated promising activity in mitigating these infestation-driven losses (AK Pandey, S Tripathi et al; 2018). Research indicates that essential oils and their isolated chemical constituents exhibit significant toxicity against both *Tribolium* species through diverse modes of action, including repellent, insecticidal, ovicidal, larvicidal, pupicidal, ovipositional, and feeding deterrent effects (AK Pandey, P Singh et al ;2018).

Several essential oil-based botanical insecticides have proven effective in protecting stored food grains from both beetle species during storage. Furthermore, these botanical insecticides have demonstrated behavior-modifying properties against both *Tribolium* species, potentially mitigating the development of pest resistance, a prevalent issue associated with conventional synthetic insecticides. Consequently, essential oil-based botanical insecticides represent a potentially advantageous alternative to conventional insecticides for safeguarding stored grains and their products against *Tribolium* infestation(AK Pandey and S Tripathi ;2018).

The problem addressed in our work is the search for a natural formulation with insecticidal activity against *T. castaneum*. To this end, our objective is to investigate several plants using different methods in order to develop a bioinsecticidal preparation against *T. castaneum*.

In this context, our thesis is divided into three parts: a bibliographic section that provides general information on pesticides and bio pesticides; a second section on materials and methods, which summarizes the materials used and the methodologies adopted during the course of this work; and a third section on results and discussion, which presents and discusses our findings.

part I: Bibliographic part

1. Pesticides definition

A pesticide is a chemical agent or mixture of chemical agents used to control or destroy pests and weeds, thereby protecting crop plants, animals, and people from damage and diseases. Their classification depends on the target organism (insecticides, fungicides, herbicides, etc.). Synthetic pesticides have become a threat to human health and the environment due to their toxicity and pollution(IC Yadav and NL Devi ; 2017).

1.1. Pesticides pollution

Pesticides represent a class of chemical compounds utilized in agriculture and public health to manage pests and vectors of disease. While their application can enhance crop yields and control disease transmission, substantial evidence indicates potential adverse effects on both the environment and human health(S Ghosh, MS Khan et al; 2024).

1.1.1. Pesticides and human health

Exposure to pesticide residues through ingestion, inhalation, or dermal absorption can induce both acute and chronic toxicological effects. The manifestation and severity of toxicity are modulated by several factors, including the specific chemical structure of the pesticide, individual metabolism, the magnitude of the dose, the route of exposure, and the potential for bioaccumulation within the organism(S Ghosh, MS Khan et al; 2024).

Acute toxicity typically arises from a singular, high-level exposure, whereas chronic toxicity develops as a consequence of repeated or prolonged exposure to lower concentrations. Furthermore, certain pesticides possess neurotoxic, immunotoxic, or endocrine-disrupting properties, underscoring their potential for systemic harm(S Ghosh, MS Khan et al ; 2024).

Comprehensive global statistics on the precise impact of pesticides on human health remain limited, it is estimated that millions of individuals worldwide experience pesticide exposure annually. Projections from the World Bank in 2008 indicated an estimated 355,000 fatalities attributable to pesticide exposure. Regionally, data from the Food and Agriculture Organization of the United Nations (FAO) in 2005, based on statistics from SriLanka, suggested that pesticide poisoning could be responsible for approximately 300,000 deaths per year in the Asia-Pacific region alone. The paucity of robust global data highlights the urgent need for enhanced surveillance and research to accurately quantify the human health burden associated with pesticide exposure (S Ghosh, MS Khan et al; 2024).

1.1.2 Pesticides and environmental pollution

Pesticides, a structurally diverse group of organic micropollutants, exert a wide spectrum of environmental consequences. The heterogeneity in their chemical properties and modes of action results in differential impacts across various taxa, complicating broad generalizations regarding their ecological effects. While terrestrial environments experience pesticide contamination, aquatic ecosystems constitute a primary route for significant ecological impact, largely facilitated by pesticide runoff from agricultural and urban landscapes(F Torrens and G Castellano; 2014).

The accumulation of pesticides within aquatic food webs is predominantly governed by bioconcentration and biomagnification. Bioconcentration refers to the direct uptake and subsequent accumulation of a chemical substance from the surrounding medium into an organism(F Torrens and G Castellano ; 2014).

Lipophilic pesticides, characterized by their high affinity for lipids, exhibit a propensity for storage in the adipose tissues of exposed organisms. Dichlorodiphenyltrichloroethane (DDT), a well-documented persistent lipophilic compound, exemplifies this phenomenon, demonstrating significant bioaccumulation in the lipid reserves of aquatic organisms, including commercially relevant fish species, with subsequent transfer and accumulation in human consumers. In contrast, pesticides such as glyphosate undergo metabolic transformation and elimination, exhibiting a comparatively lower potential for bioaccumulation. Biomagnification, the process by which the concentration of persistent organic pollutants, including certain pesticides, increases progressively at higher trophic levels, leads to apex predators, including humans, often exhibiting the highest tissue burdens(RM Gutgesell, EE Tsakiridis et al; 2020).

The ecological ramifications of pesticide contamination are multifaceted and intricately interconnected. Pesticides can contaminate various environmental matrices, including soil, vegetation, and water resources, directly inducing toxicity in non-target organisms such as fish, avian species, beneficial insects (e.g., pollinators), and non-crop flora. Beyond direct lethal or sublethal effects on individual organisms, pesticides can instigate perturbations at the ecosystem level, altering community structure, species diversity, and ecosystem functionality. The impact of pesticides on larger organisms is increasingly recognized as a significant driver of biodiversity decline. Notably, the World-Wide Fund for Nature has reported an elevated prevalence of developmental abnormalities, neoplasia, and disease in fish populations inhabiting heavily pesticide-contaminated coastal and North Sea waters of the United

Kingdom since the 1970s, findings consistent with established toxicological outcomes associated with pesticide exposure(W Zhou, M Li et al ; 2024).

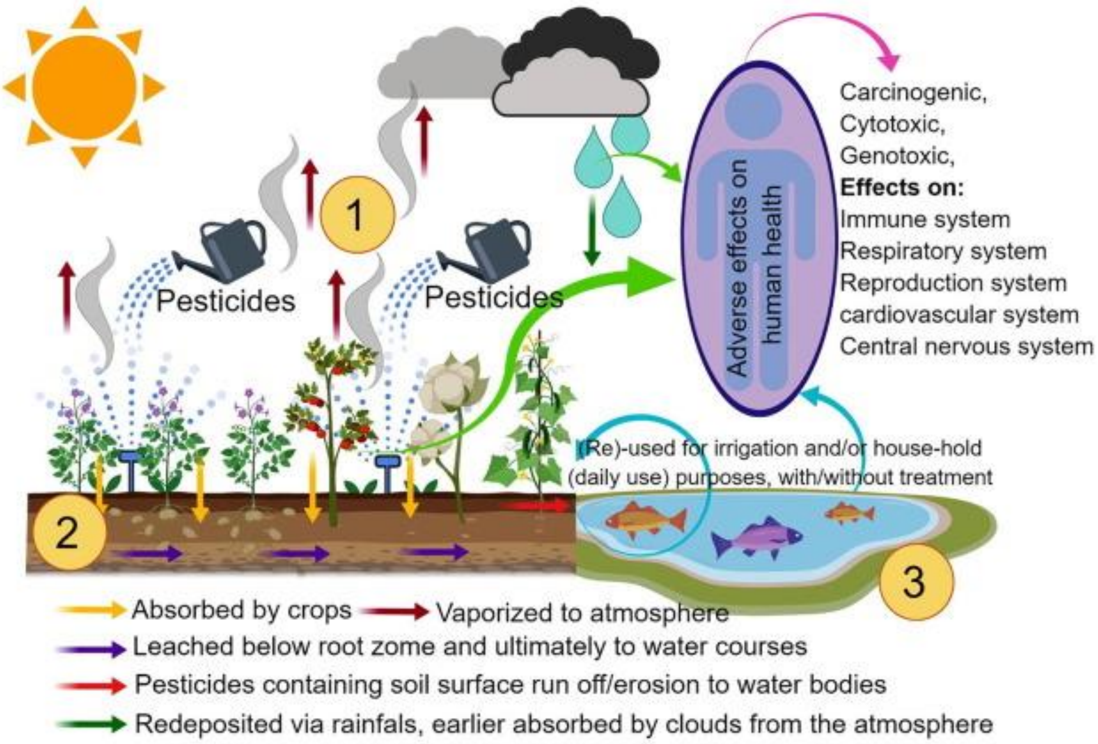


Figure (01): Schematic illustration of the hazardous impact of direct or indirect soil, air, and water pollution with pesticides on human health due to innovative agricultural practices (Liu et al; 2019).

2. Biopesticides definition

Biopesticides are derivatives of plants, microorganisms and insects. Substances from plants and animals have been used to manage diseases in crops, animals and humans (JW Muthomi; 2018).

Biopesticides are potential alternatives to synthetic pesticides. Biocides are readily available, readily biodegradable, exhibit different modes of action, are less expensive and have low toxicity to humans and non-target organisms. However, Biopesticides face challenges in their formulation, registration, marketing, acceptance and adoption(JM Roberts and TW Pope ; 2018).

2.1. Types of biopesticides

2.1.1. Biochemical pesticides

Biochemical pesticides, a category of pest management tools, are derived from naturally occurring substances. Unlike conventional chemical pesticides that typically rely on synthetic molecules with direct toxic effects, biochemical pesticides employ non-toxic mechanisms to control pests, often by modulating their behavior or physiology. This class includes diverse biologically active compounds such as pheromones and other semi chemicals (e.g., allomones, kairomones), plant-derived extracts with pesticidal properties, and naturally occurring insect growth regulators(NMA El-Ghany;2019).

Pheromones, a notable subset of biochemical pesticides, represent semiochemicals that mediate intraspecific communication, often playing a crucial role in insect mating behavior. These compounds can be naturally sourced or synthetically produced to disrupt pest populations. Strategies employing pheromones include mating disruption, where the release of synthetic pheromones confuses male insects and hinders their ability to locate mates, thereby reducing reproductive success and controlling population levels(NMA El-Ghany;2019).

Secondary Metabolites Of Plants As Biopesticides

As for the secondary metabolites of plants as biopesticides, they are: Cytochalasin, Alkaloids, Alkaloids, Terpenoids, Polyketides, Grisofulvin, Trichothecenes(AF Burlec, F Crivoi, et al ; 2021).

2.1.2. Microbial biopesticides

Microbial pesticides encompass a diverse range of pest control agents derived from living microorganisms, including bacteria, fungi, viruses, protozoa, and algae (Adeleke et al; 2022). These biopesticides exert their effects through the production of toxic metabolites or by establishing parasitic or pathogenic relationships with target pests, thereby disrupting their growth and survival. Various formulation and application techniques are employed to deliver microbial pesticides to the environment, such as emulsion, electrospraying systems, fluidized beds, spray drying, extrusion, lyophilization, spray cooling, and coacervation (De Oliveira et al; 2021).

The primary microbial taxa utilized as biopesticides include bacterial genera such as *Chromobacterium*, *Pseudomonas*, and *Yersinia*; fungal genera including *Beauveria*, *Paecilomyces*, *Verticillium*, *Hirsutella*, *Metarhizium*, and *Lecanicillium*; and nematodes belonging to the genera *Steinernema* and *Heterorhabditis* (Chang et al; 2003 Kumar et al;2021; Adeleke et al; 2022).

Among bacterial biopesticides, *Bacillus thuringiensis* (Bt) stands out as a well-established and commercially successful agent (Ujvary;2010 Ruiu;2018). This Gram-positive bacterium exhibits insecticidal activity through the production of parasporal crystals containing delta-endotoxins and endospores. Upon ingestion by susceptible insects, the alkaline environment of the midgut solubilizes these crystals, releasing the delta-endotoxin, a protein with a lethal effect on the insect (Xiao and Wu; 2019). *Bacillus thuringiensis* is employed to mitigate pest infestations in various crops, including cabbage and potato, and demonstrates control over lepidopteran pests in diverse plant species (Berini et al; 2018; Samada and Tambunan; 2020).

2.1.3. Plant-Incorporated-Protectants (PIPs)

The advent of recombinant DNA and plant transformation technologies has fundamentally reshaped insect pest management in commercial agriculture, leading to the development of plant-incorporated protectants (PIPs). This approach integrates insect control agents directly into the plant genome, offering several advantages over traditional foliar or soil-applied pesticides. These benefits include a reduced environmental footprint due to minimized off-target exposure, enhanced specificity towards target herbivore species, and simplified, season-long pest protection delivered through the plant's tissues. However, the risk of target pest resistance evolution, a significant factor limiting the long-term efficacy of conventional exogenous pesticides, remains a critical concern for PIPs. Ensuring the sustained viability of transgenic insect control strategies will necessitate the implementation of responsible integrated pest management (IPM) practices and the continuous discovery of novel insecticidal mechanisms of action for incorporation into plants. The insecticidal proteins expressed in plant-incorporated protectants(F Ali, K Neha, et al; 2023).

2.2. Botanical preparations as bioinsecticides

Botanical preparations represent a significant class of bioinsecticides with a long history of utilization in pest management, predating the advent of synthetic chemical pesticides. These bio-based agents, derived from a diverse array of plant species and their constituent parts (e.g., leaves, stems, roots, seeds), contain a wide spectrum of bioactive compounds. The modes of action exhibited by these compounds are varied and often synergistic, encompassing antifeedant, repellent, insect growth regulatory, and direct toxic effects on target insect pests (refer to the existing body of literature for specific examples and detailed mechanistic insights). The historical reliance on botanical resources for pest control across various cultures underscores the inherent insecticidal properties of numerous plant secondary metabolites. Contemporary scientific inquiry continues to focus on the identification, characterization, and optimization of these active botanical compounds, facilitating their integration into modern sustainable agricultural practices. Prominent examples of botanical insecticides include:

- **Neem (*Azadirachta indica*):** Extracts of the neem tree contain azadirachtin, a complex molecule known for its multifaceted activity as an antifeedant, insect growth regulator, and sterilant against a broad range of insect pests(JL Delgado ; 2020).
- **Pyrethrum (*Chrysanthemum cinerariifolium*):** Derived from the dried flower heads of the pyrethrum daisy, pyrethrins are characterized as fast-acting neurotoxins exhibiting efficacy against a wide spectrum of insect orders. Synthetic pyrethroid analogs are extensively utilized due to their enhanced environmental stability(F Shimira, S Uğur et al ; 2021).
- **Rotenone (various *Derris* and *Lonchocarpus* spp.):** Extracted from the roots of specific leguminous plants, rotenone acts as a respiratory disruptor and exhibits both contact and stomach poison activity. Its application is increasingly restricted due to concerns regarding its toxicity to aquatic organisms and mammals(HF Khater ; 2012).
- **Sabadilla (*Schoenocaulon officinale*):** Alkaloids isolated from the seeds of this lily-like plant demonstrate contact and stomach poisoning effects, impacting insect nerve function. Notably, sabadilla exhibits toxicity to bee populations(HF Khater ; 2012).

- **Ryania (*Ryania speciosa*):** Extracts from the stems and roots of the *Ryania* plant contain ryanodine alkaloids, which act as slow-acting stomach poisons with a relatively favorable mammalian toxicity profile(HF Khater ; 2012).
- **Essential Oils:** Volatile aromatic compounds extracted from a diverse range of plant species (e.g., *Eucalyptus* spp., *Mentha piperita*, *Citrus* spp.) possess repellent, insecticidal, and fumigant properties. Individual constituents such as limonene and linalool are also employed for pest control(HF Khater ; 2012).
- **Insecticidal Soaps:** Although often classified separately, soaps derived from plant-based fatty acids (e.g., oleic acid from vegetable oils) disrupt the integrity of insect cellular membranes, leading to desiccation and mortality, particularly in soft-bodied insect species(HF Khater ; 2012).

3. Plant secondary metabolites as potential bioinsecticides

3.1. Essential oils

Essential oils derived from aromatic plants have gained considerable attention as natural insecticides, particularly among organic growers and environmentally conscious consumers. These oils are typically obtained through steam distillation of plant materials and are composed primarily of volatile, low molecular weight compounds such as terpenes and phenolics, which contribute to their bioactive properties. (Ukoroije *et al ;*2020).

3.2. Tannins

Tannins are among the most abundant secondary metabolites in plants, comprising approximately 5–10% of the dry weight of tree leaves. They are predominantly localized in various plant tissues, including bark, wood, fruits, leaves, seeds, stems, and sap. Tannins exert significant influence on the biochemical and physiological processes of insects, contributing to plant defense mechanisms against herbivory (Ukoroije *et al ;*2020).

3.3. Alkaloids

Alkaloids represent a prominent class of naturally occurring compounds with significant bioinsecticidal activity. They are recognized as potent insecticidal agents and often act synergistically with other secondary metabolites such as Saponins and tannins. Through their repellent and antifeedant properties, alkaloids effectively deter insect pests, leading to adverse effects on their survival and development (Ukoroije *et al;* 2020).

3.4. Terpenoids

Terpenoids are a diverse class of natural plant-derived organic compounds synthesized from five-carbon isoprene units and are widely recognized for their aromatic properties. They contribute significantly to the characteristic scent and flavor of plants such as eucalyptus, clove, cinnamon, and ginger, with notable examples including menthol, citral, camphor, and salvinorin. Terpenoids also serve essential roles in traditional herbal medicine. In animals, steroids and sterols are biosynthesized from Terpenoids precursors. Based on their structural complexity, terpenoids are classified into monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, and polyterpenoids. In insect physiology, terpenoids play a critical role in disrupting endocrine functions by interfering with the biosynthesis of juvenile hormone and ecdysteroids (e.g., hydroxyecdysone), thereby affecting processes such as larval molting, pupation, and metamorphosis (Walkowiak-Nowicka *et al ;*2023).

3.5. Flavonoids

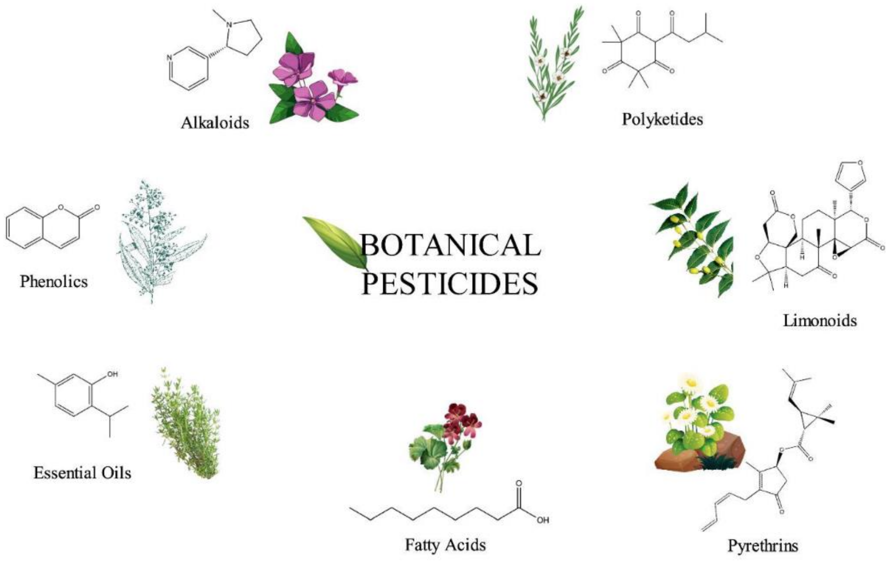
Flavonoids are plant secondary metabolites that contribute to pest management strategies due to their insecticidal properties. These compounds are widely distributed in various plants, including cocoa, citrus fruits (e.g., tangerine), parsley, blueberries, grapefruit, onions, barley, maize, sorghum, amaranth, and turnip. Flavonoids play a crucial role in plant defense by deterring herbivorous insects and reducing feeding damage (Ukoroije *et al ;*2020).

3.6. Saponins

Saponins are glycosidic compounds characterized by the presence of one or more sugar moieties linked to a non-sugar aglycone. Known for their soap-like properties, saponins exhibit bioactivity that is primarily attributed to their bitter and acrid taste, which acts as a feeding deterrent in insect herbivores (Walkowiak-Nowicka *et al ;* 2023).

3.7. Phenolics

Phenolic compounds are closely associated with plant defense mechanisms due to their involvement in interactions with herbivores and pathogens. These compounds exhibit antioxidant properties that contribute to their pesticidal activity. Upon ingestion by herbivorous insects, phenolics can exert toxic effects, thereby deterring feeding and reducing pest viability (Ukoroije *et al ;*2020).



Figure(02): Structural diversity of bioactive compounds in plant-based pesticides (Acheuk et al; 2022).

4. Mechanisms of action of bioinsecticides

The mechanisms of action of biopesticides on target insects are diverse and often exhibit greater specificity compared to synthetic chemical pesticides. These mechanisms can be broadly categorized as follows:

4.1. Toxin Production

Certain microorganisms, such as *Bacillus thuringiensis* (Bt), produce specific toxins (e.g., Cry proteins). Upon ingestion by susceptible insects, these toxins disrupt the integrity of the insect gut lining, ultimately leading to paralysis and mortality(GF Neumann and G Jetschke ; 2010).

4.2. Growth and Development Disruption

- Insect Growth Regulators (IGRs): These compounds function by mimicking or interfering with the natural hormonal processes that govern insect development, thereby disrupting molting, metamorphosis, and reproduction. Examples include:
  - Juvenile hormone analogs: Prevent the maturation of immature insects into reproductive adults.
  - Chitin synthesis inhibitors: Impede the formation of chitin, a crucial component of the insect exoskeleton.

- Ecdysone receptor agonists: Induce premature and lethal molting events.
- Azadirachtin: A compound derived from neem oil, azadirachtin acts as an IGR, disrupting the molting process and affecting insect feeding and reproductive behaviors(P Bansal ; 2019).

4.3. Neurotoxic Effects

A subset of biopesticides targets the insect nervous system, leading to various neurological dysfunctions.

- Natural pyrethrins and pyrethroids: These compounds interfere with sodium channels in insect nerve cells, causing hyperexcitation, paralysis, and subsequent death.
- Essential oils: Certain components of essential oils can interact with neurotransmitter receptors (e.g., GABA, octopamine) or inhibit key enzymes such as acetylcholinesterase, resulting in neurotoxic effects.
- Plant alkaloids (e.g., nicotine): These compounds bind to acetylcholine receptors in insect nerve cells, causing overstimulation and paralysis(N Senanayake and L Karalliedde ; 1987).

4.4. Pathogenicity

Entomopathogenic microorganisms, including fungi, viruses, bacteria, and nematodes, infect and ultimately kill their target insect hosts.

- Fungi: These organisms penetrate the insect cuticle, proliferate within the insect's body, and release toxins that contribute to mortality.
- Viruses: Insect viruses replicate within host cells, causing tissue damage and death.
- Bacteria: Certain bacteria can induce septicemia or produce toxins upon ingestion by the insect.
- Nematodes: Entomopathogenic nematodes enter the insect hemocoel and release symbiotic bacteria that cause lethal septicemia(B Deka and C Baruah ; 2021).

4.5. Repellency and Antifeedancy

Some biopesticides deter insects from feeding on treated plants or actively repel them from the vicinity of the plant.

○ Antifeedants: These compounds render plants unpalatable, thereby reducing insect feeding and ultimately impacting their survival (e.g., azadirachtin).

○ Repellents: Certain biopesticides emit odors or compounds that are aversive to insects, causing them to avoid treated plants (e.g., some essential oils)(JJ Zhu, GJ Brewer et al ; 2015).

4.6. Communication Disruption

Pheromones are employed to disrupt insect mating behavior. The release of synthetic pheromones can confuse male insects, preventing them from locating and mating with females, thus reducing reproductive success(P Witzgall and P Kirsch ; 2010).

4.7. Desiccation

Biopesticides such as diatomaceous earth exert their effect through physical means, causing damage to the insect cuticle, leading to water loss and subsequent dehydration(Z Korunić ; 2013).

5. Factors influencing the efficacy of botanical bioinsecticides

The effectiveness of biopesticides is a complex outcome of interacting environmental, biological, and operational factors. These factors can either enhance or diminish the performance of these pest management tools(MK Dhillon and C Srivastava ; 2011).

5.1. Factors positively influencing biopesticide effectiveness

5.1.1. Optimal Environmental Conditions

○ Suitable Temperatures: Matching biopesticide activity ranges with prevailing temperatures and timing application accordingly.

○ Adequate Humidity: Providing necessary moisture for microbial biopesticide activity, especially for fungi.

○ Protection from UV Degradation: Applying during low UV periods or using UV protectants in formulations(KL Bailey, SM Boyetchko et al ; 2010).

5.1.2. Favorable Biological Factors

○ Targeting Susceptible Pest Stages: Applying when pests are most vulnerable (e.g., young larvae).

- Appropriate Pest Population Density: Utilizing biopesticides early in pest infestations.
- Healthy Host Plants: Vigorous plants may support biopesticide action better.
- Absence of Pest Resistance: Using biopesticides against susceptible pest populations.
- Induction of Host Plant Resistance (ISR): Selecting biopesticides that trigger plant defenses(KL Bailey, SM Boyetchko et al ; 2010).

5.1.3. Optimized Biopesticide Formulation and Application

- Effective Formulation: Employing formulations that stabilize the active ingredient and ensure efficient delivery.
- Proper Application Timing: Applying when pests are active and most susceptible.
- Good Coverage: Thoroughly covering plant parts where pests reside.
- Correct Dosage and Concentration: Adhering to recommended application rates.
- Appropriate Application Frequency: Reapplying as needed based on persistence and pest pressure(KL Bailey, SM Boyetchko et al ; 2010).

5.1.4. Proper Storage and Handling

- Maintaining Viability of Microorganisms: Storing microbial biopesticides under recommended conditions.
- Using Before Expiration Date: Ensuring product potency by using within the shelf life(CLS Mike Johnson CMRP ; 2008) .

5.1.5. Integration with Other Strategies

- Integrated Pest Management (IPM): Combining biopesticides with biological control and good agricultural practices.
- Use of Adjuvants: Enhancing spread and adhesion to plant surfaces. (Dureja and Johnson ; 2000).

5.2. Factors negatively influencing biopesticide effectiveness

5.2.1. Unfavorable Environmental Conditions

- Extreme Temperatures: Reducing viability and activity of microbial biopesticides.
- Insufficient Humidity: Hindering germination and growth of entomopathogenic fungi.
- Intense UV Radiation: Degrading active ingredients, especially in microbial agents.
- Excessive Rainfall or Irrigation: Washing off foliar-applied biopesticides.
- Unsuitable Soil Conditions: Affecting survival and activity of soil-applied biopesticides.

○ Strong Winds: Causing spray drift and uneven coverage(G Abbaszadeh and MK Dhillon ; 2011).

5.2.2. Adverse Biological Factors

○ Mismatch in Pest Life Stage: Applying to non-susceptible life stages.

○ High Pest Population Density: Overwhelming the biopesticide's capacity for rapid control.

○ Pest Resistance: Reduced efficacy against resistant pest populations.

○ Unhealthy or Weak Host Plants: Poor support for biopesticide action.

○ Competition with Native Microorganisms: Limiting establishment and proliferation of microbial biopesticides(G Abbaszadeh and MK Dhillon ; 2011).

5.2.3. Poor Biopesticide Formulation and Application

○ Ineffective Formulation: Inadequate protection or delivery of the active ingredient.

○ Incorrect Application Timing: Applying too late in the infestation cycle.

○ Inadequate Coverage: Insufficient contact with the target pest.

○ Incorrect Dosage or Concentration: Using suboptimal application rates.

○ Insufficient Application Frequency: Allowing pest populations to rebound between applications(G Abbaszadeh and MK Dhillon ; 2011).

5.2.4. Improper Storage and Handling

○ Storage Under Unsuitable Conditions: Degrading the active ingredient and reducing efficacy.

○ Use of Expired Products: Reduced potency and effectiveness(G Abbaszadeh and MK Dhillon ; 2011)

5.2.5. Negative Interactions with Other Agrochemicals

○ Incompatibility: Antagonistic effects when combined with certain conventional pesticides or fertilizers(G Abbaszadeh and MK Dhillon ; 2011).

5.2.6. Degradation/Conversion of Phytochemicals

○ Breakdown of active ingredients in extracts, oils, or formulated products during storage due to temperature, sunlight, and microorganisms, leading to reduced bioefficacy (Gahukar ; 1998, Gahukar ; 2012). For instance, salannin and azadirachtin content can significantly decrease during storage.

6. Recent Advances and Innovations in Botanical Bioinsecticides

The escalating concerns regarding the detrimental environmental and human health impacts of synthetic pesticides have spurred a significant increase in the demand for plant-based bioinsecticides. Botanicals and essential oils (EOs) present themselves as promising, safer, and more sustainable alternatives for pest management. However, the practical application of these natural insecticides is often limited by their inherent instability under environmental stressors, high volatility, and susceptibility to thermal degradation, necessitating frequent applications and reducing their overall utility(Sarmah, K., Anbalagan et al; 2025). .

To overcome these limitations, innovative formulation strategies, including nanoemulsions, microemulsions, nanoencapsulation, and microencapsulation, have been developed. These advanced delivery systems facilitate controlled release, enhance stability, and significantly improve the efficacy of botanical- and EO-based insecticides. By enabling target-specific action, these formulations contribute to reduced application frequencies, lower dosage requirements, minimized environmental contamination, and enhanced overall pest management efficiency(Sarmah, K., Anbalagan et al ; 2025).

Experimental part II: Materials & methods

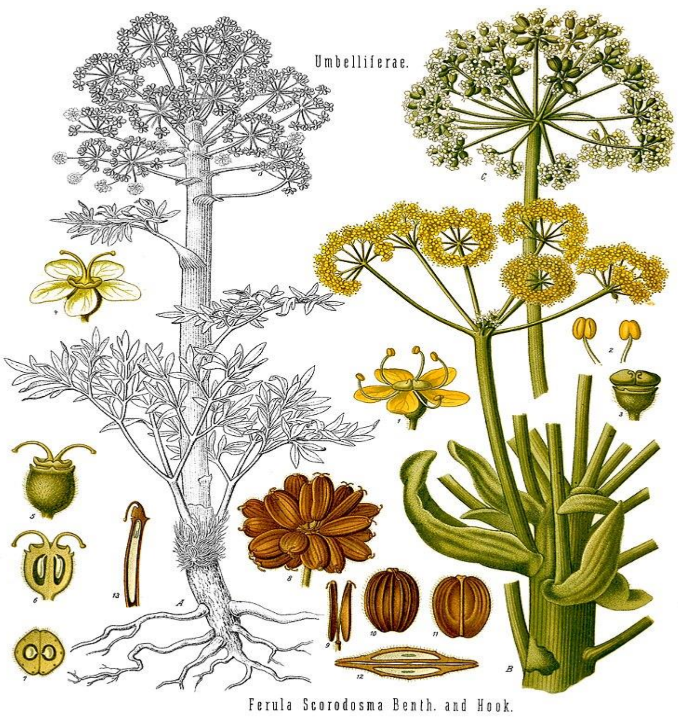
1. Material

1.1. Plant Material

1.1.1. Asafoetida (*Ferula assa-foetida*)

Ferula assa-foetida is a medicinal plant from the Apiaceae family. It is a perennial herbaceous plant with an unpleasant odor and is often considered the main source of oleo-gum resin (OGR, a milky exudate from certain plants that coagulates in the air) which has a characteristic sulfurous smell and a bitter taste (Golmohammadi et al ; 2016).

It is a plant that reaches a height of 12 feet in the wild, with a circular mass of leaves measuring 30 to 40 cm. The stem leaves have broad sheathing petioles. The flowering stems reach a height of 2.5–3 meters and a thickness of 10 cm and are hollow. These flowering stems have several schizogenous canals in the cortex containing resinous gums. The flowers are small, dull, and yellow in color, produced in large compound umbels. The fruits of this plant are oval, thin, flat, reddish-brown in color, and contain a milky substance. The roots are thick, massive, and fleshy. The nauseating odor comes from the resinous gum extracted from the stems and roots (Kareparamban et al ; 2012).



Figure(03): morphological description of *Ferula assa-foetida*(F Golmohammadi ; 2016).

- **Classification**

According to POWO (2025), the classification of the specie *Ferula assa-foetida* is as follows:

Table(01): classification of the specie *Ferula assa-foetida*

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Apiales
Family	*Apiaceae*
Genus	*Ferula*
Species	*Ferula assa-foetida*

1.1.2. Neem (*Azadirachta indica*)

The neem tree is a tropical tree found in the dry forests of India, Pakistan, SriLanka, and Burma. It grows in parts of Southeast Asia and West Africa, and is also found in the southern regions of the United States. It is a tree that is resistant to environmental and climatic conditions. Research has focused on it for its health benefits.

Neem is a tree in the mahogany family *Meliaceae*. It is one of two species in the genus *Azadirachta*, growing in tropical and semi-tropical regions. It is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. For thousands of years the beneficial properties of Neem (*Azadirachta indica A. Juss*) have been recognized in the Indian tradition. Each part of the neem tree has some medicinal property

- **Morphological description**

The neem tree (*Azadirachta indica*) stands as a remarkable fast-growing tropical evergreen, capable of reaching heights of up to twenty feet within a mere three years, and shares botanical ties with mahogany. Its resilience is notable, thriving even in regions with minimal rainfall, as low as 18 inches annually, and flourishing under intense heat reaching up to 120°F. These trees are reported to have a significant lifespan, potentially living for as long as 200 years. The leaves of the neem are compound and alternately arranged along a rachis measuring 15-25 cm in length and 0.1 cm in thickness. The leaflets exhibit an oblique base, are positioned oppositely, lack stipules, and possess a lanceolate, acute, and serrate shape, ranging from 7-8.5 cm in length and 1.0-1.7 cm in width, displaying a slightly yellowish-green hue. Their odor is indistinct, while their taste is characteristically bitter. The stem bark shows considerable variation in thickness depending on the tree's age and the specific part from which it is taken. The external surface is rough, fissured, and rusty-grey, while the laminated inner surface is yellowish and foliaceous with a fibrous fracture. It possesses a characteristic odor and a bitter taste. In early summer, the neem tree is often adorned with delicate, fragrant white flowers, arranged in axillary, typically drooping panicles that can extend up to 25 cm in length. It produces a semi-sweet, olive-sized fruit containing a seed rich in oil, renowned for its tremendous medicinal and botanical properties.

(Muñoz-Valenzuela et al ; 2007).

Figure (04): *Azadirachta indica* tree (A Balasubramanian, M Umadevi et al ; 2019)

- **Classification**

According to POWO (2025), the classification of the specie *Azadirachta indica* is as follows:

Table(02): classification of the specie *Azadirachta indica***.**

kingdom	plantae
phylum	streptophyta
class	equisetophyta
subclass	magnoliidae
order	sapindales
family	meliaceae
genus	azadirachta
species	*Azadirachta indica*

1.1.3. Harmal (*Peganum harmala*)

A perennial, woody-based, glabrous plant, 30-50 cm tall. Stems are much branched, leaves alternate, 5-10 cm long, acute-apex, 2-4 cm long. Flowers are hermaphrodite, large, long-stalked. Sepals are perennial, 5-lobed, 1.5-2 cm long, linear, sometimes trilobed, with scales between the sepals (forming a calyx). Petals are pentagonal, about 1.5 cm long and 0.5-0.7 cm wide, white or yellowish, oblong. The fruit is a capsule (0.8-1 cm), globose, compressed at the apex, many-seeded, glabrous, opening by three valves, the style is perennial. The seed is about 2 mm long, triangular, oblong-brown, tuberous. Flowering from March to May.

Figure (05): *Peganum harmala* tree (AV Ereskovsky, DV Lavrov et al ; 2011**)** .

- **Classification**

According to POWO (2025), the classification of the specie *P.harmala* is as follows:

Table(03): classification of the specie *P.harmala***.**

kingdom	**Plantae**
phylum	streptophyta
class	equisetophyta
subclass	magnoliidae
order	sapindales
family	nitrariaceae
genus	peganum
species	*Peganum harmala*

1.2. Target insects

1.2.1. Flour beetle (*Tribolium castaneum*)

Flour beetles are a group of small insects belonging to the darkling beetle family (*Tenebrionidae*). These beetles are common pests that infest and feed on flour and stored grains, as well as a variety of other dry foodstuffs such as legumes, nuts, dried fruits, and spices.

Flour beetles are small (usually 3-5 mm long) and reddish-brown or dark brown in color. Their bodies are flat and oval, and they have six legs and antennae. Some species, such as the rusty flour beetle, can fly short distances.

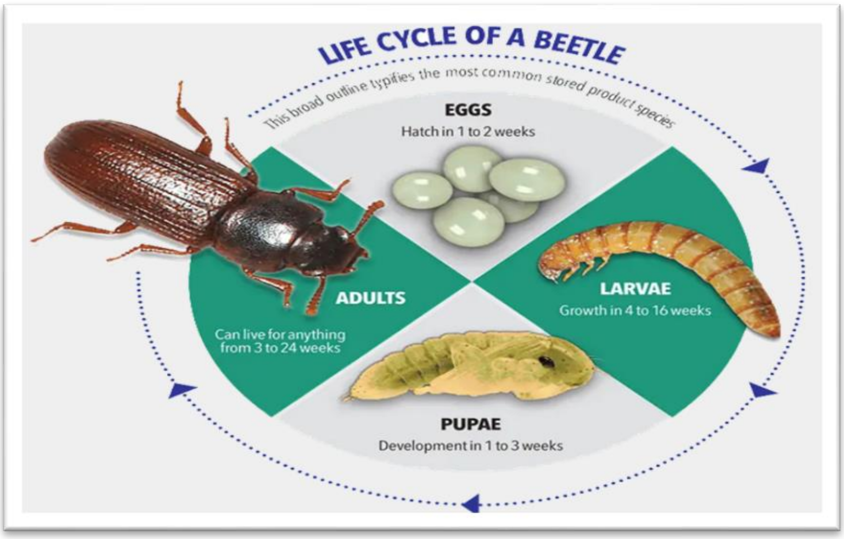
- **Life cycle**

All stages of this insect are found in warm buildings throughout the year. They live in grain stores, grocery stores, and mills. The female lays approximately 1,000 eggs between grains of flour, on containers, in cracks in the walls and floors of warehouses, or among grains.

The eggs are usually covered with a sticky substance that easily adheres to flour and other grains. The eggs hatch after approximately 5–12 days, and the larvae emerge, feeding on grain products, bran, and other materials.

Larva: Cylindrical in shape, yellowish-white in color, with two large, thick, brown spines on the abdomen. When fully grown, the larvae are approximately 6 mm in length. The larval stage lasts approximately 1–4 months (depending on temperature and food type.

Pupae: (naked, without a cocoon) white in color, turning yellow over time and eventually brown. The pupa stage lasts approximately 7–15 days. Each generation takes about 1.5 months in summer and 5 months in winter, and the adult insect may live for about two years in heated buildings. This insect has about 4-5 generations per year in heated buildings (Gao, F et al; 2024).



Figure(06): Life cycle of *Tribolium castaneum* (D Sauvard, M Branco et al ; 2010)

- **Classification**

the classification of the specie *Tribolium castaneum* is as follows:

Table(04): classification of the specie *Tribolium castaneum*

Kingdom	**animalia**
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Suborder	Polyphaga
infraorder	Cucujiformia
superfamily	Tenebrionoidea
Family	Tenebrionidae
sudfamily	Tenebrioninae
Tribe	Triboliini
genus	Tribolium
species	*Tribolium castaneum*

- **Damage caused by *Tribolium castaneum***

Flour beetles cause significant damage to stored food in several ways:

- **Feeding and spoilage:** Beetles and their larvae feed on flour, broken grains, and other food products, causing them to spoil and lose their nutritional value.
- **Contamination:** Beetles contaminate food with their remains, molted skins, and excrement, rendering it unfits for human consumption.
- **Odor**: Beetles secrete chemicals that give infected food an unpleasant, undesirable odor.
  - **Encourages mold growth:** The presence of beetles can increase moisture in food, encouraging the growth of mold and fungi(C Srivastava and S Subramanian ; 2016)**.**

2. Methodology

2.1. Extraction of essential oil

Hydro distillation is a simple yet effective method for extracting essential oils from plant materials. In this process, the plant matter is submerged in water and then heated to boiling. The steam generated carries the volatile essential oil molecules, which are then condensed back into liquid form. This resulting liquid is a mixture of water and essential oil, which are subsequently separated, yielding the pure essential oil. Hydro-distillation is particularly useful for heat-sensitive compounds as the surrounding water helps to prevent overheating and degradation.

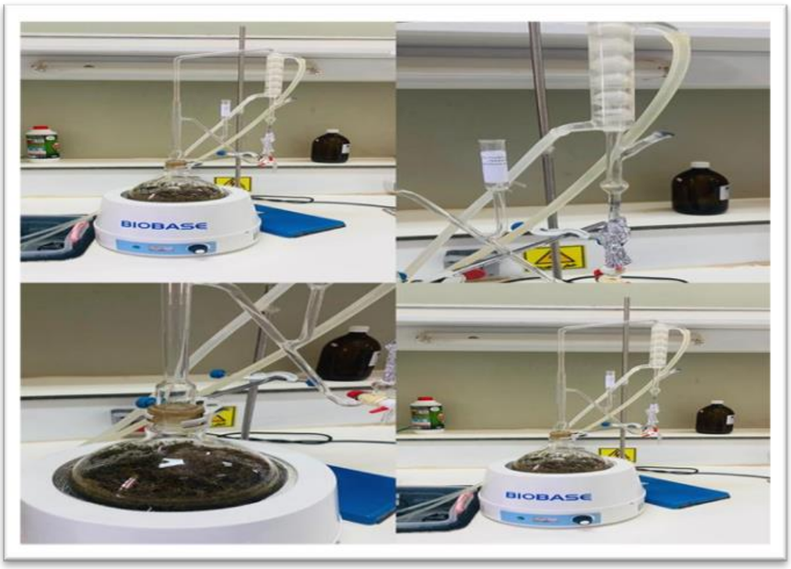


Figure (07): Hydro-distillation Clevenger apparatus system(Personal photo**)**

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In this study, essential oils were extracted from three plants species: firstly, *Azadirachta indica* (Neem) seeds, recolted from the Oum El Tyour region of El M'Ghair, Algeria. Secondly, *Ferula assa-foetida* (Asafoetida) gum resin, obtained from a plant belonging to the Apiaceae family, collected in El Fidh, Biskra , Algeria . Thirdly, *Peganum harmala* seeds, recolted from the El Bayadh Algeria.

Figure(08): Pictures of plants used to extract essential oils(Personal photo**)**

2.2. Hydro alcoholic extract

Extracts were obtained by maceration of plant material using a mixture of water and alcohol as the solvent. Powder of Neem and Harmal seeds was mixed with 1/2 methanol and distilled water. The suspension was homogenized and left covered at room temperature for 24 hours, then filtered using No. 1 filter paper. The filtrate was then concentrated under reduced pressure to remove the methanol using a Rotavap at 45°C.

Figure(09): Hydro-alcoholic extract préparation(Personal photo).

2.3. Bioassays

2.3.1. Contact toxicity

In Petri dishes, filter paper No. 1 was placed. 10 units of adult insect (*Tribolium castaneum*) were placed in each dish. Four concentrations of essential oils of seeds of *A. indica*, *P. harmala* and gum of *F. assa-foetida* (0.5 µl/ml, 1.5 µl/ml, 3 µl/ml and 6 µl/ml) diluted with acetone 5%; with control treatment using distilled water and acetone 5%.

Toxicity effects of alcoholic extracts of seeds of *A. indica* and *P. harmala* on adult insect (*Tribolium castaneum*), were evaluated on four concentrations(0.5 µl/ml, 1 µl/ml, 2.5 µl/ml and 5 µl/ml) diluted with acetone 5%. Using distilled water and acetone 5% as control.

Contact toxicity bioassays were conducted by spraying.

2.3.2. Fumigant toxicity

In glass bottles, cotton was Hangs and 10 units of insects (*Tribolium castaneum*) were placed in each bottle. Four Concentrations of oil extracts (0.5 µl/ml, 1.5 µl/ml, 3 µl/ml and 6 µl/ml) diluted with acetone 5%. And four concentrations of alcoholic extract(0.5 µl/ml, 1 µl/ml, 2.5 µl/ml and 5 µl/ml) diluted with acetone 5%. the treatment was conducted by Fumigant.

Synergic activity of *Azadirachta indica* and *Peganum harmala* seeds oils (1/1) was evaluated by the same doses and methods of oils and alcoholic extract by contact and fumigant toxicity.

Figure(10): toxicity bioassays by contact and fumigant(Personal photo).

2.3.3. Studied parameters

Following the treatment of *Tribolium castaneum* with the extract, insect mortality number were checked at 1 hour and 24 hours post-treatment. This was achieved through direct visual observation of their behavioral and movement activity, coupled with the direct application of light onto the insects.

2.4. Phytochemical screening

After obtaining the extract, we performed a chemical screening test using reagents to identify the chemical compounds of both extracts oils and alcoholics (*Peganum harmala*.

Azadirachta indica and *Ferula assa-foetida*) following the following protocol(JR Shaikh and M Patil ; 2020)

2.4.1. Saponins

Prepare 2 ml of the extract + with 1 ml of distilled water, then shake vigorously, leave it for 20 minutes, and note the result (+) or (-). The result is positive by the formation of foam.

2.4.2. Tannins

Prepare 1 ml of the extract + with 1 ml of distilled water + and add drops of FeCl3 (2 or 3 drops) and then observe the result. It is positive when a blue or black colour appears.

1.1.1. Flavonoids

Prepare 5 ml of the extract + add drops of concentrated HCl with milligrams of magnesium sulphate (mg). The result is positive with the formation of a gas and the appearance of a pink to crimson colour.

1.1.2. Terpenoids

Prepare 5 ml of the extract + add 3 ml of sulfuric acid (H_2SO_4) + 2 ml of chloroform ($CHCl_3$). Then wait a little while for the result to appear. The result is positive when a reddish-brown color appears in the chloroform. The temperature increases, and the extract becomes thicker.

1.1.3. Lipid

Prepare 1 ml of the extract + add a few drops of NaOH. The result is positive when color changes occur immediately or within a few minutes.

1.1.4. Alkaloids

Prepare 5 ml of HCl + with 1 ml of the extract, then heat in a water bath. Then, divide the extract into two equal parts:

Add 3 drops of Wagner The result is positive A brown/reddish precipitate.

Add 3 drops of Mayer The result is positive A creamy white/yellow precipitate.

1.1.5. Anthraquines

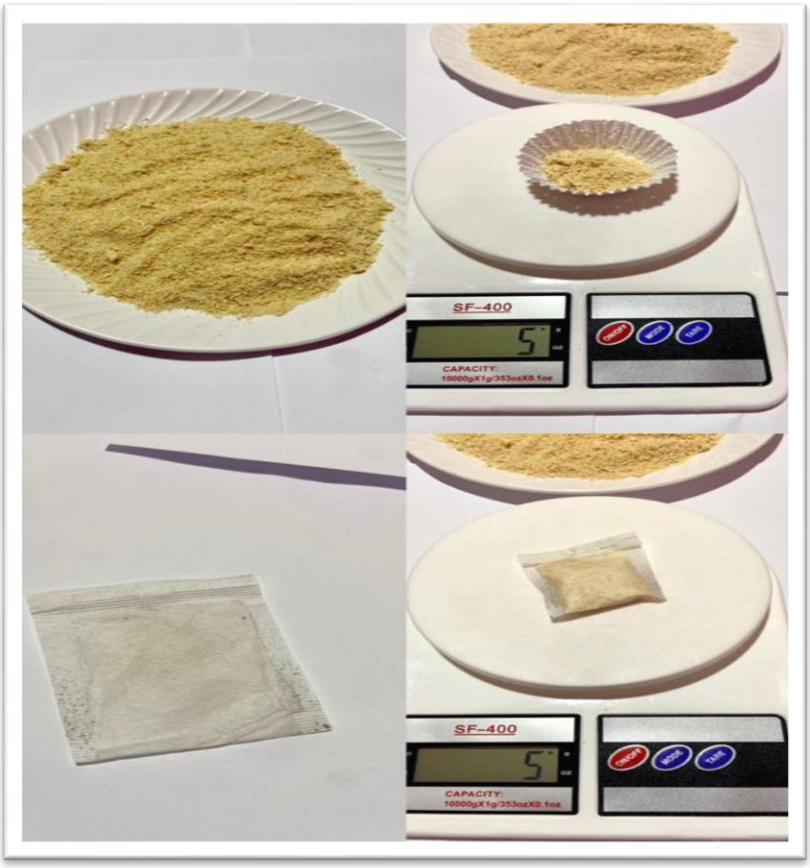
Prepare 10 ml of the extract and add 5 ml of NaOH. Wait a little while and observe the result. The result is positive when a white to reddish-yellow color appears.

1.1.6. Sugar reducer

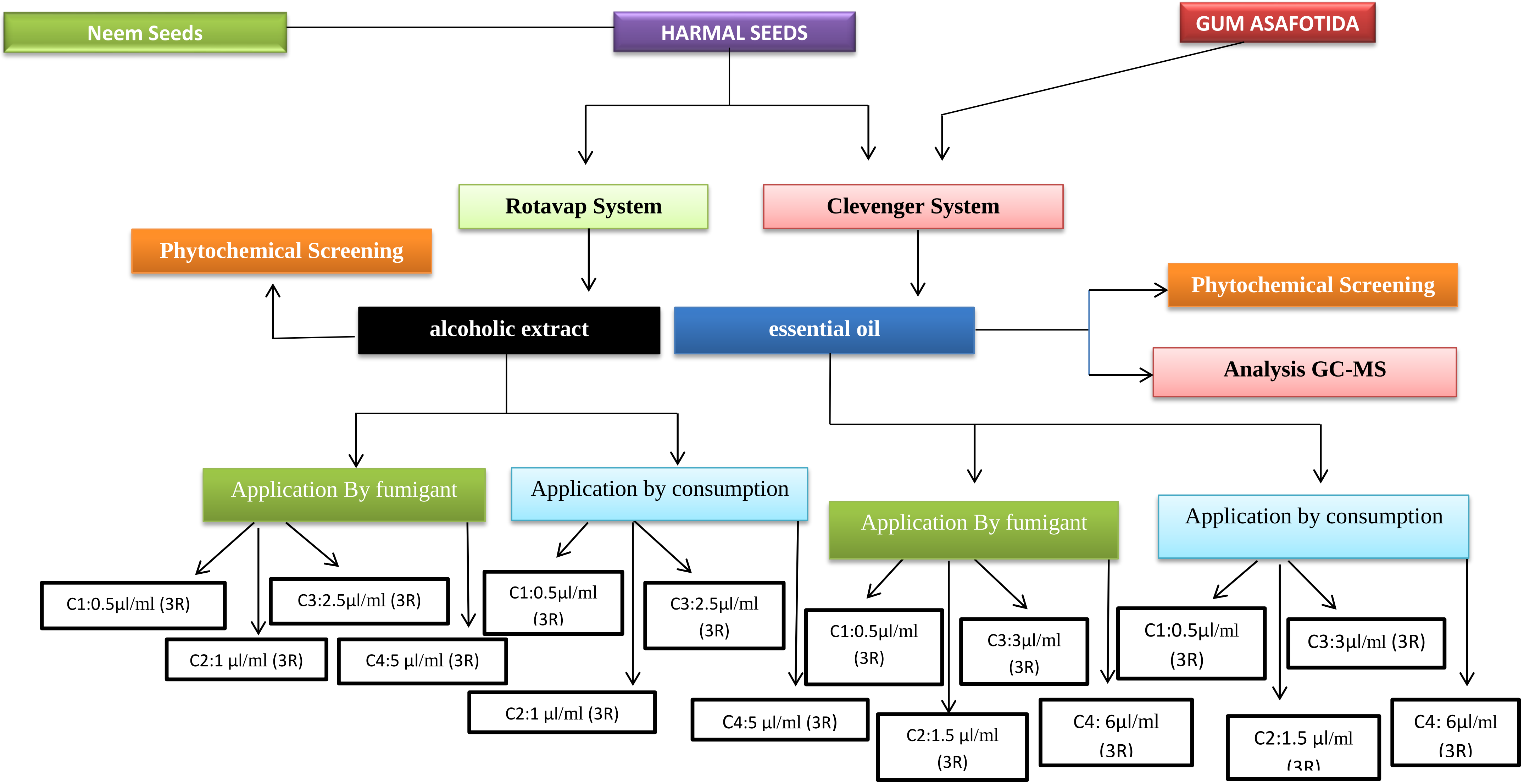
Prepare 5 ml of the extract + add 1 ml of Fehling's reagent. Wait a little while and observe the result. The result is positive. With the appearance of the blue color(JR Shaikh and M Patil ; 2020).

2.5. Insecticide product preparation

- **Preparing a mix of essential oils from three different plants:** essential oils of(*Peganum harmala*. *Azadirachta indica* and *Ferula assa-foetida*) were mixed.
- **Preparation of oil extract-starch capitation:** oil mixteur was mixed with starch as a supporting
- **Packaging:** the resulting mixtur (5g starch with ml From a mixture of oil) was placed into small and odor-permeable sachets to exert its effect on *T. castaneum.* From the exterior the small sachets were then enclosed in aluminum foil to avoid the evaporation of oils.



Figure(11): Steps for preparing pesticide products(Personal photo).



Figure(12): Metodology Chart

Part III: discussion

Discussion

1.The effect of essential oils from different plants on the *Tribolium castaneum*

Aromatic plants can offer alternative solutions to currently used insect control methods because they contain a rich source of *bioactive molecules* (Table 05). Our experiment revealed that the *essential oils* of *Neem* (*Azadirachta indica*), *Harmal* (*Peganum harmala*), and *Asafoetida* (*Ferula assa-foetida*) are rich in Saponins, flavonoids, triterpenoids, alkaloids, Lipid, anthraquinones, and sugars. This confirms the effectiveness of these oils in controlling the stored-product pest, the red flour beetle (*Tribolium castaneum*).

It has been established that many plant-derived products have *insecticidal* effects on stored-product pests. In our current research, we tested the efficacy of these oils using two methods: direct contact and fumigation and increasing doses (0.5 µl/ml, 1.5 µl/ml, 3 µl/ml and 6 µl/ml), against the red flour beetle(*Tribolium castaneum*).. The results demonstrated a 100% mortality rate within the first 24 hours of treatment with the oils. This includes the essential oils of Neem, Harmal, Asafoetida, and their mixture. Furthermore, statistical analysis showed that the mortality rate depends on the concentration (dose) used and the exposure time.

Our findings are supported by results obtained by other researchers who have demonstrated the effectiveness of *insecticides* derived from the essential oils of plants against stored-product insects. For example:

1. (A. Hameed, S. Freed, et al., 2012) demonstrated the effectiveness of the essential oil of Neem (*Azadirachta indica*) seeds in controlling the red flour beetle. The *bioactivity* of Neem essential oil was evaluated against *Tribolium castaneum* on stored wheat grains. Five concentrations (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) were used at different exposure times (24, 48, 72, and 168 hours). The *filter paper impregnation method* was employed. The results showed that Neem achieved a 45% mortality rate at 168 hours of exposure at a concentration of 2.5%, and 16.67% at a dose of 0.5% within 24 hours. *LC50 values* were calculated using *Probit analysis* for Neem after 24, 48, 72, and 168 hours. *LT50 values* for this time period of the *bioinsecticides* at a concentration of 2.5% were also calculated. The results indicated that bioinsecticides are a better approach for controlling red flour beetle infestation in stored wheat grains.

2. (R. C. Saxena, B. P. Rueda et al 1988) In a food preference chamber, fewer adults of red flour beetle (*Tribolium castaneum* Herbst) settled in rice grain treated with 100, 500, or 1,000 ppm of the oil of turmeric (*Curcuma longa* (L.)), sweetflag (*Acorus calamus* (L.)), or neem (*Azadirachta indica* A. Juss), or "Margosan-O" (a commercial neem-based insecticide). *Repellency* increased with increasing concentrations of the oils and Margosan. In another choice test, filter paper strips treated with turmeric oil or sweetflag oil at 200, 400, or 800 µg/cm² repelled insects during the first 2 weeks; thereafter, repellency decreased more rapidly than with neem oil or Margosan-O. *Tribolium castaneum* adults fed wheat flour, which had been treated with the test materials at 200 ppm, produced fewer and underweight *larvae*, *pupae*, and adults compared with the control.

3. (S Ali, M Sagheer et al ; 2015)Study was aimed on use of indigenous medicinal plants in Entomological Research Institute, AARI, Faisalabad, Pakistan during 2010 to check the invasion of red flour beetle, Tribolium castaneum (Herbst) to estimate their effects on larval, pupal and adult emergence against wheat grains. Acetone leaf extracts of Peganum harmala and Syzygium aromactium were used to evaluate their antifedant, toxicant, and growth regulatory effects against adults of Tribolium castaneum. Four concentrations (5, 10, 15 and 20 %) of each extract along-with a control treatment with five replications were applied. Thirty adults of *Tribolium castaneum* were subjected to different concentrations of botanical extracts for bioassay. Results showed that percentage of mortality was directly proportional to increasing concentration of extracts. Both Peganum harmala and Syzygium aromactium significantly reduced the larval, pupal and adult emergence as well as percentage of weight loss (at α 5 %). Peganum harmala performed better as compared to the Syzygium aromactium. Hence it was concluded that both the botanicals extracts can be used as effective tool against T. castaneum along-with other IPM practices.

4. (M Fazeli-Dinan, SH Osia-Laghab et al ;2023) S. oryzae is a serious pest of stored rice and is usually controlled using aluminum phosphide, which is hazardous to humans. Botanical insecticides are promising alternatives for controlling this pest. In this study, seed extracts of Peganum harmala were evaluated against adult S. oryzae. Phytochemical analyses revealed that the extract contained steroids, terpenoids, tannins, alkaloids, and flavonoids. Bioassays revealed repellent effects for all extracts, and S. harmala extract showed a significant mortality rate (93.33±6.6%, LC50: 521.06 mg/ml) and may therefore have potential applications in preventing S. oryzae infestation of stored rice.

The plant powders and extracts tested in this study proved effective in repelling and inducing vapor toxicity in fifth-instar larvae of T. granarium. Based on previous research on the effectiveness of some plant products in safely controlling human food pests in stores and fields without leaving any residues harmful to humans, animals, plants, and environmental health, this activity is attributed to the bioactive content of the tested plants and their toxic effects on insects (Ansari et al., 2000; Choubey, 2007; Sultana et al., 2016; Schuler, 2017). The repellent efficacy was evident from the beginning of the experiment, especially for F. assa-foetida, and persisted over time. Regarding F. assa-foetida, Ravi (2002) evaluated the high repellent and toxic activity of F. assa-foetida resin extract against Tribolium castaneum and Ephestia kuebneilla. Peruvi et al; (2011) also reported strong repellent and toxic activity of F. assa-foetida essential oil against adult carob moth. Balerao; (2014) reported toxic efficacy of aloe vera gum extract when mixed with 4 g/4 g Tribolium castaneum food after 72 hours of treatment. Plant extracts exhibit rapid activity within the first hours of exposure for all tested plants. According to Lui et al. (2007) (N Al-Fuhaid ;2017).

2.The effect of alcoholic extracts of different plants on the *Tribolium castaneum*

Insecticide formulation is a physical process consisting of mixing a chemical compound as a solvent, which has a biological effect, using inert products in an effective and more toxic manner against stored product pests.

In this work, we produced a bio-insecticide formulation using methanol as a solvent with seed powder from two plants *Azadirachta indica* and *Peganum harmala*. We tested the effectiveness of the bio-insecticide formulation and proved that the powder of both *Azadirachta indica* and *Peganum harmala* seeds is toxic and effective against the *Tribolium castaneum*. This was achieved through Phytochemical screening analyses of the alcoholic extract plants of *Azadirachta indica* and *Peganum harmala* ,(table06) where the results indicated the presence of flavonoids, terpenoids, sugars, and lipid. In addition, we conducted an experiment to test the toxicity of the alcoholic extracts on adult *Tribolium castaneum* through two methods: contact and fumigation.

The experimental results showed that the extract formulation exhibits insecticidal activity against *Tribolium castaneum* insects, and this depends on the concentration, dose, and exposure time. We used four increasing concentrations: (0.5 µl/ml, 1 µl/ml, 2.5 µl/ml, and 5 µl/ml). The product is highly effective against the red flour beetle, allowing for 100 percent elimination within the first 24 hours after the start of treatment, and the results apply to both seed *Azadirachta indica* extract, *Peganum harmala* seeds extract, and the mixture together.

The effectiveness of this bio-insecticide formulation was confirmed by referencing our experimental results to researchers who have proven the effectiveness of an alcoholic extract in eliminating the *Tribolium castaneum*:

1. (F Ahmad d, A Javaid ; 2023). Increasing grain production is of little significance unless it is protected from post-harvest losses. One insect pest that causes significant losses in stored grain is Tribolium castaneum. Insecticides have been used for decades to control insect pests, but they pose significant environmental risks. Here, we test plant extracts as alternatives for grain protection and environmental conservation. Methanol extracts of Peganum harmala at five different concentrations were used to test their insecticidal activity against Tribolium castaneum. The extract showed significant effects on pest mortality, with a concentration of 40 × 103 ppm of each of the two methanol extracts resulting in larval mortality rates of over 80%. These results were time-dependent, with LD50 values decreasing with increasing exposure duration, and the lowest concentrations (6.9 and 6.1 × 103 ppm) of the two methanol extracts of P. harmala were obtained on the 15th day after treatment. The analyzed results showed that both P. harmala extracts exhibited good insecticidal activity on T. castaneum and could be used in the development of new drugs in the search for novel insecticidal compounds

2. (R Jbilou and F Sayah ; 2007)The effects of a methanol extract of *Peganum harmala L.* seeds on *Tribolium castaneum* (Herbst) were evaluated. Larval growth was inhibited and food intake reduced when the extract was incorporated into the diet at concentrations of 2.5, 5, and 10%. All doses prolonged the larval period and completely prevented F1 offspring production. Major body metabolites were determined in last-instar larvae after feeding for 8 days on the treated diet at different doses. Protein and lipid contents decreased at concentrations >2.5%. Glycogen content decreased at a concentration of 10%. Tribolium castaneum larvae possess two major and one minor isoform of α-amylase, as determined by gel assays. Larvae fed the treated diet showed reduced activity of the major isoforms, while the activity of the minor isoform was completely inhibited. The activity of this enzyme was not inhibited in vitro by seed extracts. Histological studies have shown that harmala extracts cause severe toxicity to midgut epithelial cells, resulting in the formation of large cytoplasmic vacuoles and extensive intercellular space. These results indicate that harmala seed extracts exert their toxic effect by severely damaging midgut epithelial cells. This natural plant extract may be useful in combating *T. castaneum*.

3. (A Sharma ; 2011)The present study deals with the chemistry and evaluation of neem extract against Tribolium castaneum (Herbst). Different extracts were obtained by Soxhlet

extraction from dried and powdered Azadirachta indica seed pods using methanol and chloroform as solvents, separately. The hexane extract was subjected to fractionation using methanol. Different extracts were subjected to thin layer chromatography to determine the number of compounds present in the different extracts. Adult rearing of Tribolium castaneum (Herbst) was carried out. Different neem seed pod extracts were tested for their bioactivity against Tribolium castaneum (Herbst) adults by releasing them into wheat grains supplemented with different concentrations of the experimental extracts: 20,000, 15,000, 10,000, 5,000, and 1000 µg/g, respectively. Corrected mortality percentage was calculated using Abbott's formula. The methanol-soluble hexane fraction was found to be more active than the original hexane fraction, while the methanol-insoluble hexane fraction showed negligible biological activity. . . Rearing of Tribolium castaneum was carried out to raise the first generation of Tribolium castaneum. Different neem seed extracts were subjected to bioactivity studies against Tribolium castaneum (Herbst) adults. Wheat grains were inoculated with different concentrations of the test extracts, namely 20,000, 15,000, 10,000, 5,000, and 1,000 µg/g of wheat. There were three replicates for each treatment, and for the control treatment, only wheat and acetone were used. The bottles were placed in an electric shaker for 5 minutes to enable complete mixing of the extract with the wheat grains. Ten adults of the same age were released into each bottle, and the bottle mouth was covered with gauze. Observation of T. castaneum mortality was recorded every 24 hours until complete or consistent mortality was obtained. Larval emergence and growth were also observed for 45 days. The corrected percentage of mortality was calculated using the Abbott formula. The results indicate that mortality increases with increasing concentration of the extract used and with increasing duration of application, then stabilize

4. (M Khalequzzaman ; 2006) Cypermethrin was applied either alone or in combination with neem (Azadirachta indica A. Juss) leaf and seed extracts to four strains of adult red flour beetle Tribolium castaneum (Herbst), and mortality was assessed 24 and 48 hours after treatment. Median lethal dose (LD50) values were calculated as follows: 0.0072, 0.0105, 0.0056, and 0.0124 µg/cm² at 24 hours, and 0.0041, 0.0076, 0.0046, and 0.0097 µg/cm² at 48 hours after treatment for Local, CR-1, FSS-II, and CTC-12 strains, respectively. The results showed synergism when applied at combined doses of cypermethrin and leaf and seed extracts with petroleum alcohol, ethyl acetate, and methanol. The leaf extract in acetone showed little effect, showing inactivity with cypermethrin, except for 24 hours after treatment of the local strain and 48 hours after treatment of the CTC-12 strain. The seed extract in

methanol of the local strain showed little effect after 24 hours of treatment, but a synergistic effect was demonstrated after 48 hours.

Final product bio insecticide

Figure (27): Final product bioinsecticides.

Directions for use

The bag most be hanged in home storage and small flour stores and replaced weekly.

Conclusion

Conclusion

This work primarily aimed to address the challenge of developing a natural and effective insecticidal formulation for controlling stored product pests, specifically targeting the flour beetle *Tribolium castaneum*. In this context, the individual toxicity of plant extracts from Neem (*Azadirachta indica*) seeds, Harmal (*Peganum harmala*) seeds, and Asafoetida (*Ferula assa-foetida*) gum was evaluated at increasing concentrations, alongside the assessment of a mixture of these extracts.

The findings revealed that the tested plant extracts exhibited insecticidal efficacy against *Tribolium castaneum*, with observed mortality rates varying based on the dosage, exposure duration, and plant species.

Notably, the formulation containing a mixture of Neem (*Azadirachta indica*), Asafoetida (*Ferula assa-foetida*), and Harmal (*Peganum harmala*) extracts demonstrated superior potency and a faster action compared to the individual extracts, achieving a 100% mortality rate within 24 hours using both contact and fumigant methods for both essential oil and alcoholic formulations.

These results open promising avenues for future research towards developing natural alternatives to chemical insecticides in stored product protection. Future studies could focus on identifying the specific active compounds responsible for the enhanced efficacy of the mixture, optimizing their extraction and formulation to improve potency and stability.

Furthermore, broader investigations are suggested to evaluate the impact of these natural extracts on other stored product pest species and on the safety of treated products and the environment. Exploring these research directions can significantly contribute to achieving more sustainable and safer practices in the management of stored food product pests.

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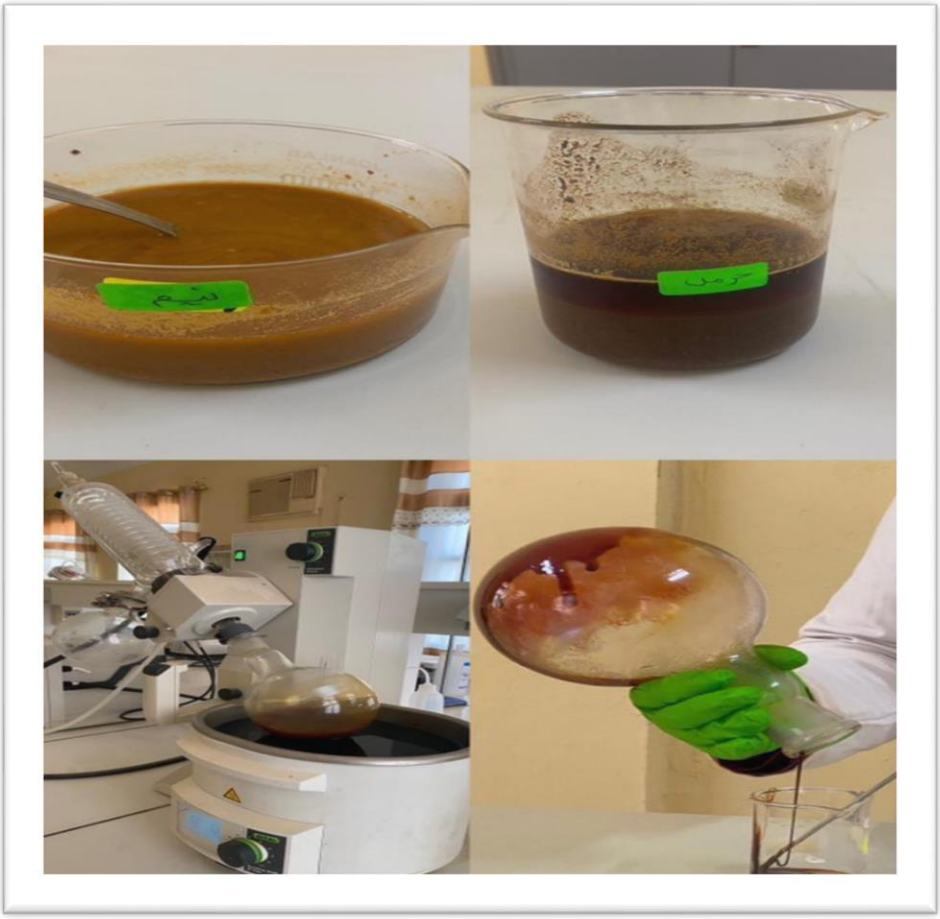
Annexes

Steps to prepare the alcoholic extract(personal photo)



Treating Spodoptera lituralise with *Azadirachta indica* and asafoetida gum *Ferula assa-foetida* essential oils(personal photo)