Evaluation of insecticidal and repellent effects of *Mentha x piperita* and *Cymbopogon citratus* on economically important stored grain against *Tribolium castaneum* (Red flour beetle)

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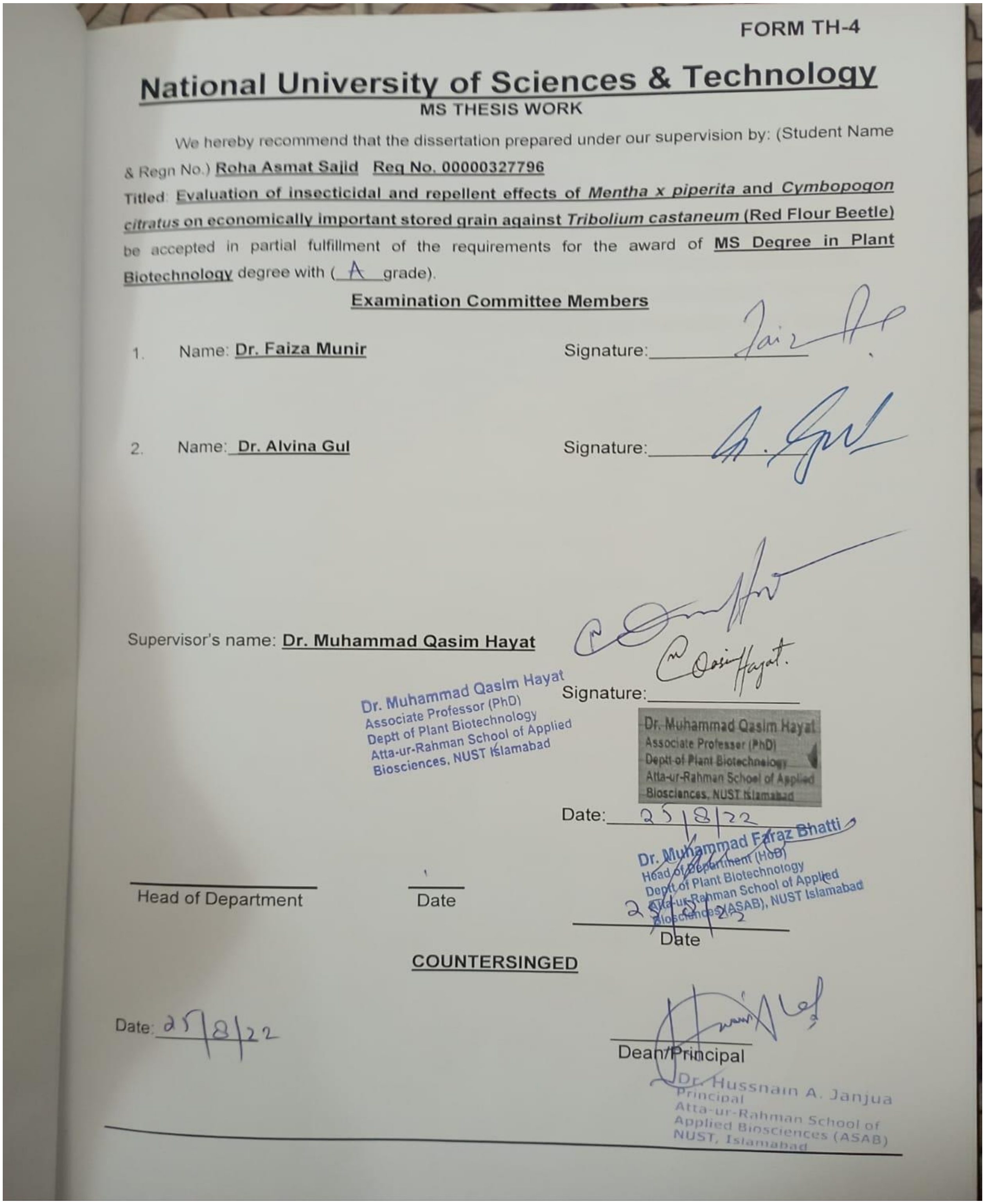

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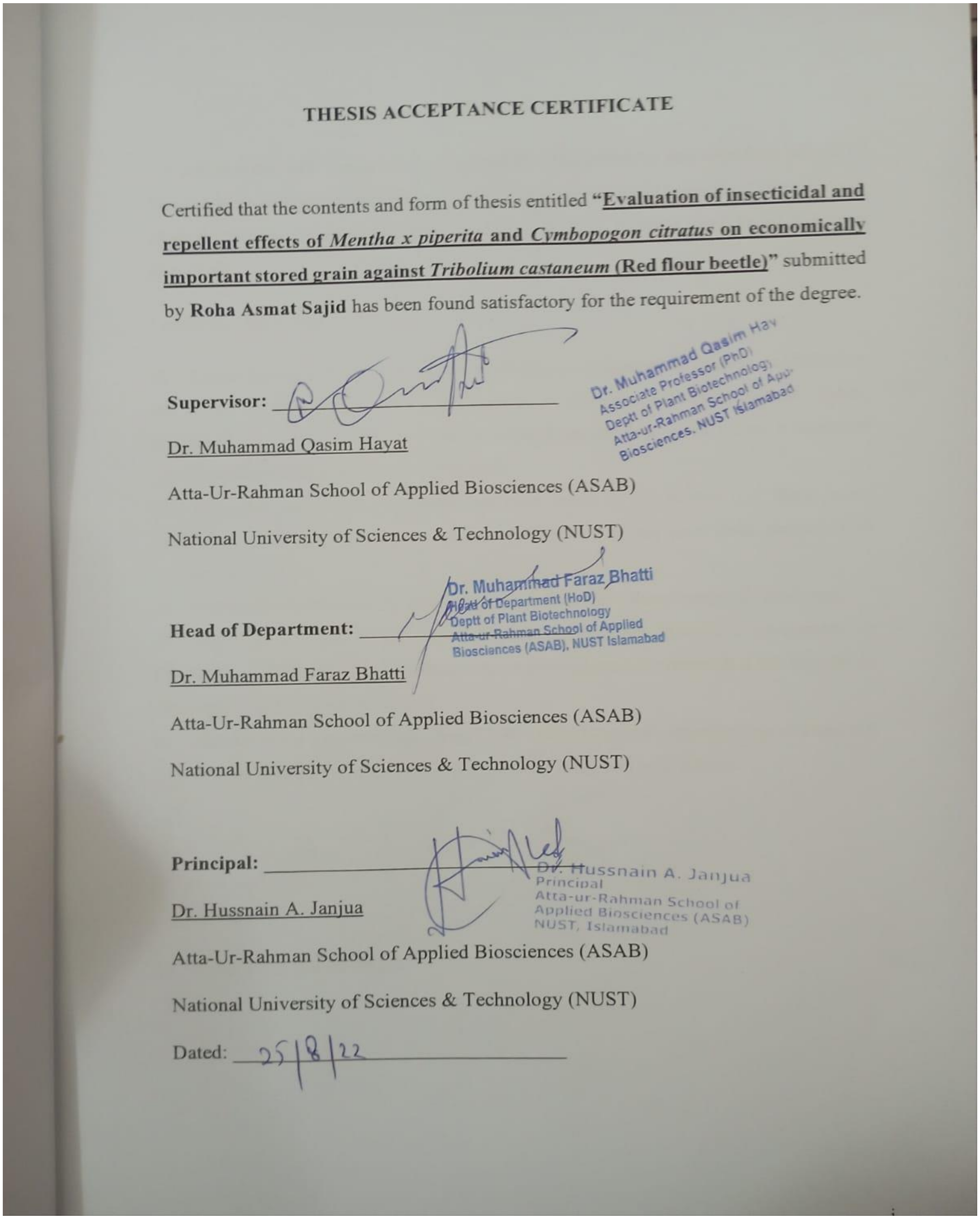

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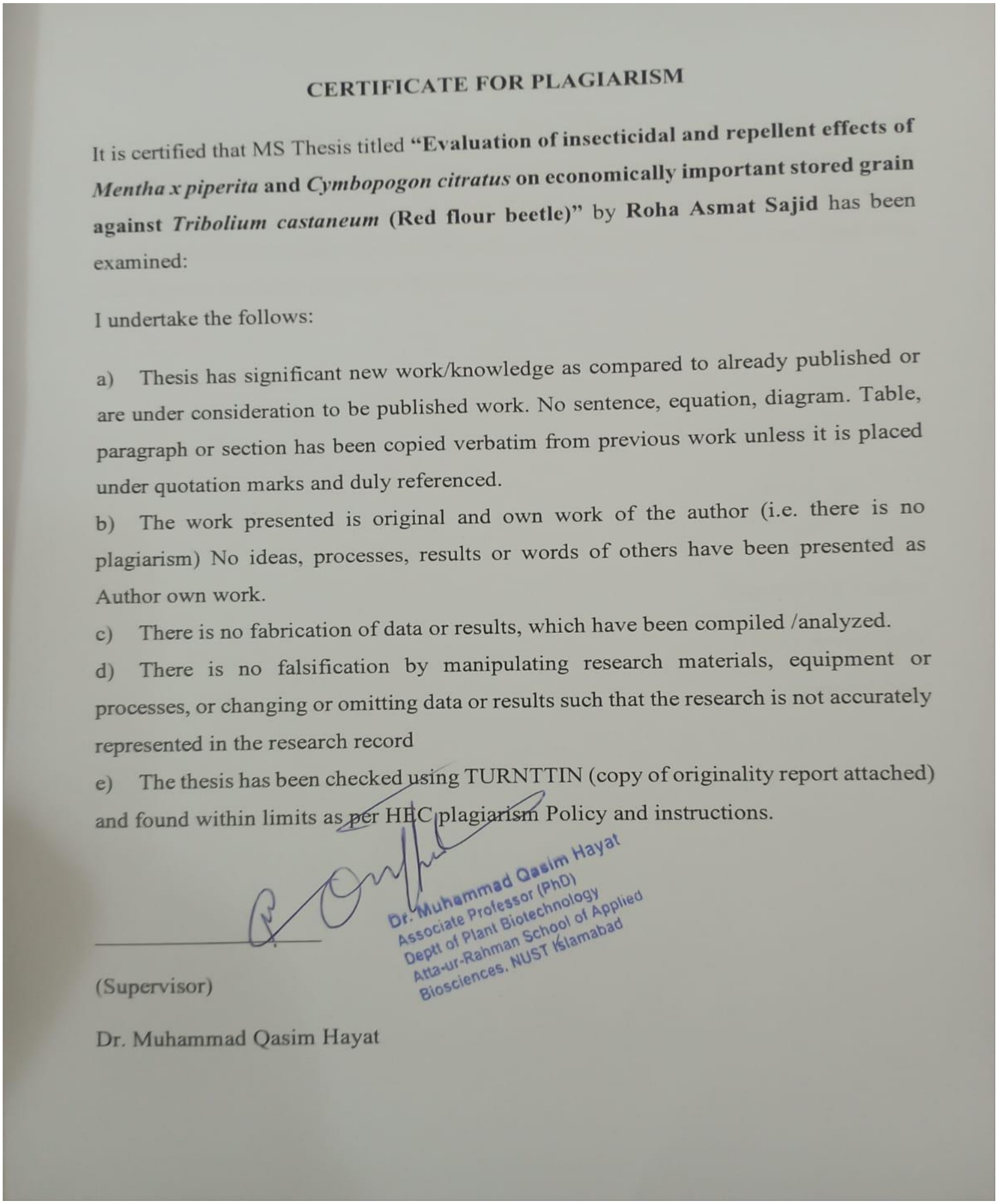

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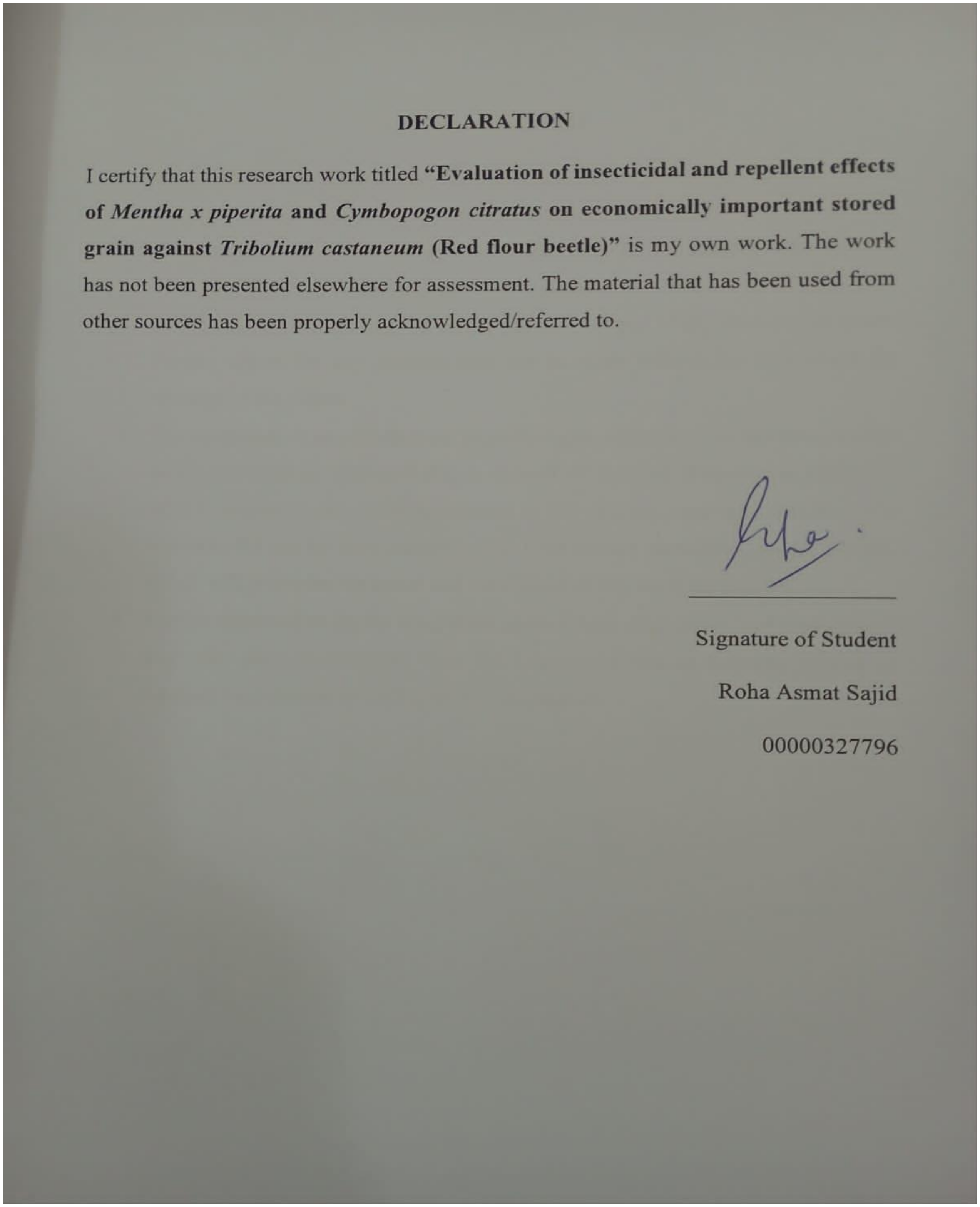

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I certify that this research work titled **"Evaluation of insecticidal and repellent effects of *Mentha x piperita* and *Cymbopogon citratus* on economically important stored grain against *Tribolium castaneum* (Red flour beetle)"** is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged/referred to.

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Dedication

To my late grandfather and beloved family for their constant support and encouragement throughout my pursuit of education

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ABSTRACT

The excessive use of synthetic insecticides poses serious damage to the environment, crops, and human health. These chemical control strategies lead to resistance to pests, environmental contamination, human intoxication, and toxic residues in the food. Due to these alarming issues, the natural insecticides of botanical origin have attracted arch interest in these recent years as eco-friendly alternatives to their synthetic pesticidal ancestors. These are biodegradable, reduce crop losses, are environmentally friendly, and are much cheaper than conventional pesticides. Plant extracts have several bioactive compounds that are tremendously fruitful for plant defense against insect pests. The prime objective of this study was to evaluate the insecticidal and repellent effects of leaf extracts of two plants i.e., *Mentha x Piperita* and *Cymbopogon citratus* against the worldwide pest of the stored products *Tribolium castaneum,* and to conduct the qualitative analysis for the screening of active chemical compounds present in both the extracts. Mortality and repellent assays were conducted using different concentrations of the extract and results were evaluated at different periods of exposure. LT50 for both extracts was also calculated to estimate the time required by both extracts to kill and repel 50% of the target pest. As per the data obtained, the relationship between exposure time and extract concentration on mortality and repellency of target pest indicated that mortality was increased by increasing the extract concentration and exposure time, and it also differed from one plant to the other. *Mentha x Piperita* proved to be a better repellent than *Cymbopogon citratus* exerting a percentage mean repellency of 80 ± 0%, 87 ±11.5%, and 100 ± 0% at 1.5%, 2.5%, and 3.5% concentration treatment at 75 mins of exposure period. On the contrary, *Cymbopogon citratus* methanolic extract proved to be a better insecticide than that of *Mentha x Piperita* because of its earlier response against *T.castaneum* at every concentration applied. 5%, 10% and 15% of concentration treatment of lemongrass exerted 86.7 ± 5.77%, 96.7 ± 5.77%, and 100 ± 0% percentage mean mortalities respectively after 30 hours of exposure. Additionally, the preliminary phytochemical screening as a qualitative identification of chemical groups also confirmed the presence of terpenoids present in both the sample plants, which happen to be major constituents acting against *Tribolium castaneum.* The results of the study provided sufficient scientific support for preliminary screening bioassays using natural

plant products in the form of extracts as control agents rather than synthetic ones to minimize the resistance mechanism, pesticide intoxication, and environmental toxicity. These plants in the form of botanical insecticides can fit well in the Integrated Pest Management programs designed for the control of various pests causing losses in food commodities.

1. INTRODUCTION

Food supply is in greater demand than ever before, resulting in increased crop yield and warehouse storage of food grains. Due to the unavailability of food and access to it, food security all around the globe is affected. This adds to the difficulty of resolving global problems such as malnutrition and hunger. (Storage of Grains, n.d.).

Stored grain infestation is one of the intense issues since there is economic damage and decays in food quality due to various stresses. Because of the poor storage conditions and structures, severe food grain losses are inflicted annually. (Sial & Abid, n.d.). This includes factors such as biotic and abiotic stresses that can cause damage and deterioration of the food grains.

Abiotic factors include moisture, air, and temperature. Whereas biotic factors like rodents, insects, bacteria, fungi, mites, and birds potentially contribute to the losses. (*Storage of Grains*, n.d.) Among the abiotic factors, temperature and moisture play quite significant roles in grain storage. The extent of food loss is particularly determined by the temperature of granaries and warehouses. Higher temperature leads to rapid pest reproduction, mold growth, and deteriorated grain quality. Moisture, on the other hand, determines the safety of stored grains. Greater moist and warm conditions provide a favorable environment for biotic components such as insects, molds, fungi, etc. (Apporva Bali, n.d.)

1.1. Damages to the Stored Food Grains

The damages to the food grains during storage are classified into two major categories as direct damage and indirect damage. The direct damage is caused by the insects and the pests that eat grains from the inside. This leaves the grains hollow, reducing their weight, and causing discoloration and a foul smell. (*Storage of Grains: Importance, Factors, Damages, Sample Questions*, n.d.). On the other hand, indirect damage is induced by consuming these infected grains from the direct damage. Indirect damage causes food poisoning, worm infections, and other harmful effects on human health.

On a global scale on average, around 10% to 28% (Nelson et al., n.d.) of crop production is lost to pests, causing loss of yield, and leading to social unrest and starvation. Post-harvest losses are yet another dilemma observed in developing countries. Around 9% of the post-harvest losses occur in developed countries, and 20% of these occur in developing ones. (Van der Fels-Klerx et al., 2016)

1.2. Dispersal of Pests and Concept of Coevolution

The dispersal of pests occurs through two main processes, including anthropogenic and natural ones. The spread was facilitated during the past decades of market globalization for plants and plant products. Due to this global trade of agricultural products, many insect pests were moved away from their native environment to new ones. This diminishes the concept of coevolution, which is one of the important phenomena recognized for plants and their respective pests. It is known to create a stable balance between both the pests and hosts within their endemic ecosystems. (Deutsch et al., 2018; Nelson et al., n.d.).

Around 10,000 years ago since their domestication, the crops have been vulnerable to a multitude of pests. Any newly introduced crop is equally threatened by the introduction of new pests into a completely new ecosystem leading to serious damage, since hosts and the pests may not have coevolved together. As per the definition in the International Standard for Phytosanitary Measures No. 5 (ISPM), ***“A plant pest is any species, biotype, or a strain of plant, pathogenic agent, or animal that causes damage to the plant or the plant products”.*** In addition, climate change is another causative factor that poses potential effects on plant pests, leading to the deterioration of plant health. The increased globalization of the market in these recent years, along with the rising temperatures, led to providing a favorable environment for pest movement and establishment, resulting in severe yield losses. (Deutsch et al., 2018; Nelson et al., n.d.)

In the Food and Agriculture Organization of the United Nations (FAO) review on ***The Impact of Climate Change on Plant Pests***, it is recorded that up to 40% of the global crop production is lost to pests annually. Moreover, every single year, plant diseases cost over $220 billion of the global economy, whereas invasive insects cost at least $70 billion. (*Scientific Review of the Impact of Climate Change on Plant Pests*, n.d.)

A study published in the Journal Nature, Ecology & Evolution reported that five major food crops including rice, maize, wheat, potato, and soybean are facing a severe yield shortage. It reported that pests and pathogens are causing 10% to 28% of wheat losses, 25% to 41% loss in rice, 20% to 41% of maize losses, 8% to 21% of potato losses, and 11% to 32% of losses in the soybean crop. (Nelson et al., n.d.)

Rice is the staple food for around more than half of the world’s population. Approximately 500 MTs of milled rice is produced annually at the global level. It is mostly grown in the Asian regions and numerous types of them are produced worldwide. The average rice yield is a derivate of a range of factors such as seed quality, climatic conditions, agricultural techniques, and farmer skillset. In terms of the highest average yields, Australia ranks the highest as it produces approximately 10MTs tons of rice per hectare. Whereas, among the regional players, Pakistan and India register a yield at par of around 4 MTs per hectare.

1.3. Objective of the Study

- Screening of eco-friendly and easily biodegradable plant products with natural insecticidal activity and low toxicity.

2. LITERATURE REVIEW

2.1. Rice Production and Consumption in Pakistan

The economy of Pakistan is categorized into three significant segments Agriculture, Industry, and Services. The agricultural sector of Pakistan grew by ~2.7% during 9MFY20 despite the negative estimated GDP growth of 0.4%. Among the group of 155 countries, Pakistan is ranked 77th with a per capita consumption of 18kg of rice. It exports different qualities of rice to different countries and various regions, and Pakistan is recorded to have been historically self-sufficient in meeting its in-house demand for rice.

Oryza sativa, commonly known as Asian Rice is the Kharif or Summer crop in Pakistan and its sowing begins in April and goes up to the month of June. It is then harvested from October to December. In Pakistan, the export of rice is one of the major sources of foreign exchange earnings. (FAS Islamabad, n.d.) It is also the second major exportable commodity after cotton. Rice contributes a percentage of 0.5% to the GDP of this country, whereas it contributes 2.4% of value added to the agricultural sector. (Pakistan Economic Survey (2021-2022), n.d.)The exports of Pakistan make up more than 8% of the total trade of rice in the world and are ranked in the 10th position in the largest rice-producing country.

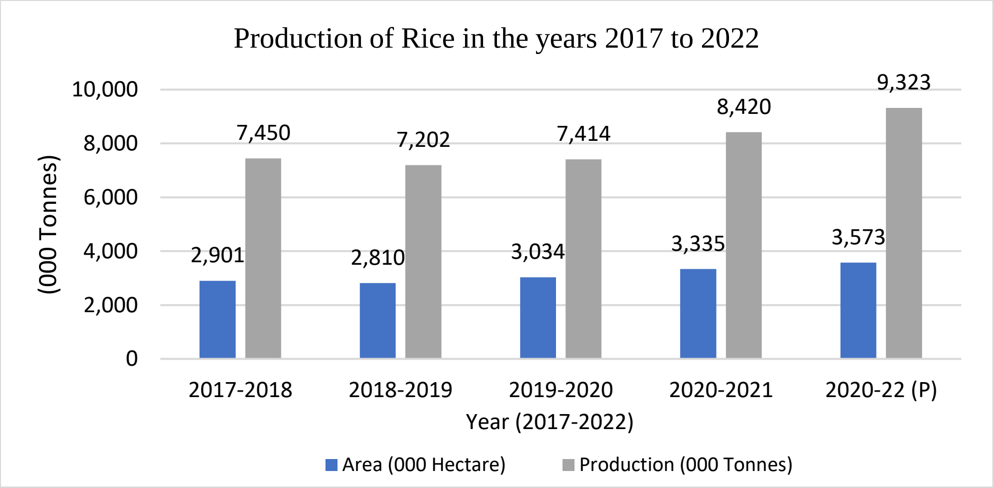

Figure 2.1.: Production of Rice in Pakistan in the Years 2017-2022

Source: Pakistan Bureau of Statistics

2.2. Production of Rice Varieties in Pakistan

Pakistan produces different varieties of rice among which some popular export varieties are Basmati 2000, Basmati–198, Basmati–385, Basmati 370, Kernal, Shaheen (Basmati) and IRRI – 6, IRRI – 9, PG (IRRI), KS 282, DR (REAP, 2010). (Punjab Could Increase the Annual Rice Production of the Country to Double Digit.). Two of the major rice-producing provinces of Pakistan include Punjab and Sindh, both of which account for around 90% of the total rice production in the country.

The province of Punjab has favorable agro-climatic and soil conditions, allowing a 100% production of Basmati rice in Pakistan. This type is known to be of premium quality and expensive too in comparison with the non-Basmati rice. Sindh, on the other hand, is enriched with the cultivation of non-basmati rice, which is IRRI-6, majorly exported to the African regions. One of the famous soil types known as the **Kalar** bowl area is efficient and suitable to produce Basmati rice. It is between the rivers of Chenab and Ravi in Punjab. The IRRI rice, however, is grown in both Sindh and Punjab. (Rice Sector an Overview, 2020b).

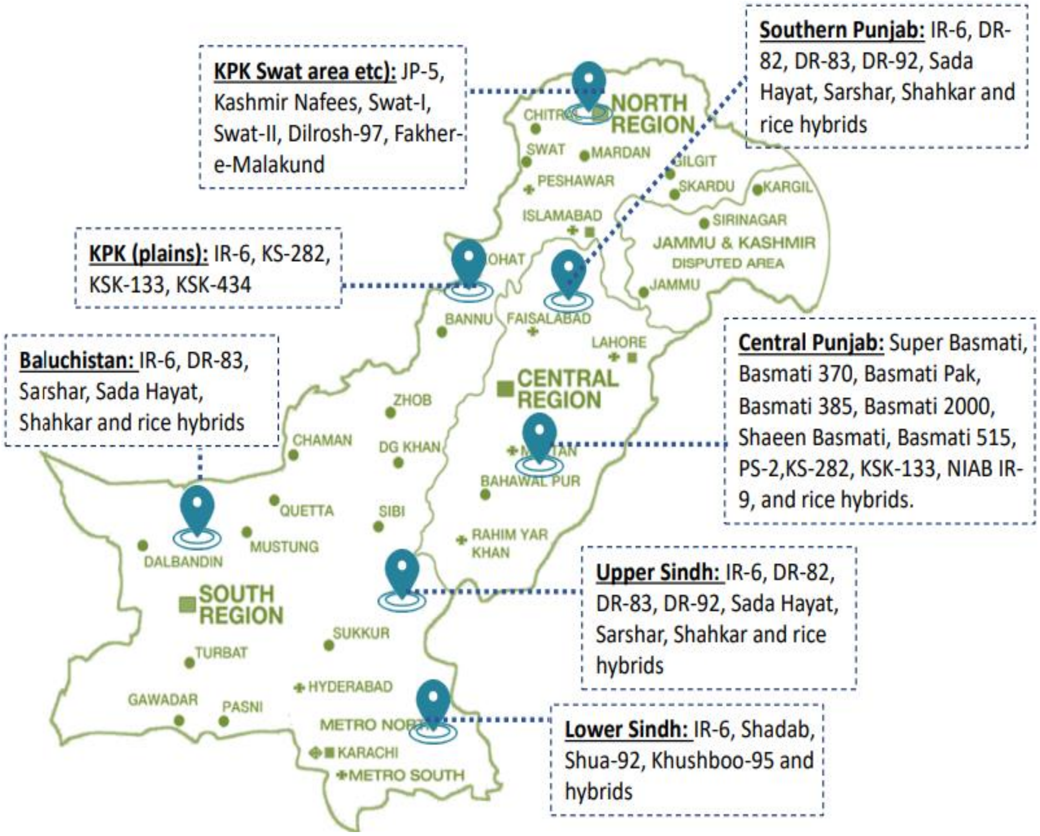

Figure 2.2: Rice Producing Areas of Pakistan

Source: (FAS Islamabad, n.d.)

Even though Pakistan’s second largest exportable crop after cotton is rice, the country has massively underutilized its potential. The long hauling challenges hinder crop growth as there is an insufficient check and balance to preserve crop quality. Basmati rice of Pakistan is considered to be one of the premium quality kinds of rice in the International Market, but the country has been unsuccessful in its shares due to a lack of policy framework to boost the yield. (*About Rice Crops*, n.d.)

2.3. Insect Pests of Stored Food Products

Several insects in stored food products are distributed widely and attack many other commodities. Most of these pests damage the food grains during storage because of contamination and heating that promotes fungal growth. (*Storage Insects - University of California Rice On-Line*, n.d.). The Food and Agriculture Organization of the United Nations (FAO) states ***“Global yield losses of major staple crops such as wheat, rice, and maize are projected to increase by 10 to 25 percent per degree of***

global average surface warming. Crop losses will be most severe in areas where warming accelerates plant pest and disease population growth and their metabolic rates.” (*Climate Change Has an Impact on Pests, Pathogens, and Plant Physiology*, n.d.). Among these, rice grain faces several biotic and abiotic problems during the pre-harvesting and post-harvesting stages. Many insect pests affect the quality of rice during storage conditions leading to severe infestation.

2.4. Stored Rice Insects

Stored rice insects are classified into two groups Internal Feeders and External Feeders. Internal feeders are the insects that would develop inside the rice kernel and consume the endosperm. Whereas the external feeders develop outside the rice kernel and feed on dust, broken kernels, mold, or bran.

- **Internal feeders**: Rice weevil, Lesser grain borer, and Angoumois grain moth.
- **External feeders**: Flour beetles, Indian meal moth, Saw-toothed grain beetle, Grain beetles, foreign grain beetle, and Hairy fungus beetle.

2.4.1. Internal Feeders

a. Rice Weevil

Rice weevil, known as *Sitophilus oryzae (L.)* is the primary pest and grain feeder belonging to the order Coleoptera and the family Curculionidae. The adult is small ranging in between 2.5 to 4mm and has deep brown with 4 distinct reddish yellow patches on the elytra. The larvae are white, and they are legless grubs that develop within the kernel. The adults can fly and live up to 4 to 5 months. Female adults lay multiple eggs within the kernel and more than one larva can develop within one kernel. (*Rice Weevil*, 2022).

b. Lesser grain borer

Lesser grain borer, known as *Rhyzopertha dominica* infests many kernels and develops within the grain. The adults are 2.3 to 3.0 mm in length and are dark brown. These are also primary grain pests and attack undamaged grains making them susceptible to attack by secondary pests. The female lesser grain borer lays eggs in between the range of 300 to 500 for approximately three weeks. The eggs typically hatch in 7-18 days giving white larvae with small legs and brown heads. The total life cycle of this pest lasts from 24 to 133 days depending upon the temperature. (*Lesser Grain Borer*, n.d.)

c. Angoumois grain moth

Angoumois grain moth is commonly known as the rice grain moth, and it belongs to Gelechiidae moth family. These are found as pantry pests in the home but are also recognized as hazardous pests in grain storage on commercial levels. The adults are of tan or golden color and about 1/3 inch long. Larvae are white and have a yellowish to brown head, with dark reddish-brown mouthparts. They bore into the kernels and emerge through the hole cut after pupating. The rice grain moth has a complete development in about 5 weeks. (Jackman John, n.d.)

2.4.2. External Feeders

a. Flour beetles

The two most common *Tribolium* species include *Tribolium castaneum* (Red Flour Beetle) and *Tribolium confusum* (Confused Flour Beetle), both of which are common secondary pests of all the plant commodities in stores worldwide. (Jackman John, n.d.). Both species are 1/8 to 3/16 inches long and have flattened bodies that are well adapted to crawling in the tiny crevices. The only difference between both is their antennas, as the last three segments of the antennae on Red Flour Beetle are enlarged to form a club. On the contrary, the Confused Flour Beetle is enlarged towards the tip. Both the species feed on the broken, brans, and dust, and their larvae and pupae develop among the rice grains, and they can live several months up to three years. (*Red Flour Beetle*, n.d.)

b. Saw-toothed grain beetle

The sawtoothed grain beetle, scientifically known as *Oryzaephilus surinamensis* has a flat body and is well adapted to crawling into the crevices. Their thorax margins are sawtoothed and have six projections on each side. Sawtoothed grain beetle larvae are yellowish white in color, with a brown head. The full-grown larvae are around less than 1/8 inch. The female grain beetle lays around 375 eggs on average which hatch n 3 to 5 days. These beetles are typically found in rice, walnuts, dried fruit, cereals, tobacco, sugar, breakfast foods, and a few other products. (Sawtoothed Grain Beetle Oryzaephilus Surinamensis (L.), n.d.).

c. Grain beetle

Grain beetles belong to the family Silvanidae and the genus Cryptolestes. Various such species carry six blade-like projections on their body. Few of them have large prominent eyes which makes their head very pointed. Most of the grain beetles range between 2 and 3mm in the length and are brown or dark red. They lay their eggs in the grain mass or the grain cracks and cervices. The grain beetles are usually encountered in moist places such as warehouses, home pantries, mills, and cargo ships that promote viable environments for them.

2.5. Threats to Stored Grain Products

The pests incur great losses, both qualitatively and quantitatively to the stored grains. One of the prime reasons for these stored insect pests is the presence of favorable and optimum growth conditions, increasing the reproduction rate. A number of these insect pests gain access to the stored grains during the development of seeds, the maturation stage, the transmission of seeds, or during storage. It is reported that almost one thousand diverse kinds of grain pests exist all around the world, leaving undesirable flavor and smell in food products.

Among all the existing pests, Lepidoptera and Coleoptera are two major orders that consist of most stored grain pests. These cause serious threats to the dried, stored, and perishable agricultural products. (Ahmad et al., 2021a). Not only this, but they also pose damage to the non-food derivates of these agricultural products around the world.

The Diversity of insect pests and food weight losses of stored cereal grains were investigated in Bahawalpur (Punjab, Pakistan). The study concluded that the order Coleoptera had the most diversity among many other common insect pests of the stored grains. The weight losses observed due to the insect pests in different grains were 5.6% in rice, 4.56% in pulses, 4.65% in wheat, and 3.55% in maize. In addition, more than 18.36% loss in the seed weight was observed during the storage period of six months, under natural optimum conditions. The reason for the higher percentage recorded was the result of rainy summer season and a warmer climate, favoring the progeny of insect pests in the stored food. (Sial Nuzhat & Abid Sobia, 2021)

2.6. Climatic Conditions of Pakistan Favor Pest Growth

The climate of Pakistan favors the growth of such pests leading to major economic losses. These pests not only consume the actual grain products but also contaminate them with their feces, making them unfit for human consumption. The most prevalent of these pests affecting the yield of rice in Pakistan include the red flour beetle, grain moth, rice weevil, and khapra beetle, where the maximum infestation reported is by the red flour beetle, scientifically known as *Tribolium castaneum.* (Grünwald et al., 2013)

2.7.Red Flour Beetle

Tribolium castaneum, is a worldwide pest of the stored products, especially of food grains including biscuits, rice, flour, cereals, nuts, pasta, and beans. It is of Indo-Australian origin and found in temperate regions and is more common in warmer climates. The species require an optimum temperature of 22°C to 40°C, whereas their development occurs in the temperature range of 32°C to 35°C.

Table 2.1: Scientific Classification of Red Four Beetle (*Tribolium castaneum*)

Scientific Classification of Red Flour Beetle	
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Tenebrionidae
Genus	*Tribolium*
Species	***T.castaneum***

2.7.1. Life Cycle of Red Flour Beetle

The Red Flour Beetle, belonging to the family of darkling beetles, is also recognized as a model organism for various food safety and ethnological research. The female Red Flour Beetle deposits around 200-450 eggs in the food during its 1-3 years life span. These eggs then hatch in 5 to 12 days, and the larvae mature within 30 days in the warmer months, or around 120 days in the cooler months. The surface of their eggs is usually sticky, to which food particles can easily attach.

Every egg then hatches a beetle larva which is usually white, slender, tinged with yellow, and cylindrical in its visual appearance. Each larva consists of six legs and has two forked projections at the back. This is the stage in which beetles then molt as many as twelve times and grow up to the length of at least one-quarter inch. The feed of larvae eventually grows to the size of that of rice grains. The complete larva period lasts for up to 15 days, and then it closes itself into the pupal case, whose outing covering is known as the pupal cuticle. The pupal stage is known to last up to five days or even more. (Sreeramoju & Msk, n.d.)

At this stage, the identification of female and male beetles becomes easier. The female papillae are larger than the male with two finger-like structures just anterior to the paired horns. On the other hand, the male papillae are smaller and look like fingertips. This is the stage where beetles recognize themselves to become an adults. The newly emerged adult is flat, brown, shiny, and has antennae and six legs. Adult beetles are highly active and move about in irregular patterns. These can last up to several years too if present in the ideal and most optimum conditions. They have a shorter life cycle and a higher reproduction rate. (*Flour Beetles*, n.d.)

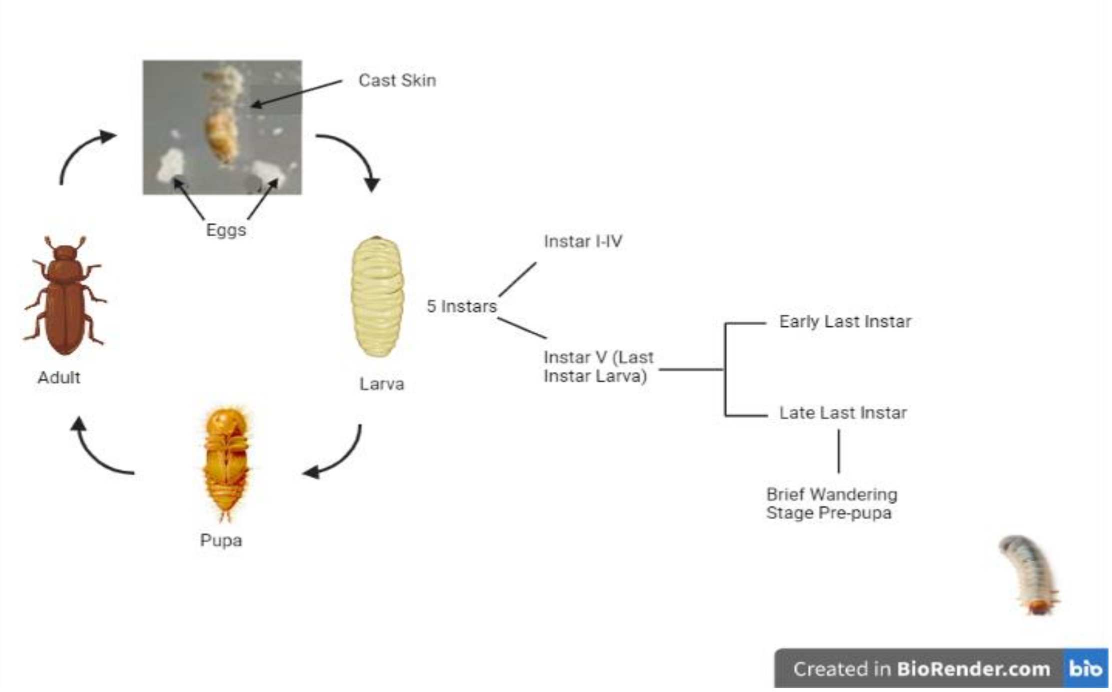

Figure 2.3: Classification of different developmental stages of the red flour beetle

2.8.Current Control Strategies for the Control of Pests

Stored grain insects are notorious enough to cause damage to pulses, grains, and many other food products in one way or the other. The damage can be done either directly or indirectly by consuming these grains or their products. It can also occur through cadavers, webbing, and accretion of the exuviae. (Ahmad et al., 2021b). Storage of grains is one of the important activities that enhance marketing efficiency by providing. (Ejaz Ashraf et al., 2014)

Different control strategies are implemented across the world to prevent the loss of stored grain to a maximum level. Currently, synthetic insecticides belonging to distinct groups of pesticides are the prime source of controlling these pests. (Ejaz Ashraf et al., 2014).

2.8.1. Chemical Insecticides

Chemical insecticides are still considered to be the most effective methods for pest control, but these chemicals are also meant for toxicity and cause a severe imbalance of the microorganisms in the ecosystem. (Garbi et al., 2006). They degrade some of the toxic compounds, the residues of which remain in the soil, water, air, and in food commodities. (Anacapa et al., 2016). Therefore, the excessive use of insecticides, particularly on cash crops leads to the killing of significant fauna, including human intoxication.

Various fumigants, pyrethroids, organophosphates, organochlorides, and insect growth regulators are used to control the spread of *Tribolium* species. But the excessive and irrational use of these insecticides leads to various detrimental effects on both the crops and human health. In Pakistan, the *Tribolium sp.* has become resistant to many of these insecticides. In addition to insect resistance, they lead to toxicity to the non-targets, environmental contamination, and an increased production costs. (Koçak et al., 2015)

The resistance mechanism was studied in the four different field populations of *Tribolium castaneum (*Red flour beetle) from four distinct regions of Pakistan. The sample was collected from Faisalabad, Jhang, Sahiwal, and Sargodha districts to evaluate insecticide resistance to the pest. Bioassays were conducted with two frequently used insecticides i.e., permethrin and deltamethrin, in addition to one biological insecticide, Spinosad. The study reported the resistance level and/or tolerance in various populations of the red flour beetle. The toxicological studies demonstrated that three strains (Faisalabad, Jhang, and Sahiwal) showed higher tolerance ratio of 26.5-fold, 21-fold, and 18.6-fold respectively, in relevance to the Sargodha strain against deltamethrin. This evolution of the resistance in the *Tribolium sp* against various insecticides threatens the sound storage of products. (Riaz et al., 2018).

Among many existing pesticides, some of them including endrin, heptachlor, chlordane, hexachlorobenzene, and propanil have persistent organic pollutants (POPs). These pollutants resist degradation and stay in the environment for a long period leading to disruption in the food chains. In addition, these compounds also bioaccumulate and reach up to the concentration of 70,000 folds in comparison to their original concentration. (Kim et al., 2017) Overuse of these insecticides leads to the development of insect resistance and results in pest resurgence. (Damalas &

Eleftherohorinos, 2011). Due to these potentially detrimental effects, humans are vulnerable to diseases such as cancer, gastrointestinal issues, diabetes, leukemia, and (Damalas & Eleftherohorinos, 2011) many others after pesticide intoxication.

2.8.1.1. Exposure of Pesticides in Pakistan

Pakistan happens to rank in the second position in the overall consumption of pesticides among South Asian countries. The major use of these pesticides is in the agricultural sector (Waheed et al., 2017; Yadav et al., 2015) and as per the available data, a significant amount of these pesticides was used in the years the 1980s to 1990s. They are used for different crops in Pakistan leading to the accumulation of toxic active ingredients in the food chain leading to devastating health issues. They are used for different crops in Pakistan leading to the accumulation of toxic active ingredients in the food chain leading to devastating health issues. (Damalas & Khan, 2017)

According to the FAO, previously cultivated lands are used for around 80% of food production in developing countries. Whereas only a small percentage i.e., 20% of new food production comes from the expansion of new farming lands. (*Pesticide Residues in Food*, n.d.). This increases the chances of exposure to pesticides in those agricultural lands, especially for higher crop production. Numerous data have been reported on the pesticide poisoning in surface and ground waters of different areas of Pakistan, in addition to increased levels in soils, sediments, and air with hazardous classes

Table 4.2: Detection of pesticides in the ground waters and surface of Pakistan

Area	Year	Pesticide	Conc (μg/L)	LD50 mg/kg	Class	Reference
D.G. Khan, Muzaffargarh, Rajanpur	2004	Bifenthrin Carbofuran Endosulfan Monocrotophos	4.3 23.1 2.8 8.3	55 8 80 14	II Ib II Ib	(Tariq et al., 2004)
Rawal Lake, Islamabad (Capital of Pakistan)	2006	Azinophos-methyl Parathion-methyl Fenitrothion α-Cypermethrin	0.06-13.3 0.02-2.7 0.08-8.3 0.2-18.8	NA 13 503 144	NA Ia II II	(Ahad Karam et al., 2009)
Rawal Lake and Simly Lake, Islamabad	2009	2,4-DDT Diazinon	0.9-2.9 1.8-3.6	113 300	II II	(Iram Shazia et al., 2009)
Southern Punjab	2012	Imidacloprid	0.6	450	II	(S. Baig & Ahmad Niaz, n.d.)

Table 2.5: Detrimental health effects on Pakistani population due to exposure of pesticides in different areas

Location of affected	Affected %	Pesticide type	Harmful impacts	Reference
Hospital patients, Karachi	100	General	Low AChE level	(Azmi & Naqvi, n.d.)
Female cotton pickers, Khairpur (Sindh)	18 (22% for married whereas 9% for unmarried)	Methamatophos Baythroids Cypermethrin Endone, Aldrin	Significant increase in reproductive hormones (LH and FSH, PG and estradiol).	Rizwan et al, 2005
Factory areas in Lahore, Multan, and Karachi	29% small and 8% medium industrial workers	Endosulfan Imidacloprid Thiodicarb Carbofuran Methamidophos	Serum AST, ALT, Creatinine GGT, Malondialdehyde, and CRP significantly higher	Khan et al, 2010
Male, female, and children from three different farm locations, Vehari, Punjab	NA	Endosulfan Aldrin Dieldrin pp-DDT	DDT, 0.92 ngmL−1 aldrin, 0.68 ngmL−1 dieldrin and 1.96 ngmL−1 endosulfan in their blood samples.	Saeed *et al*, 2017

The excessive overuse of pesticides is harmful to agricultural production, especially when it causes both environmental and health issues in farming communities. The careless handling of these chemical control strategies leads to contaminations in the water, air, and land, which in return enter the food chain and human body. This sole reliance on pesticides emphasizes the need for substantial research work for the development of eco-friendly and cost-effective methodologies as an alternative to reduce environmental contaminations.

2.8.2. Physical methods

Physical methods are used for the management of stored grain pests after they have infested the grains or their respective products. This includes physical exclusion, grain distribution, regulation of temperature, sanitation, aeration, hermetic sealing, and oxygen saturation. They prevent damage to grain products and keep pests at bay.

2.8.3. Traditional methods

Some of the traditional practices also exist that are followed to control the attack on food grains by pests. Now and then, man has developed conventional methods for the prevention of stored grains to maximize their quality and quantity. These include the use of bamboo, straw, cow dung, wooden plank, mud, and leaves of many plants.

2.8.4. Biological control

The biological control strategy includes the use of some predatory insects or microbes that control the pests. These viable organisms include bacteria, mycoflora, viral strains, and parasitoids that are eco-friendly and already present in our ecosystem. (Islam, 2017). These parasites are indeed killing many grain pests, but still, they do not provide complete protection as grains themselves are damaged. The scope of biological control approaches in stored grain management is exceptionally low. One of the major drawbacks of this method is that it is expensive and requires the maintenance of culture for pest control. (Ahmad et al., 2021c).

2.9.Botanicals: Alternative Approach to Synthetic Insecticides

Synthetic insecticides have highly discouraging aspects including toxicity, residual effects, harmful effects on plants and animals, and nonbiodegradability. Since the beginning of the 21st century, researchers are looking toward finding cheaper, safer, and locally available botanical derivates for the management of stored grain pests. One such suitable method is the use of plant volatile organic compounds that exhibit insecticidal or repellent properties. It is one of the most viable options for effective control of grain pests that exhibit less toxicity and persistency, have fewer or no threats to the environment. (Damalas & Eleftherohorinos, 2011)

It is true to say that only a small number of chemical insecticides and pesticides have been fully authorized for stored grain protection. Concerns such as resistance issues and ecotoxicity emphasize the need for the development of eco-friendly agents to control the pests in stored grain products. Among many other existing control measures, pure plant-derived substances with promising biological activities have been found effective in pest control. They have a high potential of being used as an eco-friendly for pest control since they pose no harm to human health and the environment. Most of the reported plant species for this purpose are a part of the human diet and are not harmful to humans. Edible plant extracts produced using various solvents are safe candidates for use as fumigants, contact pesticides, and repellents too. (Nikolaou et al., 2021)

The modes of pesticides are not always species-specific, hence affecting the non-target organisms. Due to this several pesticides have been withdrawn and banned from the markets. In the year 2001-2008, it was reported that European Union banned 26% of insecticides because of damaging the entire ecosystem. (Karabelas et al., 2009). Therefore, natural insecticides or botanicals have attracted arch interest in these recent years as safer alternatives to their synthetic pesticidal ancestors.

2.9.1. Bioactivates Associated with Different Botanicals

Plants are a rich source of secondary metabolites that hold foremost importance in therapeutics and have constituted the origin of pharmacology. World Health

Organization (WHO) launched a Traditional Medicine Strategy, which included herbal medicines as medicinal therapies. This was done to ensure the safety, quality, and effectiveness of these traditional medicines. In the early ages, they were used as preparations. But now they are dominantly used as the isolated molecules or the extracts that are characterized phytochemically. (*WHO.Indd*, n.d.)

Due to several beneficial reasons, plants are gaining much international popularity as a source of medicine. These include their natural origin, cost-effectiveness, lesser side effects, availability, especially in the local communities, and ease of administration. To obtain the bioactive compounds responsible for various bioactivities, the procedure of extraction is performed. It includes the separation of active plant materials or the secondary metabolites from the plant source. The secondary metabolites are classified into several categories such as alkaloids, flavonoids, steroids, tannins, saponins, glycosides, terpenes; separated from the plant material using right solvent and optimized extraction procedure. (Abubakar & Haque, 2020a)

2.9.2. Plant-Based Extracts

Plants are a useful source of many bio-products such as food, medicines, cosmetics, biopesticides, cosmetics, and many others. To manufacture these products, various parts of the plants are used such as leaves, roots, stems, flowers, and fruits, depending upon the research objective and outcome. All the plant-based extracts are multi-compound products and natural, with numerous bioactivities. Most of these extracts are antioxidant, antiviral, antifungal, antimicrobial, anti-inflammatory, antiparasitic, aromatic, and antiprotozoal. These natural products have the potential to add to the benefits of sustainable agriculture, as they pose low toxicity to humans and the environment. In addition, they have greater resistance to biotic and abiotic stresses, increase crop yield, and alleviate the use of mineral fertilizers and pesticides for pest prevention and crop damage. (Godlewska et al., 2021)

There is a dire need for suitable and sustainable management of agricultural land areas to reduce the unavailability of resources and the degradation of ecosystems. Due to these reasons, great efforts are being put forward towards decreasing the dependency on chemical insecticides and pesticides, since their misuse poses multiple threats to human health and the environment. (*Role of Bio stimulant Formulations in Crop Production: An Overview*.). Instead, cheap, and safer alternatives are being brought into usage, derived from the plant sources that act as natural pest management agents and improve agricultural productivity preserving the natural physiology of the plants.

2.9.3. Plant Protection Products

Various plant protection products are currently used for the prevention of plant diseases, which involve the use of pesticides. However, the widespread use of these can lead to adverse damage to the environment leading to quality deterioration. (Suteu et al., 2020; Zioga et al., 2020). In addition to this, the pesticides also immobilize the soil and affect the organic matter and composition of the microbial community there (Jouini et al., 2020). Another prime factor to making the minimal use of pesticides is that it causes contamination to both the food and groundwater, the quality and safeness of both of which are crucial to human health and agricultural purposes. (Suciu et al., 2020; Suteu et al., 2020)

At present, the main aim of plant protection is to introduce and implement safer, harmless, and novel methods that would restrict the growth of pests in the cultivation of crops, and post-harvest food storage. (Hassauer & Roosen, 2020; Kopacki et al., 2021). This is only possible if there is a mindful approach and rational management of the natural resources and decrement in the use of synthetic products on the crops. Eliminating and limiting the attack of such destructive pests is the desired goal for the sustainable production of high-quality food.

Even though the development of alternative control strategies to chemical ones has been in practice since time immemorial, further research and efforts continue to grow for seeking novel plant-based products for pest control. (Tembo et al., 2018). The bioactive compounds from the plants are extracted and can be used as an eco-friendly agent to fit the best as an alternative for synthetic pest control products. (Du Jardin, 2015; Singh Gurjar et al., 2012). The major advantage of these botanicals is that they are biodegradable, reduce crop losses, are environmentally friendly, and are much cheaper than conventional pesticides. (Pylak et al., 2019)

These botanicals have high efficacy against stored grain pests and diseases, and little to no toxicity against the non-target organisms. The ever-growing interest in the food that is produced using environmentally friendly methods offers potential for commercialization of the natural bio-products.

Leaves of *Bridelia ferruginea, Blighia sapida*, and *Khaya senegalensis* were used to test the repellent activities of its powders and extracts against *Dinoderus porcellus. D. porcellus is* considered one of the most important pests of the stored yam chips. All the extracts were found effective as repellents and the findings suggested that the powders and extracts of these three plants were sources of botanical insecticides and may be used for effective integrated management of *Dinoderus porcellus* as an alternative to synthetic insecticides. (Loko et al., 2017)

Another study revealed the adulticidal and repellent activities of the leaf extracts of *Eclipta alba* and *Andrographis paniculata.* The extracts were prepared using different solvents including hexane, chloroform, ethyl acetate, benzene, and methanol. The extracts were assayed for their toxicity against the vector mosquitoes; *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). All the extracts had moderate adulticide effects on the target insects. The results suggested the use of leaf solvent plant extracts to have the potential as an eco-friendly tool for the control and management of mosquitos. (Govindarajan & Sivakumar, 2012).

Red flour beetle *(Tribolium castaneum),* one of the major stored grain pests has been a major problem over the years. The leaf extracts of few plants were found to have effective insecticidal activity against the respective pest. Methanolic, water, and n-hexane extracts of *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalyptus species*, *Swietenia mahagoni*, and *Psidium guajava* were used in the assay. Among all the plants, *Cucumis sativus* had the highest mortality rate and its n-hexane extract was most toxic to the red flour beetle. (M. Mostafa et al., 2012)

Insecticidal and growth inhibitory impacts of five different plant extracts were tested against different life stages of the red flour beetle (*Tribolium castaneum).* The extracts of plants *Melia azedarach, Salsola baryosma, Azadirachta indica, Pegnum hermala, and Zingiber officinale* showed good potential as a bioinsecticide, with the highest mortality rate by *A. indica*. The study concluded that plant extracts are a safer substitute for synthetic insecticides and can be used in integrated pest management for the control of pests, both in the stored commodities and crops grown in the field. (Sehrish Kanwal et al., 2021)

In a study involving the pests of cabbage (*Brassica oleracea* L.), including green peach aphid (*Myzus persicae*), cabbage aphid (*Brevicoryne brassicae*), and the diamondback moth (*Plutella xylostella*), *Nicotiana megalosiphon* aqueous extract was studied to access its insecticidal properties against the respective pests. Different concentrations of the extract were applied to the population of pest species, and the highest mortality (25 ± 0.03%, 90 ± 0.04%, and 100 ± 0.00%) of the extract was observed against diamondback moth (*Plutella xylostella*) in a period of 24 hours. (Amoabeng et al., 2018). The data explicitly demonstrates that the use of botanical plant protection products can play a great role in integrated pest management and the natural plant chemicals can work the best in future pest control.

2.10. Extraction of Medicinal Plants

The following steps are required for the extraction of medicinal plants: (Extraction Technologies for Medicinal and Aromatic Plants, n.d.).

2.10.1. Size reduction

To reduce the size, the dried plant material is ground in the hammer mill or a disc pulverizer with in-built sieves. The purpose of bringing the dried plant to a powder form is to rupture the cell structures and let its medicinal ingredients get exposed to the extraction solvent. The smaller the size, the greater the surface area, greater the medicinal ingredients are exposed to the solvent for extraction.

2.10.2. Extraction

The extraction of plants is subject to different methods and techniques. The extraction involves the separation of medicinally active plant portions or tissues. The standard extraction procedure requires the use of selective solvents on the principle of solubility. The obtained extracts can then be used as medicinal agents or can be incorporated into the dosage forms such as capsules or tablets.

2.10.3. Filtration

After the extraction, the extract obtained is separated from the plant material. The extractor has a built-in false bottom which is covered with a filter cloth and allows the extract to trickle down into the holding tank. The extract is then collected in the holding tank and then pumped into the filter to eliminate any colloidal particles from the extract.

2.10.4. Concentration

The extract is then subjected to a rotary evaporator to remove the solvents from samples at low pressure by evaporation. It consists of a round bottom flask where evaporation occurs, and it keeps rotating at a constant speed. The main components of the rotary evaporator include the rotary motor, vacuum source, glass tube, heating bath, condenser, and the receiving flask that receives the distilled sample. The solid mass obtained is used for the desired pharmaceutical formulations or processed for phytoconstituents isolation.

2.11. Various Methods Used for Extraction of Medicinal Plants

(Abubakar & Haque, 2020b).

Various methods are utilized in the preparation of medicinal plant extracts depending on the targeted outcome of the research. It involves both the determination of quantity and quality of the bioactive constituents to be obtained.

2.11.1. Maceration

Maceration is the extraction method suitable for the thermolabile plant material in which the powdered plant material is placed inside a container. The menstruum (solvent) is then poured over the plant material until it is completely immersed. The container is then placed in the dark for at least two to three days and stirred periodically to ensure efficient extraction. Once the extraction is complete, filtration or decantation is done to separate the micelle from the marc. It is further followed by the separation of menstruum by evaporation using a water bath or rotary evaporator.

2.11.2. Infusion

The infusion extraction method is also like the maceration. The plant material is ground into a very fine powder before extraction. The solvent for extraction is poured into the plant material. The powdered is then immersed in the solvent and kept for a short period for extraction purposes. Unlike maceration, this method is more suitable for bioactive constituents that are readily soluble. The infusion extraction method involves a solvent sample ratio of around 4:1 or 16:1 typically based on the research purpose.

2.11.3. Decoction

Decoction involves the use of water as a solvent within a specific volume. This method works best for the plant materials that are readily soluble in water and are heat stable too. The ground plant material of suitable particle size is placed into the container and water is poured over the sample. Continuous hot extraction is done by applying heat throughout the process to speed up the extraction. The time required for decoction extraction is usually about 15 mins with a solvent sample ratio of around 4:1 or 16:1.

2.11.4. Digestion

Digestion extraction methods work best for readily soluble plant materials. It involves the use of moderate heat of around 50° C during extraction. The extraction solvent is added into the container, after which the powdered plant material is added. Heat is applied consistently during the process which decreases the viscosity of the extraction solvent. It also helps in the removal of secondary metabolites.

2.11.5. Soxhlet extraction

Soxhlet extraction is one of the commonly used extraction methods and is best for plants that are partially soluble in the select solvent and with insoluble impurities. The apparatus used is referred to as the Soxhlet apparatus consisting of the round bottom flask, extraction chamber, condenser, and siphon tube. The dried and powdered plant material is placed inside the thimble (porous bag), tightly closed, and placed inside the extraction chamber. The solvent of extraction is then poured into the round bottom flask and placed on the heat source. The solvent gets heated up, evaporates, and then passed through the condenser. After condensation, it flows down to the extraction chamber containing the plant material. The solvent drips on it and extraction begins. The level of a solvent when reaches the top of the siphon, the extracted plant material along with the solvent flow back to the flask. The extraction occurs until the solvent in the siphon becomes colorless and there is no residue behind it.

2.11.6. Supercritical CO_2 extraction

The supercritical CO_2 extraction method uses controlled temperature and pressure to create phase changes in carbon dioxide. The plant material is ground and placed into the extraction vessel to perform an extraction. The pump forces the CO_2 into the extraction vessel where it encounters the plant sample. The extraction occurs and the pressure release valve allows the material to flow in the separate vessel which adjusts the pressure and temperature helping specific molecules to bond with CO_2 and separate from the plant. The extract is then collected in the collection jar. In this method, carbon dioxide reaches the temperature of 31.1°C and a pressure of 1071 psi. in this supercritical state, it works both as a liquid and a gas. (*Supercritical Carbon Dioxide (CO2) Extraction Method from Cole-Parmer*, n.d.)

2.11.7. Ultrasound-assisted extraction

The ultrasound-assisted medicinal plant extraction method involves the usage of sound energy at a frequency greater than 20 kHz. This helps in the disruption of plant cells to assist the extraction process and increases the surface area for the solvent to penetrate. The plant material is dried and ground to a fine powder, mixed with the right solvent, and packed into the ultrasonic extractor. The sound energy then carries forward the extraction proceeds by reducing the heat requirements.

Table 2.6: Commonly Used Solvents for Extraction of Medicinal Plants

Sr No.	Solvents	Polarity
1	n-Hexane	0.00
2	Petroleum ether	0.1
3	Diethyl ether	0.117
4	Ethyl acetate	0.228
5	Chloroform	0.259
6	Dichloromethane	0.309
7	Acetone	0.355
8	n-Butanol	0.586
9	Ethanol	0.654
10	Methanol	0.762
11	Water	1.000

2.12. Bioactivities of Lemongrass and Peppermint

2.12.1. *Cymbopogon citratus*

Lemongrass (*Cymbopogan citratus),* a member of the Poaceae family is a tall plant that has several striped leaves with an uneven edge. It is grown in different parts of tropical and sub-tropical Southeast Asia and Africa, native to Pakistan, Sri Lanka, and India. (Rehman et al., 2016). Lemongrass is a tall perennial grass belonging to the genus *Cymbopogon* of aromatic grasses and has essential oils with lemon favor of fine taste. Some of its species are cultivated as medicinal herbs because of the scent it possesses or as culinary too.

Table 2.5: Scientific Classification of Lemongrass

Scientific Classification	
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Tracheophytes
Division	Magnoliophyta
Class	Loliopsida
Order	Poales
Family	Poaceae
Genus	*Cymbopogon*
Species	*Cymbopogon citratus*

The plant lemongrass is known to have a mechanism of action that is non-toxic and safe both for the environment and human health. The essential oil of lemongrass is used as a food additive and is Generally Recognized as Safe (GRAS) under the REACH which is a regulation by the European Chemical Agency (ECHA). (Lemongrass Oil Profile, n.d.).

The bioefficacy of lemongrass and tea tree essential oils has shown significant results against the control and management of House Fly (*M. domestica)*. The activity of tea tree essential oil was found to be more lethal against the larvae and adult stage of *M.domestica* with LC50 at 14.88mg/dm3. On the other hand, the lemon grass essential oil was more lethal against the pupicidal stage of house flies with LD50 of 14.99 µl/0.25L in comparison to the tea tree essential oil. (Chintalchere et al., 2021).

The insecticidal property of lemongrass extract is accredited to the various secondary metabolite. It is composed of bioactive cyclic and acyclic terpenes, which disrupt the neurotransmitter in insects. In addition, various alkaloids, flavonoids, and carotenoids have been found in lemongrass extracts, indicating its potential as a bio-insecticide, and tannin compounds may be used as inhibitors of the enzyme activities in insect digestion. Citral is considered for the insecticidal activity of lemongrass extract and essential oil. (Moustafa et al., 2021).

2.12.2. *Mentha x piperita*

Peppermint is a hybrid herb, scientifically known as *Mentha x piperita*, firstly defined in 1753 by Carl Linnaeus. It was created by the combination of two varieties of the mint plant i.e., spearmint and water mint. The plant is green in color with purple and violet flowers, and the stem has a square shape. The plant is known to survive freezing conditions and can grow up to the height of 2 and a half feet. Peppermint being a perennial plant can live up to more than two years of span and can be grown in shady areas and in full sunlight too. The peppermint plant, being a hybrid, does not produce any seeds and reproduced by spreading its rhizomes.

Table 2.6: Scientific Classification of Peppermint

Scientific Classification	
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Mentha
Species	*M. x piperita*

The plant grows a hardy fibrous root system that supports the plant, absorbs water from the soil, and dissolves minerals for the plant. The roots are covered with small root hairs and have root caps made up of dead cells that push through the soil. On the other hand, the stem of the plant provides support to the plant, conducts materials from the leaves to be used for photosynthesis, and displays the leaves. Peppermint has an herbaceous dicot stem with vascular tissues present on the edge of the stem arranged in fibrovascular bundles.

The leaves of peppermint are dicot leaves, attached to the stem helping in the light absorption and turning it into energy for the plant. The leaf has a flattened part referred to as the blade, connected to the petiole. The leaves of peppermint are shiny with violet blossoms that bloom in early summer and late spring. The flowers, however, are tall, and slender, with small violet buds around them. They play a major role in the reproduction and characteristics of the plant.

The peppermint plant is herbaceous, vascular, and perennial and its life cycle includes several steps. The plant reproduces sexually, and the pollinators are attracted to the sweet smell and violet colors of the plant. The flower has different parts including the pistil and the stamen, where the stamen is the male reproductive part of the flower. The stamen further includes the anther, which is responsible for holding the pollen grains. It is long and has skinny filaments sprouting on the pistol side, which is a female reproductive part of the plant. It consists of the ovary and the ovule.

The pollinators when land on the flower of peppermint leave small particles of the pollen, that land on the stigma tip forming a pollen tube. The pollen tube then reaches the ovary and the sperm from the pollen fuses with the egg cell. The zygote is then formed within the pistol, which after being fertilized develops into the seed and continues to grow further. This seed is the beginning of each new plant. (*Peppermint - CreationWiki, the Encyclopedia of Creation Science*, n.d.).

Peppermint is widely distributed in regions across Asia, Africa, Europe, Australia, and North America. All the species have been found to have a diverse commercial role in various industries, including food, ornamental, and medical. (Nieto, 2017). Its role engages in multiple herbal therapeutics, such as against anorexia, liver diseases, nausea, flatulence, bronchitis, and ulcerative colitis. (Ouakouak et al., 2019). Various bioactivities are associated with the *Mentha* species such as they have anti-obesity, anti-inflammatory, anti-bloating, insecticidal, anticancer, analgesic, and antispasmodic actions. (Farzaei et al., 2017)**.**

2.12.2.1. Bioactive constituents of *Mentha* species

The leaves of *Mentha piperita* have different compositions of various constituents, among which they include 1.2-3.9 % (w/v) of the essential oils. The other classes of constituents present in the mint leaves are 52% of monoterpenes, 9% sesquiterpenes, 9% aromatic hydrocarbons, 9% aldehydes, 7% lactones, and 6% of alcohols. One of the major constituents of monoterpenes is menthol, which is around 35% to 60%. While other components of the monoterpenes include 0.1-6% limonene, 3-4% neomenthol, 2-5% isomenthone, 1-13% 1,8-cineole, 0.3-14% menthofuran, 0.7-23% menthyl acetate, and 2-44% menthone. (Eftekhari et al., 2021).

Mint is known to exhibit efficient response against various insects helping in their control and management. *Mentha* species have been used as insecticides and repellents majorly in the form of essential oils and polar extracts in contact, fumigation, and repellency bioassays. (Brahmi et al., 2017)

Mentha piperita plant extract was tested against the cowpea seed beetle (*Callosobruchus maculatus)* and it was found that it significantly affected the relative growth rate, feeding deterrence index, and relative consumption rate of the insect. The oil had strong repellency at 90, 180, and 360 μL/L air concentration evident from the repellency index and it could be used as a low-risk insecticide. (Saeidi & Mirfakhraie, 2017).

The insecticidal activity of *Mentha rotundifolia* essential oil was evaluated under laboratory conditions against two aphids namely *Rhopalosiphum padi* and *Sitobion avenae.* At all the concentrations for essential oils of both the *Mentha* species, the mortality rate increased with the time of exposure. The results further showed that the essential oil of *M. rotundifolia* was toxic to *R.padi* and *S. avenae*, but the former species responded better than the latter one. The study hence suggested the use of essential oil as an alternative strategy in the integrated biological control against the cereal aphids. (Leblalta et al., 2020)

The mint extracts are widely used for the management of stored grain pests population throughout the world. The repellent and insecticidal properties of the *Mentha* species have been investigated against the stored grain pests through a series of experiments. The essential oils and the extracts affect the physiology of insects in diverse ways, leading to the disruption of the balance of insects endocrinologically. Monoterpenoids are the major class of compounds reported to be more toxic than oxygenated compounds against pests. However, the effectiveness of *Mentha* extracts also depends upon the solvent utilized for the extraction. The chosen solvent decides the plant activity of the extract by extracting the insecticidal component of the designated plant. (Kumar et al., 2011).

The current study was conducted to evaluate the response of two most commonly available plant species i.e., *Mentha x piperita* and *Cymbopogon citratus* to evaluate their efficacy against the *Tribolium sp.*

3. MATERIALS AND METHODS

3.1. Chemicals and Reagents

Analytical grade reagent including methanol was used as a solvent for extraction as per the literature. In addition, Dimethyl Sulfoxide (DMSO) was used to prepare dilutions of the plant extracts.

3.2. Pest Collection

T. castaneum was collected from the grain market located in Islamabad.

3.3. Insect Rearing

The insect cultures were reared on a wheat flour in the jars and kept in the incubators at a temperature of 30±2ºC and a relative humidity of 70±5%. Each jar had thirty insects inside it containing 200gm of the wheat flour as a diet. To avoid the insect escape, the jars were covered with a muslin cloth. Then, right after five days the adults were sieved out from the grain flour. The remaining flour that contained eggs were kept in the incubator under same conditions to get another generation of the pest. However, the flour that contained the adults were also transferred into the jar to get homogenous population.

3.4. Collection of Plant Samples

Dried lemongrass leaves and fresh peppermint leaves were obtained from the local market in Islamabad as there as they were the plants under the investigation studies.

Figure 3.1.: Dried Lemongrass Sample and Fresh Peppermint Leaves

3.5. Drying and Grinding

Fresh peppermint leaves were separated from the stems and both the plant samples were thoroughly washed under water and rubbed with fingers to remove any dirt and debris on them. The samples were then dried before further processing. For drying, samples were kept on a newspaper sheet in an open environment for two days and then dried using an Arshia FD-130 Food dehydrator to remove any extra water content for effective extraction for about 3 hours.

Once the plant samples were completely dried, they were ground to a fine powder using a pestle and mortar, and electrical blender. They were ground to an extent to provide more surface area for the extraction of active constituents from the plant samples. After proper grinding, the powder was sieved to obtain the finest particle size. The powder obtained from each plant species was then packed in an airtight container and stored in a cool dry place until further use. The silica gel beads (desiccant) were placed with the sample to prevent them from attaining unwanted moisture and keeping the sample dry.

3.6. Solvent Selection

For the selection of appropriate solvent, literature was thoroughly consulted. Most of the papers cited the use of methanol or ethanol to be highly effective for the extraction of under-study plants. Therefore, methanol was used as an extraction solvent since most of the plant constituents were soluble in it.

3.7. Maceration

Methanolic extracts of both the respective plant samples were prepared using Maceration as an extraction technique. Both the powdered dry plant samples were equally weighed individually, and the extraction was carried out with a 1:10 (sample to solvent) ratio. 23 grams of both powders were taken in two conical flasks separately, and 230 mL of methanol was added to dissolve the plant powders. The content was then allowed to stand for 7-10 days, covered with an aluminum foil to prevent contact with light, and kept in the dark until the extraction was complete.

To maximize the effectiveness of the extraction, the sample and solvent solution was transferred to the 50 mL falcon tubes, covered with aluminum foil, and placed on the tube rollers for a couple of hours.

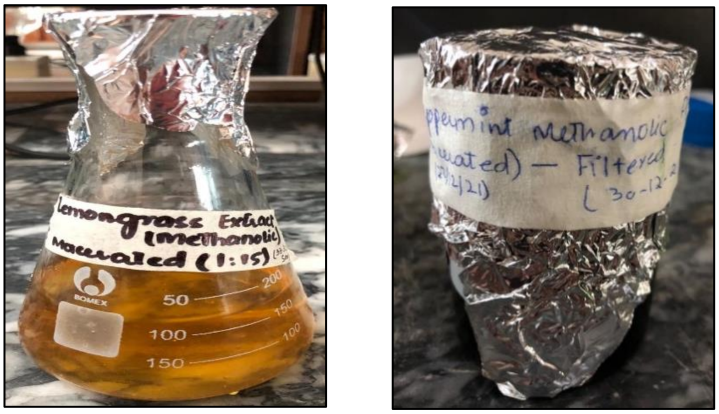

Figure 3.2: Maceration of Plant Materials in Methanol

3.8. Filtration

Once the extraction was complete, the filtration process was performed to separate the extract and the solid tailings of the plant sample. To do so, a separating funnel was taken, above which a Whatman filter paper was placed. The mixture was poured over the filter paper and sieved through it to remove the plant particles. The obtained extract was then used for the respective purpose and the remaining was stored up until further use.

Figure 3.3: Filtration of the extract to separate solid tailings of the plant sample

3.9. Qualitative Screening of the Plant Extracts

The preliminary screening of the plant extracts was done to evaluate the phytochemical characteristics of them isolated from the leaf samples of both the plants. Following are the tests that were performed. (Al-Harbi et al., 2021).

3.9.1. Screening for Tannins

Braymer’s Test was performed for the screening of tannins. 2mL of the plant extract was taken in which 2mL of water was add. Few drops of 5% of FeCl3 were then added. Transient greenish to the black coloration confirmed the presence of tannins.

3.9.2. Screening for Steroids

Salkowski’s Test was performed for the screening of steroids. 2mL of plant extract was taken, into which few drops of cH_2SO_4 were added. It was allowed to stand for few minutes. The presence of red color at the lower layer confirmed the presence of steroids.

3.9.3. Screening for Glycosides

Liebermann’s Test was performed for the screening of glycosides. 2mL of the plant extract was taken, into which 2mL of $CHCl_3$ was added, in addition to 2mL solution of CH_3COOH. Violet to blue to green coloration confirmed the presence of glycosides.

3.9.4. Screening for C. Glycosides

Cardiac glycosides were also tested in the extracts as part of preliminary screening. 2mL of plant extract was taken and 2mL acetic acid was added into it. After that, few drops of $FeCl_3$ were added along with 1mL of CH_2SO_4. Violet coloration indicated the presence of cardiac glycosides.

3.9.5. Screening for Terpenoids

2mL of the extract was taken and dissolved in chloroform, followed by the addition of 2mL sulphuric acid. If a reddish and brown layer was formed at the junction of both the solutions, the presence of terpenoids was indicated.

3.9.6. Screening for Proteins

Xanthoproteic test was performed to confirm the presence of proteins in both the plant samples. 1mL of extract was taken into which 1mL of cH_2SO_4 was added. Formation of white precipitates that turned yellow upon heating was used as an indicator for the presence of protein in sample.

3.9.7. Screening for Phlobatannins

To confirm the presence of phlobatannins in the plant extracts, precipitate test was performed. In this, 2mL of the plant extract was taken into which 2mL of 1% HCl is added. The mixture was heated until it boiled. The presence of red precipitates means that the sample contains traces of phlobatannins.

3.9.8. Screening for Phytosterols

Salkowski's test was performed to confirm whether the sample contained phytosterols or not. 1mL of the plant extract was taken and few drops of cH_2SO_4 were added into it. The mixture was shaken and allowed to stand fora couple for minutes. The appearance of red color at the lower layer indicates the presence of phytosterols in the plant extracts.

3.9.9. Screening for Phenols

To confirm the presence of phenols in both the extracts, 1mL of plant extract was taken into which few drops of $FeCl_3$ were added. The appearance of bluish-black color is an indication of presence of phenols in the extracts.

3.9.10. Screening for Alkaloids

Three tests were performed to confirm the presence of alkaloids in the plant extracts. Procedure followed for these tests is mentioned as below.

3.9.10.1. Mayer's Test

2mL of plant extract was taken into which few drops of Mayer's reagent were added. Yellow precipitates indicate the presence of alkaloids.

3.9.10.2. Wagner's Test

2mL of plant extract was taken into which 2 drops of 1.5% HCl were added. Then, 3 drops of Wagner's reagent were added into the solution. The formation of brown precipitates indicates the presence of alkaloids in the sample.

3.9.10.3. Hager's Test

2mL of the plant extract was taken into which few drops of Hager's reagent were added. The formation of yellow precipitates here serves as an indicator for alkaloids presence.

3.10. Drying and Scraping - Rotary Evaporator

The extract was further concentrated under dried vacuum on the rotary evaporator at a temperature of 50-55℃ for 1 hour. Another alternate method used was where the extracts were placed in a petri dish for quick drying. The petri plates were covered and put in the dark for a couple of days. Both the samples dried out and sticked to the petri plates, after which they were scraped with the help of a steel spatula. The extracts were then saved in a dark place for later use.

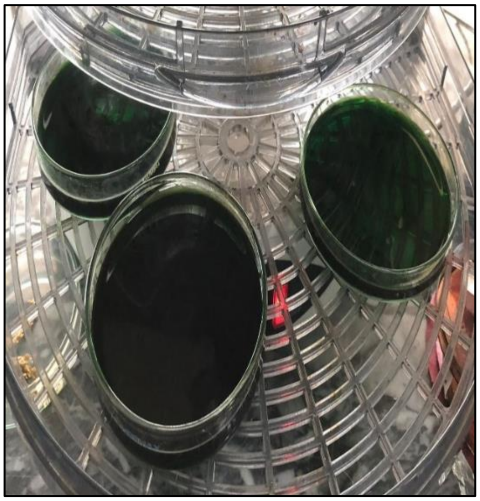

Figure 3.4: Solvent Drying Using Dehydrator

Figure 3.5: Solvent Drying Using Rotary Evaporator

3.11. Preparation of Different Concentrations of Extracts in Dimethyl Sulfoxide (DMSO)

The extract was then scraped off the Petri plates and dissolved in solvent dimethyl sulfoxide (DMSO) to prepare different concentrations for the treatment. (Khan, 2021).

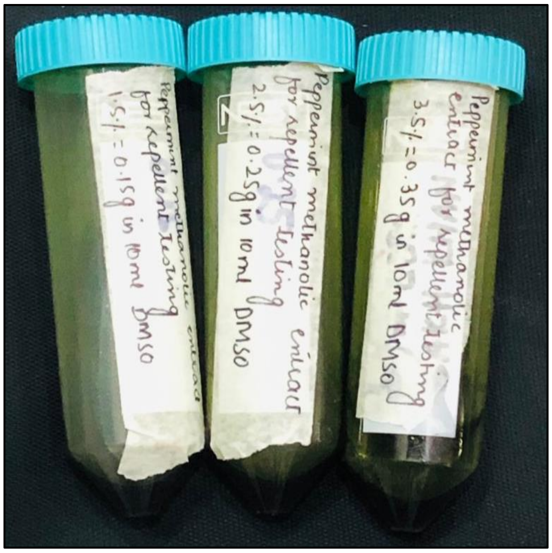

Figure 3.6: Different Concentrations of Plant Extracts in DMSO

3.12. Experimental Setup for Bioassays

Both the assays i.e., Insecticidal activity and Repellency tests were carried out using Completely Randomized Block design. The collected data was then analyzed using the computer program GraphPad Prism 9.3.1.

3.12.1. Mortality Assay

To access the percentage mortality of both the plant extracts under study, different concentrations were prepared i.e., 5%, 10%, and 15% of the extract. Negative control was used as DMSO, containing no amount of plant extract it. In addition, synthetic insecticide (0.02g of permethrin) was used as a positive control to evaluate its effects on the insect mortality. To do so, filter papers were taken and cut into sizes that would fit inside the petri plates. Each filter paper was treated with 2mL of different concentration of plant extracts, negative control, and positive control with three replicates each. The filter papers were then dried at the room temperature for about approximately 30 mins to let any excess amount of solvent evaporate. Once dried, they were placed inside the petri plates, and 10 insects were release in each petri plate. Small amount of diet was added too in the plates to prevent the insect death from starvation. After introduction of the insects, the petri plates were covered with a cling film food wrap to avoid insect escape, and tiny holes were made for the air to circulate. The plates were then kept at 29° C, 70% RH and in darkness. The mortality of the insects was recorded after regular intervals starting from 6 hours, 12 hours, 18 hours, 24 hours, up to 30 hours of exposure to the treated discs. The mortality was then assessed by means of direct observation, and when no leg or antennal movements were observed, the insects were considered.

To calculate the percentage mortality of different treatments, following formula was used.

Percent mortality = Number of dead insects / Number of introduced insects* × *100

The data were then corrected to eliminate the natural mortality in control using the Abbott's formula (Abbott, 1925) as.

% Corrected mortality = % Mortality of treated adult - % Mortality of control adult / 100 - % Mortality of control adult* × *100

Figure 3.7: Experimental Setup for Testing % Mortality of Pest Using Different Concentrations of Plant Extracts with reference to Controls

3.12.2. Repellency Assay

The repellency of the extracts was tested according to (Lehman L. McDonald et al.) method with few changes. Both the plant extracts under study were dissolved in DMSO to prepare different concentrations of the treatments. Various concentrations prepared were 1.5%, 2.5%, and 3.5% to access their repellency effects on the insects. For repellency testing, the filter paper disks were prepared and divided into two equal halves but cutting them from the center. One half of the filter paper disk was treated with plant extract concentration, while the other half was left blank. Equal volumes (2mL) of all concentrations were applied on the paper disks, with three replications per treatment. Controls were added which contained no amount of either of the plant extracts. Both the filter paper halves were then dried for 30 mins at the room temperature and then joined using a sellotape. The disks were then placed inside the petri dishes and 10 insects were released right in the center. Insects were free to move on either side of the disks i.e., the treated half and the untreated half (control). The experiment was maintained at 29° C, 70% RH and in darkness. The repellent behavior of the insects was observed under every treatment after regular intervals. The readings were recorded after 15 mins, 30 mins, 45 mins, 60 mins, and 75 mins.

The percentage repellency of both the extracts was finally calculated using the following formula by (y Lehman L. McDonald et al., n.d.)

$$\textit{Percentage Repellence} = (Nc - Nt) / (Nc + Nt) \times 100$$

Where Nc = number of insects on the untreated (control) half

Nt = number of insects on the treated half

The mean repellency value of each of the extract concentration was calculated using the above formula and assigned to repellency classes starting from 0 to V. Classes were differentiated based on the % repellency range as Class 0 (PR ≤ 0.1%) , Class I (PR = 0.1-20%), Class II (PR = 20.1-40%), Class III (PR = 40.1-60%), Class IV (PR = 60.1-80%), and Class V (PR = 80.1-100%).

Figure 3.8: Experimental Setup for Testing % Repellency of Pest Using Different Concentrations of Plant Extracts with reference to Control

3.13. Statistical Analysis

The results of this study were presented as means ± standard deviation. The data were then subjected to Probit Analysis to determine the LT_{50} and RT_{50} values of both samples at various concentrations used. All the experimental data were analyzed using the statistical software GraphPad Prism 9.3.1. and evaluated by Two-Way ANOVA. Mean differences were tested using Tukey's and the statistical differences at the $p < 0.05$ were considered statistically significant results. In addition, the correlation coefficient ($R^{2)}$ was calculated using the same statistical analysis program.

4. RESULTS

4.1. Qualitative Screening of the Plant Extracts

The following groups were identified using different phytochemical screening tests for both the plants extracts under study.

Table 4.1: Confirmatory analysis of chemical groups present in both plant extracts

Sr no.	Phytochemical Test	*Mentha x piperita*	*Cymbopogon citratus*
1	Tannins	+	+
2	Steroids	+	+
3	Glycosides	+	+
4	C.Glycosides	+	+
5	Terpenoids	+	+
6	Proteins	-	+
7	Phlobatannins	-	-
8	Phytosterols	-	-
9	Phenols	+	+
10	Alkaloids		
	Mayer’s’ Test	+	+
	Wagner’s test	+	+
	Hager’s Test	+	+

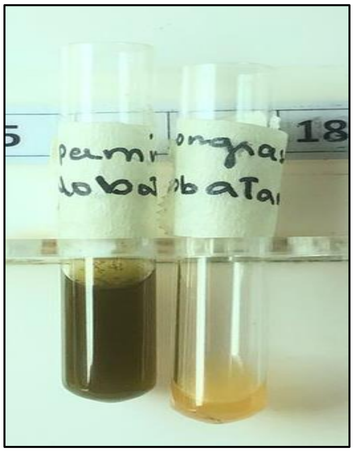

Figure 3.9: Test for Phlobatannins

Figure 3.10: Test for Phytosterols

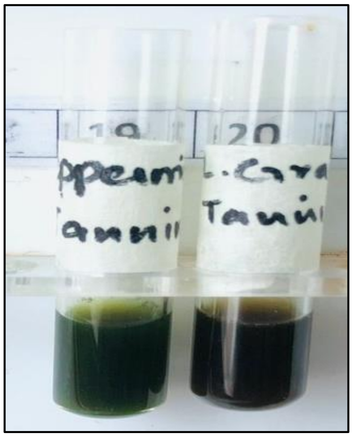

Figure3.11: Test for Tannins

Figure 3.12: Test for Proteins

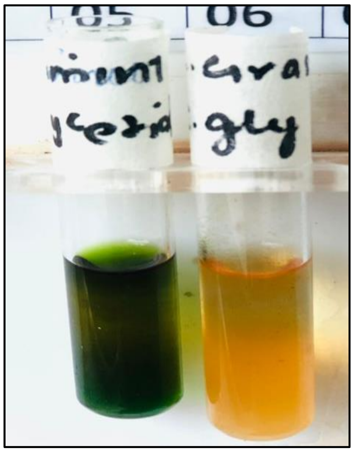

Figure 3.13: Test for Glycerides

Figure 3.14: Test for Terpenoid

Figure 3.15: Test for Steroids

4.2. Insecticidal Efficiencies of Plant Extracts

4.2.1. Insecticidal Activity of *Mentha x Piperita* Plant Extract

The mortality of *Tribolium castanuem* (Red flour beetle) was determined by using the crude methanol extracts from two plants including *Cymbopogon citratus* and *Mentha x piperita.* The findings of the experiment revealed that the percentage mortality of the insect was dependent on the plant tested, the concentration of the extract, and the exposure time. Both the botanical extracts showed adverse effects against the pest with the increase in concentrations and prolonged exposure times.

Percent mortality of *Tribolium castaneum* with the interaction of time interval and different concentrations of *Mentha x piperita* plant extract are shown in the figure 19. The mortality was determined by applying three concentrations i.e., 5%, 10%, and 15% of the extract over 30 hours of close exposure. Mortality was recorded after every interval of 6 hours up to a total of 30 hours to evaluate the response of each concentration in each amount of time. The mortality effects of 5 %, 10%, and 15% *Mentha x piperita* extracts on *Tribolium castaneum* were significantly different from the effect of synthetic insecticide Permethrin at 6 hours of the treatment ($p<0.05$, $q=5.576$ (5%), $q=5.576$ (10%), $q=4.055$ (15%)). However, any significant difference was not observed between the concentrations themselves within 6 hours of exposure to the treatment ($p>0.05$). After 12 hours of exposure, 15% plant extract was not significantly different from the synthetic insecticide Permethrin ($p>0.05$, $q=2.027$) in terms of mortality. The same trend was observed for up to 30 hours where both the extract and Permethrin exerted 100% adult mortality against the target insect.

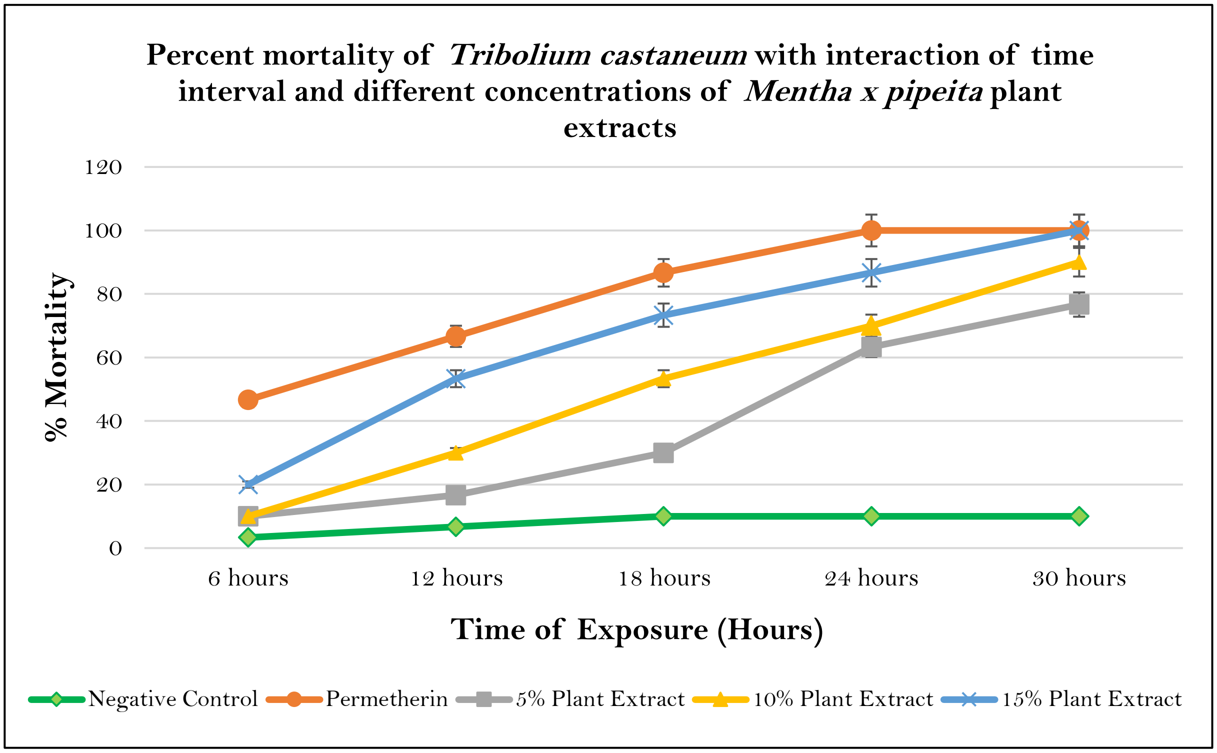

Figure 4.1: Percent mortality of Tribolium castaneum with the interaction of different time intervals and different concentrations of Mentha x piperita

In terms of the various concentrations used, the mortality effects exerted by 5% and 15% of the plant extract differed significantly (p<0.05) at 18 hours of exposure. However, there was not any significant difference observed between 10% and 15% after 24 hours of exposure for up to 30 hours. In contrast, there was a slightly significant difference in adult mortality caused by 5% plant extract (30% ± 20) and 10% plant extract (53.33% ± 23.09) at 18 hours of exposure. In general, 5% of the *Mentha x piperita* extract caused mean adult mortality of 76.7% ± 25.2, 10% of the extract caused mean mortality of 90% ± 17.3, whereas 15% of plant extract killed 100% ± 0 of the target insects at 30 hours of the treatment. Therefore, the results show that mortality increases with the increase in concentration (powder dose) and with the length of hours. However, the adult mortality of *Tribolium castaneum* caused by various concentrations of the tested extracts was higher than those of the negative control (p<0.05) throughout the exposure time of the treatment since there was little to no mortality at all in the control.

All the concentrations of the prepared extracts gave variable impacts on the mortality of the target insect with respect to different intervals of time and increases in dosage. The bar chart given below indicates the mean mortality effect of *Mentha x piperita* extracts at three different concentrations i.e., 5%, 10%, and 15% where each of the treatments shows the trends of percent mortality after the interval of every 6 hours. Probit analysis was also performed to determine the Lethal Time (LT_{50}) required by every concentration to cause 50% mortality in a group of insects under study. The Probit Regression Analysis for LT_{50} revealed that the greater the concentration of plant extracts, the shorter the time required to kill 50% of the target insects.

The lowest concentration i.e., 5% of *Mentha x piperita* extract required the greater time to kill 50% of the target pest (LT_{50}=21.20 hrs., $y = 3.0169x + 0.9998$). On the contrary, 10% plant extract and 15% plant extract had LT_{50} values of LT_{50}=15.8 hrs., $y = 3.6148x + 0.6656$ and LT_{50}=11.6 hrs., $y = 4.8989x - 0.2303$, respectively. Synthetic insecticide Permethrin, used as a positive control had the lowest LT_{50}=7.8 hrs., $y = 6.2552x - 0.5907$ since it was significantly most effective of all the treatments at 6 and 12 hours of exposure.

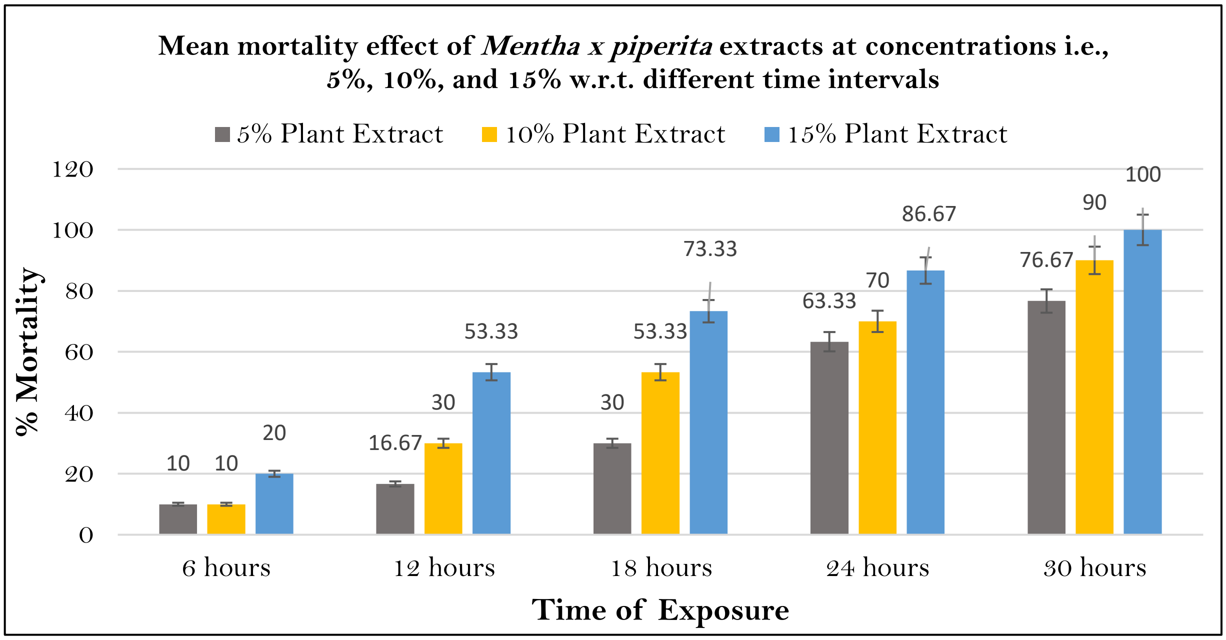

Figure 4.2: Mean mortality of Mentha x piperita plant extracts at different concentrations w.r.t. different exposure periods

Table 4.2: Means and Standard Deviation of Mortality Rates After Different Exposure Periods

		Means and standard deviation of mortality rates after different periods of exposure (hours)				
	Treatments	**6**	**12**	**18**	**24**	**30**
Peppermint	**Positive control**	46.7 ± 5.77	66.7 ± 11.5	86.7 ± 5.77	100 ± 0	100 ± 0
	Negative control	3.33 ± 5.77	6.67 ± 5.77	10 ± 0	10 ± 0	10 ± 0
	5%	10 ± 10	16.7 ± 20.8	30 ± 20	63 ± 15.2	76.7 ± 25
	10%	10 ± 10	30 ± 10	53.3 ± 23.1	70 ± 10	90 ± 17.3
	15%	20 ± 0	53.3 ± 11.5	73.3 ± 11	5.77	0

4.2.2. Insecticidal Activity of Cymbopogon citratus Plant Extract

The mortality effect on the target pest Tribolium castaneum was evaluated also evaluated using the indigenous crude methanol extract of Cymbopogon citratus. The extract showed efficient activity against the insects at different concentrations and exposure periods at different intervals of time. The concentrations were applied at three different dose ratios i.e., 5%, 10%, and 15%. According to the obtained results, both the concentrations (dose rate) and exposure periods had significant effects on the mortality of the pest as it increased with the increase in dose and exposure time. Each dose showed different percent mortalities at every interval and their significance was recorded with respect to the synthetic insecticide Permethrin, used as a positive control in the experiment.

The results of the Two-Way ANOVA showed that the effect of mortality significantly varied with two tested variables i.e., time and concentration. The results of various concentrations of the extract were compared with the percent mortality caused by synthetic chemical Permethrin in the same amount of time. At the exposure of 6 hours in the beginning, there was high significant difference ($p<0.05$) between Permethrin and each concentration of the extract used (5% $p<0.05$, $q=12.90$; 10% $p<0.05$, q=10.92; 15% $p<0.05$, q=6.947). The mean percentage mortality at 6 hours of exposure for 5% extract was 6.67% ± 5.77, for 10% it was 13.33% ± 5.77, and for 15% it was 26.7% ± 5.77. Whereas synthetic chemical used as a positive control showed mean percentage mortality of 50% ± 10. There was no significant difference ($p>0.05$) between the response of 5% and 10% dose concentration of the extract at 6 hours, however, slight significance was observed between the respective concentrations at the exposure period of 12 hours where 5% showed percent mortality response of 13.7% ± 5.77, and 10% showed mortality of 33.3% ± 5.77. High significance was recorded in the responses of concentrations with an increase in the exposure period. At 18 hours of exposure, 5%, 10%, and 15% extract showed mortality of 46.7 ± 5.77%, 57% ± 5.77, and 73.3% ± 5.77. The results depict that the effect of plant extract on the mortality rate depends on multiple factors, most important of which include the applied dosage/concentration and period of exposure. The bar chart given below indicates the increase the mortality with every concentration of extract applied. The highest efficacy shown by 5%, 10% and 15% was 86.7% ± 5.77, 96.7% ± 5.77, and 100% ± 0 respectively at 30 hours of exposure time. The adult mortality of Tribolium castaneum caused by various concentrations of the tested extracts was higher than that of the negative control ($p<0.05$) throughout the exposure time of the treatment since there was little to no mortality at all in the control. On the contrary, no significant difference ($p>0.05$) was observed between the mean percentage mortalities of 10% (96.7% ± 5.77) and 15% (100% ± 0) extract with that of the synthetic chemical (100% ± 0).

Probit analysis was also performed to determine the Lethal Time (LT50) required by every concentration to cause 50% mortality in a group of insects under study. The Probit Regression Analysis for LT50 revealed that the greater the concentration of plant extracts, the shorter the time required to kill 50% of the target insects.

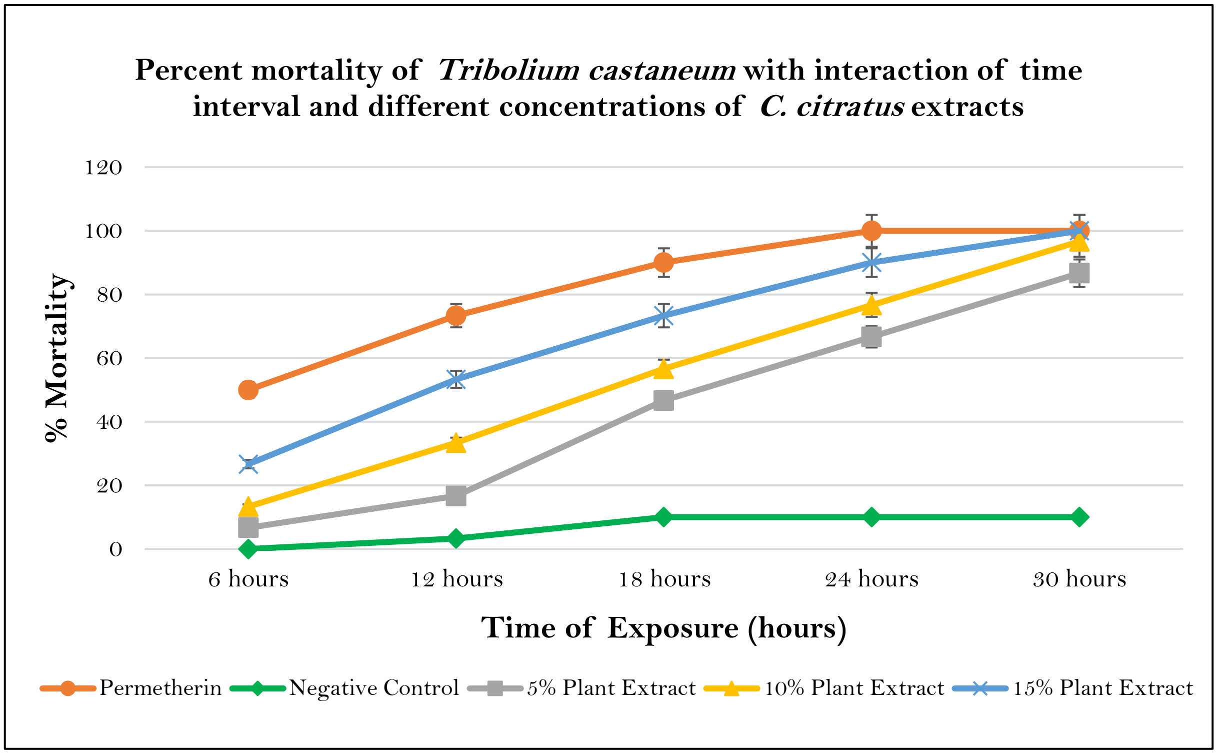

Figure 4.3: Percent mortality of Tribolium castaneum with interaction of time interval and different concentrations of Cymbopogon citratus plant extracts

The lowest concentration i.e., 5% of *Cymbopogon citratus* extract required a greater time to kill 50% of the target pest (LT_{50}=18.5 hrs., y = 4.1654x - 0.2805). On the contrary, 10% plant extract and 15% plant extract had LT_{50} values of LT_{50}=14.9 hrs., y = 4.0038x + 0.2942 and LT_{50}=10.8 hrs., y = 6.4037x - 1.55, respectively. Synthetic insecticide Permethrin, used as a positive control had the lowest LT_{50}= 6.04 hrs., y = 6.1988x + 0.1567 since it was significantly most effective of all the treatments at 6 and 18 hours of exposure.

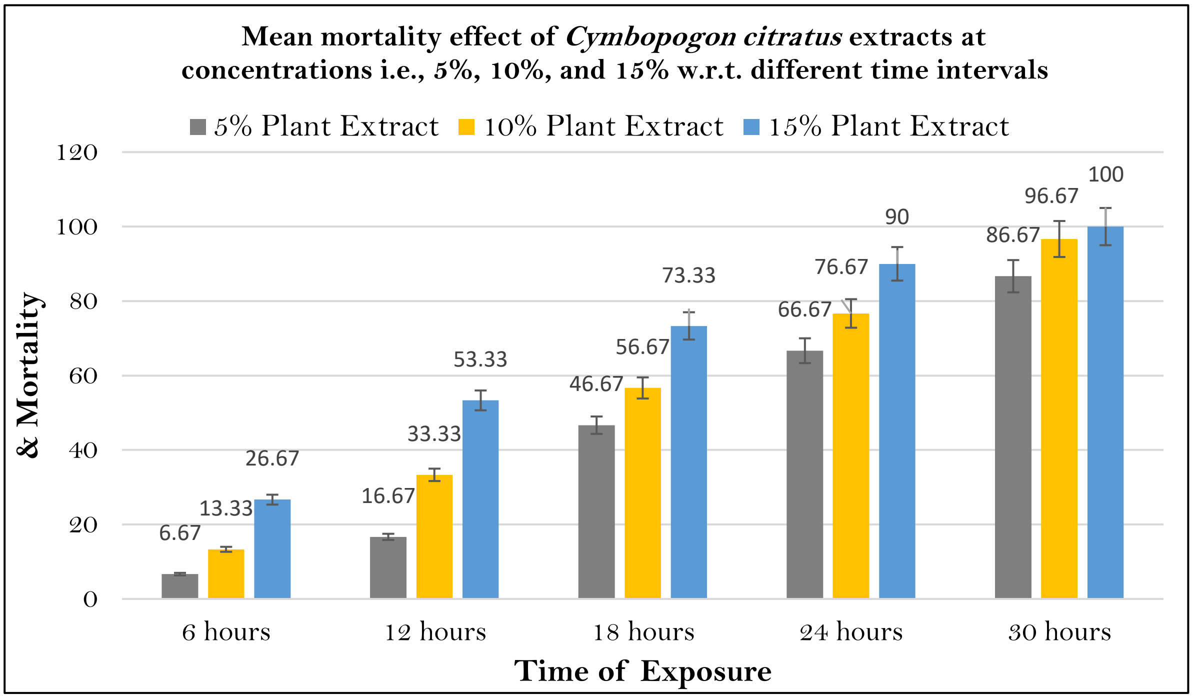

Figure 4.4: Mean mortality effect of Cymbopogon citratus plant extracts at three different concentrations w.r.t. different time intervals

The mortality effect of each plant showed different efficacies under different intervals of time. The values of maximum mortality differed from one plant to the other when noted at all the exposure times. On comparison of equal concentrations of extracts from both the plants, it was found that *Cymbopogon citratus* showed better results against *Tribolium castaneum* at every concentration applied than that of mortality exerted by *Mentha x piperita* in the same exposure time. After 30 hours of exposure, the percentage mean mortality caused by 5% *Cymbopogon citratus* extract was 86.7 ±

5.77%. whereas, under the same exposure time 5% *Mentha x piperita* extract had a mean mortality of 76.7 ± 25.2%. As the concentration increased up to 10%, so did the percentage mean mortality. 10% of *Cymbopogon citratus* and *Mentha x piperita* extracts showed a percentage mean mortality of 96.7 ± 5.77%, and 87 ± 11.5% respectively. Lastly, the treatment of the highest concentrations of both the extracts had mean mortality of 100 ± 0%, giving no significant difference at 30 hours of exposure time. Since *Cymbopogon citratus* extract had greater efficiency than *Mentha x piperita*, it was also able to induce 50% mortality of *Tribolium castaneum* earlier at every concentration applied.

Table 4.3: Means and Standard Deviation of Mortality Rates After Different Exposure Periods

		Means and standard deviation of mortality rates after different periods of exposure (hours)				
	Treatments	**6**	**12**	**18**	**24**	**30**
Lemongrass	**Positive control**	50 ± 10	73.3 ± 5.77	90 ± 0	100 ± 0	100 ± 0
	Negative control	0 ± 0	3.33 ± 5.77	10 ± 0	10 ± 0	10 ± 0
	5%	6.67 ± 5.77	13.7 ± 5.77	46.7 ± 5.77	66.7 ± 15.2	86.7 ± 5.77
	10%	13.3 ± 5.77	33.3 ± 5.77	57 ± 5.77	76.7 ± 5.77	96.7 ± 5.77
	15%	26.7 ± 5.77	53.3 ± 11.5	73.3 ± 5.77	90 ± 10	100 ± 0

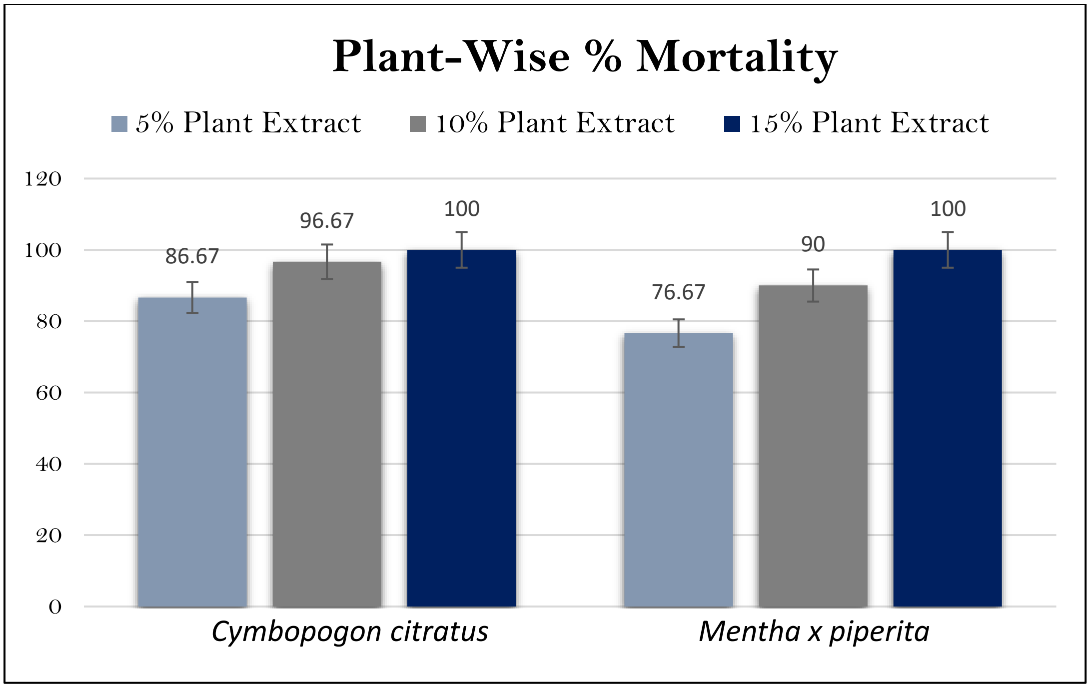

Figure 4.5: Comparison of mortalities exerted by all three concentrations 5%, 10%, and 15% of Mentha x piperita and Cymbopogon citratus plant extracts

Table 4.4: **LT50 values and regression equations of both plants from mortality assay at different concentrations**

Mentha x piperita		
Treatments	**LT_{50} (hours)**	**Simple Linear Regression**
5%	21.2	$y = 3.0169x + 0.9998$
10%	15.8	$y = 3.6148x + 0.6656$
15%	11.6	$y = 4.8989x - 0.2303$
Cymbopogon citratus		
Treatments	**LT_{50} (hours)**	**Simple Linear Regression**
5%	18.5	$y = 4.1654x - 0.2805$
10%	14.9	$y = 4.0038x + 0.2942$
15%	10.5	$y = 6.4037x - 1.551$

4.3. Repellency of Plant Extracts

4.3.1. Repellency Efficiency of *Mentha x piperita* Plant Extract

The repellency of *Mentha x piperita* plant extract was tested against *Tribolium castaneum* at different periods of exposure and under different prepared concentrations of the extract. Results of the present study revealed significant effects of the peppermint extract in causing the repellency of the target pest. The highest repellency was observed in the positive control treatment i.e., Permethrin, and no repellency was observed in the solvent only (negative control) treatment. The insects were exposed to the treatment of extract starting from 15 mins up to a total of 75 mins, and % repellency was observed after the interval of every 15 mins in each of the given concentrations of the extract.

Initially, there wasn’t any significant repellent activity observed, but the response increased with the increase in exposure period and increased concentration of the extract. At 15 mins of exposure time, 1.5% treatment of the extract showed the lowest repellency of 20 ± 0%, whereas 2.5% and 3.5% extracts had a repellent response of 33.3 ± 11.57% and 53.3 ± 11.54% respectively. On the other hand, the % repellency given by the positive control in the same amount of time i.e., 15 mins of exposure was up to 80 ± 0%, the highest of all the plant extract treatments. This showed great significance ($p<0.0001$) in between each concentration of the extract and positive control. The same trend followed for up to 15 mins, after which there was no to slight significance between the peppermint extract and synthetic chemical treatment. After 30 mins of exposure, the response of *Tribolium castaneum* against the plant extract increased with the increase in repellency in each given treatment. The repellency of 1.5%, 2.5%, and 3.5% increased up to 46.7 ± 11.5%, 53.3 ± 11.6%, and 53.3 ± 11.54% respectively. The results at this given amount of time revealed no significant difference between 1.5% and 2.5% of extract concentration, however, there was high significance observed between 1.5% and 3.5%, and 2.5% and 3.5% dosage of the extract used. Chemical insecticide permethrin had 93.33 ± 11.55% repellency at this point which wasn’t distant from that of the repellency caused by the 3.5% treatment of the extract (86.7 ± 11.54%, $p>0.05$). Further response of the target insect was monitored at 45 mins of the exposure period where repellency by each dosage increased with respect to its effectiveness against the insect. Positive control gave complete repellency of 100 ± 0% at 45 mins, whereas there was no significant difference ($p>0.05$, q=1.374) between 1.5% and 2.5% of the plant extracts with reference to their percentage repellency of 60 ± 0% and 66.7 ± 11.6% respectively. On the contrary. 2.5% and 3.5% extract concentrations had mean repellency of 66.7 ± 11.6% and93.3 ± 11.54% with significant difference ($p<0.05$, q=5.494).

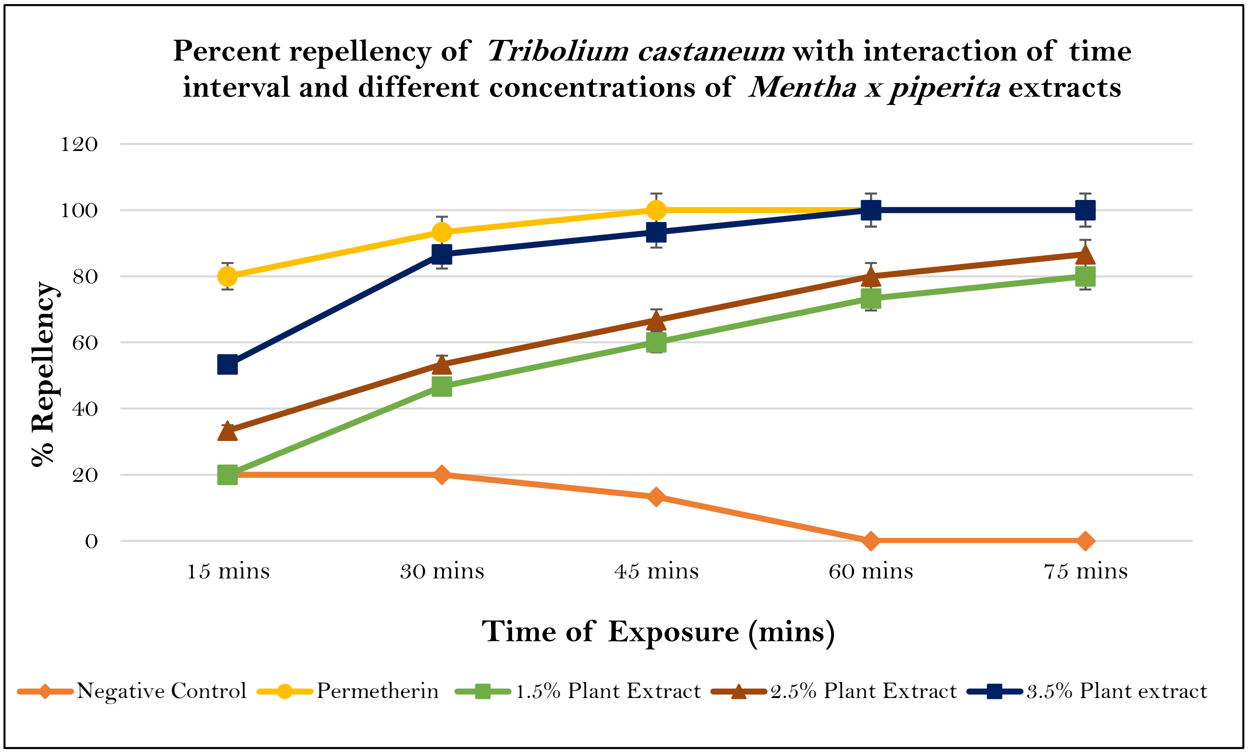

Figure 4.6: Percent repellency of Tribolium castaneum with interaction of time interval and different concentrations of Mentha x piperita plant extracts

With the increase in the exposure period, the extracts showed a somewhat similar response as that posed by the synthetic chemical Permethrin on the target pest. After 75 mins of exposure, there was no significant difference between the repellency caused by 2.5% and 3.5% of plant extracts to that of positive control. The mean repellency after 75 mins of exposure observed was 100 ± 0% for positive control, 80 ± 0% (Class IV) for 1.5% plant extract, 87 ± 11.5% (Class V) for 2.5% plant extract, and 100 ± 0% (Class V) for 3.5% treatment of the extract. These were the highest repellencies caused by each respective dosage of the extract in the same amount of time.

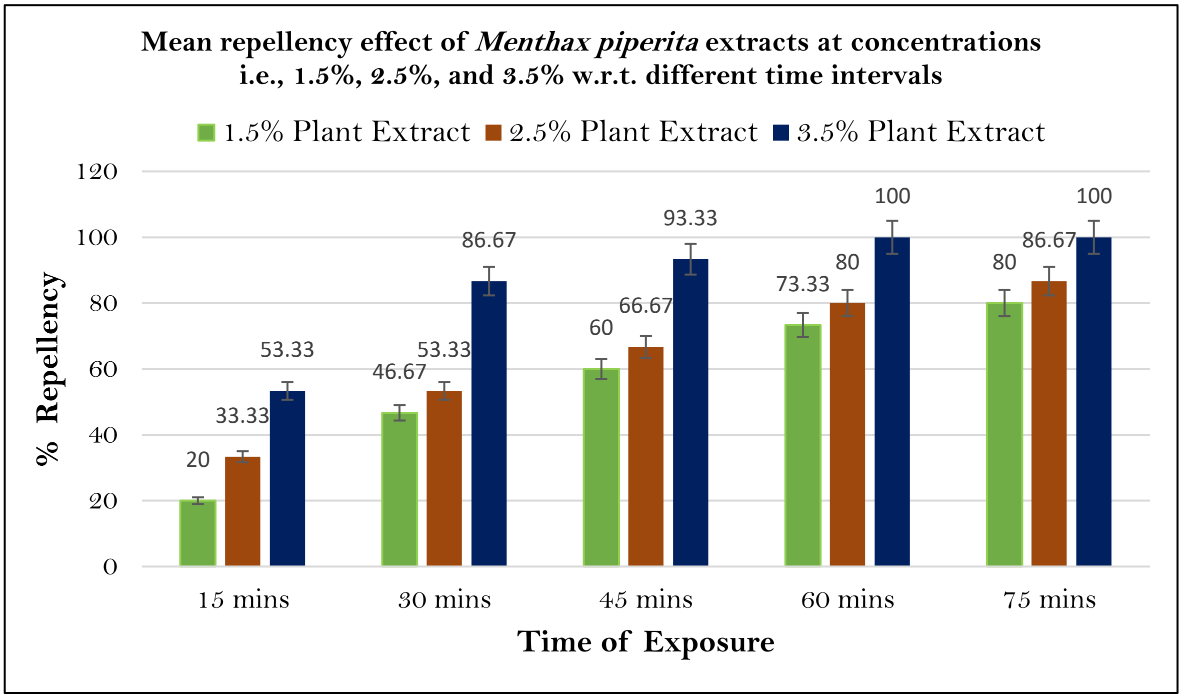

Figure 4.7: Mean repellency of Mentha x piperita plant extracts at different concentrations w.r.t. different time intervals

Each concentration of the extract showed a different repellency percentage by the end of treatment time; however, each exerted an increase in the response by the *Tribolium castanuem* at different times of exposure (in mins). Probit analysis was also performed which determined the Median Repellent Time (RT_{50}) of each concentration to know at what amount of time 50% repellency in each of the given treatments was observed.

The value for RT_{50} varied with each concentration and the exposure period and the highest RT_{50} was required by 1.5% of the plant extract which was approximately 34.03mins., $y = 2.3676x + 1.373$, and the lowest time required to repel 50% of the test subjects in the experiment was by synthetic repellent i.e., 11.07 mins., $y = 5.175x - 0.4042$. Among the concentrations of plant extracts used, 3.5% had the lowest RT_{50} of 15.2 mins., $y = 5.356x - 1.3363$, and 2.5% had the RT_{50} of 26.51 mins., $y = 2.1977x + 1.8715$. The data indicate that among all the applied concentrations, 3.5% plant extract was the only one to have repelled 50% of the insects in the initial 15 mins of the treatment, while the lower concentrations had a lower amount of repellency when compared to the of 3.5% extract (p<0.05).

Table 4.5: Means and standard deviation of repellency after different periods of exposure (mins)

		Means and standard deviation of repellency after different periods of exposure (mins)				
	Treatments	**15**	**30**	**45**	**60**	**75**
Peppermint	**Positive control**	80 ± 0	93.3 ± 11.5	100 ± 0	100 ± 0	100 ± 0
	Negative control	20 ± 0	20 ± 0	13.3 ±5.77	0 ± 0	0 ± 0
	1.50%	20 ± 0	46.7 ± 11.5	60 ± 0	73.3 ±11.5	80 ± 0
	2.50%	33.3 ± 11.5	53.3 ± 11.6	66.7 ± 11.6	80 ± 20	87 ±11.5
	3.50%	53.3 ± 11.5	86.7 ± 11.5	93.3 ±11.5	0	0

4.3.2. Repellency Efficiency of *Cymbopogon citratus* Plant Extract

The extract of *Cymbopogon citratus* showed significant results on the repellency of *Tribolium castaneum* at different periods, as there was an increase in the response of the target insect with the increase in length of hours and the concentration of extracts applied. All three concentrations of the lemongrass extract exerted an increase in the percentage repellency. In the initial 15 mins of the treatment, 1.5% extract showed repellency of 26.7 ± 11.55%, 2.5% had a repellency of 33.3 ± 11.54, and 3.5% showed the greatest repellency of all the treatments at 15 mins of exposure time i.e., 46.7 ± 11.5%. There was no difference in the response of 1.5% extract after 30 mins of exposure. On the contrary, 2.5% and 3.5% extract concentration increased the response up to 40 ± 20% and 73.3 ± 11.54% respectively after 30 mins of treatment. There was no significant difference (p>0.05, q=3.560) observed between the highest concentration of extract i.e., 3.5%, and the synthetic chemical used as a repellent in the experiment at 30 mins of treatment. However, the response exerted by 1.5% and 3.5% extract had a great significance ($p<0.05$, $q=8.307$) in terms of mean repellency caused by them at 30 mins exposure time. The same trend for significance was followed for up to maximum exposure time i.e., 75 mins, which depicted that mean repellency caused by the plant extract was dependent on the dosage used and the time of exposure. All the concentrations had an individual impact on the target insect but there was no significance observed between the treatment of synthetic chemical used and 3.5% of plant extract at 75 mins of the exposure period ($p>0.05$, $q=0.000$). Whereas the results of 1.5% and 2.5% extract did show significant differences when compared to that of the synthetic repellent at 15 mins, 30 mins, 45 mins, and 60 mins of the exposure time, which, however, decreased at 75 mins exposure time.

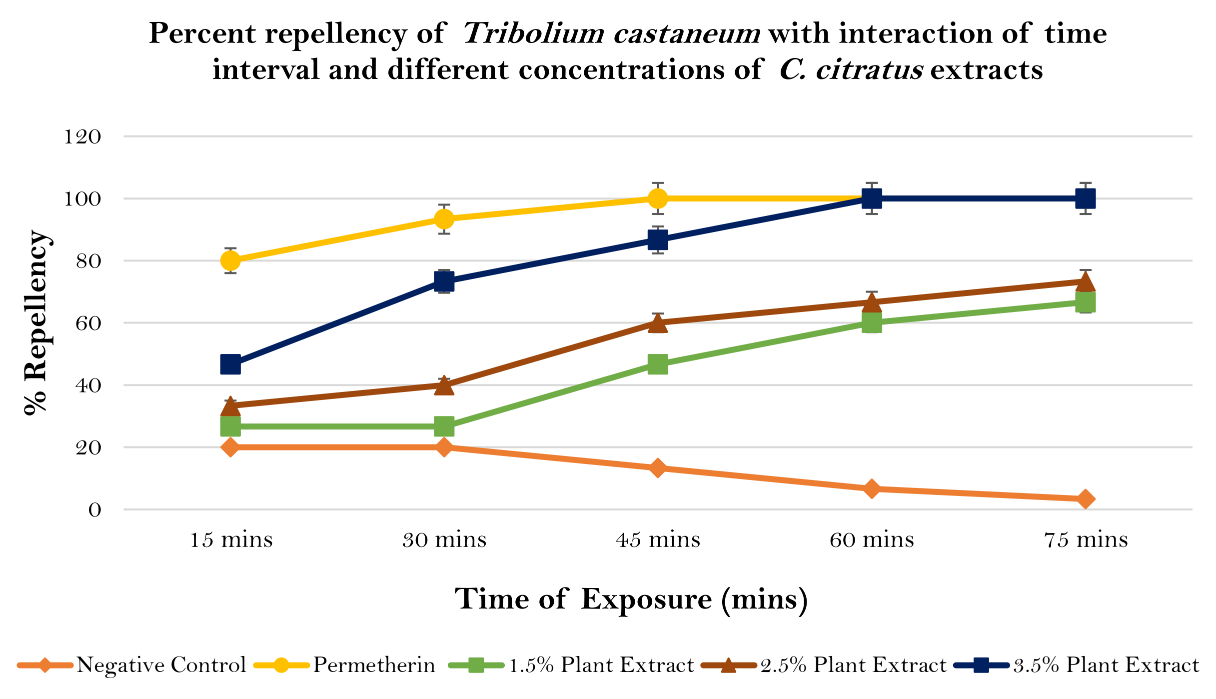

Figure 4.8: Percent repellency of Tribolium castaneum with interaction of time interval and different concentrations of Cymbopogon citratus

The bar chart given below depicts the mean repellency caused by every concentration in a specific time and the maximum response exerted by each of the concentrations in the total amount of exposure time. The percentage repellency for 1.5%, 2.5%, and 3.5% of extracts applied increased from 46.7 ± 11.5% to 66.7± 11.55% (Class IV), 60 ± 0% to 73.3 ± 23.09% (Class IV), and 86.7 ± 11.54% to 100 ± 0% (Class V) from 45 mins to 75 mins of exposure time respectively. Throughout the treatment time, there was no significance observed between 1.5% and 2.5% of the extracts used (p>0.05).

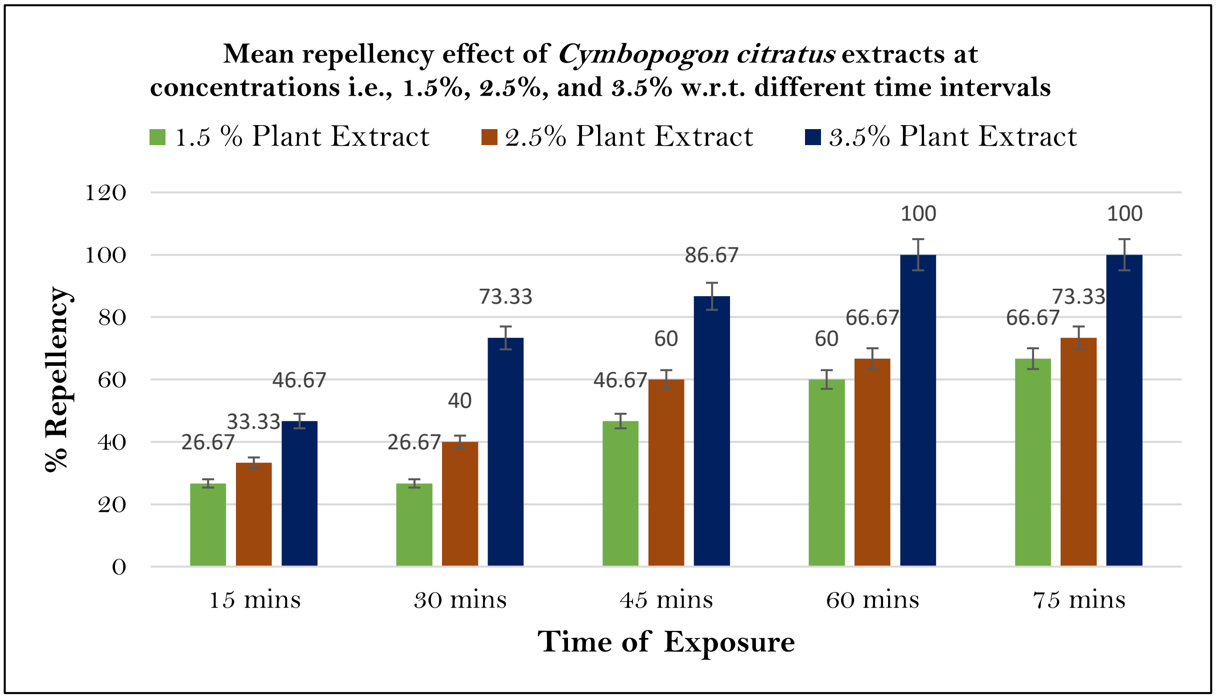

Figure 4.9: Mean repellency effect of Cymbopogon citratus plant extracts at three different concentrations w.r.t. different time intervals

Probit analysis confirmed the Median Repellent Time (RT_{50}) of each concentration at which 50% repellency in each of the given treatments was observed. 1.5% of plant extract concentration was able to exert 50% repellency in the test subjects after 49.15 mins of exposure with a simple linear regression equation of y = 1.5737x + 2.3388. On the other hand, the time required by 2.5% extract to repel 50% of the insects was 36.05 mins., y = 1.4304x + 2.7729, lesser than that required by the lowest concentration treatment (1.5%). Lastly, 3.5% extract required the lowest time of all extracts due to its strong dosage and more efficacy to cause 50% repellency at 16.7 mins., y = 5.4815x - 1.7102.

Table 4.6: Means and standard deviation of repellency after different periods of exposure (mins)

		Means and standard deviation of repellency after different periods of exposure (mins)				
	Treatments	**15**	**30**	**45**	**60**	**75**
Lemongrass	**Positive control**	80 ± 0	93.33 ± 11.5	100 ± 0	100 ± 0	100 ± 0
	Negative control	20 ± 0	20 ± 0	13.3 ± 5.77	6.67 ± 5.77	3.33 ± 5.77
	1.50%	26.7 ± 11.5	26.7 ± 11.5	46.7 ± 11.5	60 ± 0	66.7± 11.5
	2.50%	33.3 ± 11.5	40 ± 20	60 ± 0	66.7 ± 11.5	73.3 ± 23.1
	3.50%	46.7 ± 11.5	73.3 ± 11.5	86.7 ± 11.5	100 ± 0	100 ± 0

The results of percentage repellency of *Cymbopogon citratus* and *Mentha x piperita* shown in the table 8 and 9 respectively represent that response obtained by *Tribolium castaneum* was not only dose and time dependent but also varied from one plant to the other. The active ingredients present in the medicinal plants have different modes of action which determines the severity of response of the target insect. As per the data obtained, *Mentha x piperita* had better repellent property to that of *Cymbopogon citratus* under the same exposure time. However, the repellency did increase with the increase in length of exposure period, but *Mentha sp.* attained 50% repellency earlier at every concentration than that of *C.citratus*. The bar chart given below shows the effect of each concentration of both plants on the target insect where 1.5% of *Mentha piperita* and *C.citratus* showed mean repellency of 80 ± 0% and 66.7± 11.55% respectively after 75 mins of exposure. The response increased with the increase in concentration and 2.5% *Mentha piperita* and *C.citratus* extract gave percentage mean repellency of 87 ± 11.5% and 73.3 ± 23.09% respectively. Lastly, the treatment of the highest concentrations of both the extracts had mean repellency of 100 ± 0%, giving no significant difference at 75 mins of exposure time. Since *Mentha x piperita* extract had greater efficiency than *Cymbopogon citratus*, it was also able to induce 50% repellency of *Tribolium castaneum* earlier at every concentration applied.

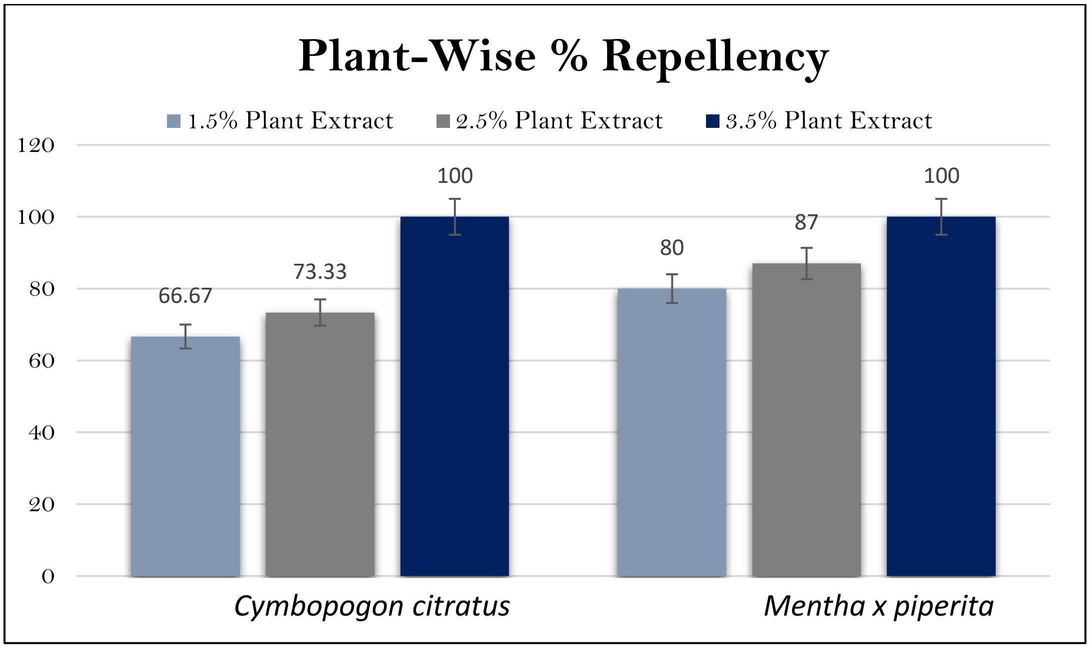

Figure 4.10: Comparison of repellencies exerted by all three concentrations 1 5%, 2.5%, and 3.5% of Mentha x piperita and Cymbopogon citratus plant extracts

Table 4.7: LT50 values and regression equations of both plants from repellency assay at different concentration

Mentha x piperita		
Treatments	**RT$_{50}$ (mins)**	**Simple Linear Regression**
1.5% (Class IV)	34.05	y = 2.3676x + 1.373
2.5% (Class V)	26.5	y = 2.1977x + 1.8715
3.5% (Class V)	15.2	y = 5.356x - 1.3363
Cymbopogon citratus		
Treatments	**RT$_{50}$ (mins)**	**Simple Linear Regression**
1.5% (Class IV)	49.1	y = 1.5737x + 2.3388
2.5% (Class IV)	36.05	y = 1.4304x + 2.7729
3.5% (Class V)	16.7	y = 5.4815x - 1.7102

5. DISCUSSION

The management of stored-grain pests has always been a constant challenge to agricultural researchers as stored grain infestation is one of the intense issues. This causes severe economic damage and decays in food quality due to various stresses leading to annual losses of food grains. These insects are widely distributed and notorious enough to attack many commodities leading to severe infestations and making them highly unfit for human consumption. (Ahmad et al., 2021b). At present, various control strategies are put into action across the world for the prevention of loss of stored grain to a maximum level. (Ejaz Ashraf et al., 2014). Most of these methods include chemical strategies including various fumigants, organophosphates, insect growth regulators, and organochlorides to minimize the spread of stored-grain pests (Riaz et al., 2018). One of the major concerns with the use of these control measures is that they lead to the development of insect resistance leading to pest resurgence. Not only this, but they also pose detrimental effects on human health due to pesticide intoxication. This sole reliance on pesticides emphasizes the need for substantial research work for the development of eco-friendly and cost-effective methodologies as an alternative to reduce environmental contaminations.

For this purpose, various botanicals have been found effective as the researchers are looking for safer, cheaper, and locally available derivatives for the management of stored grains. These botanicals have the potential of being suitable candidates for pest control as most of the reported plant species for this purpose are a part of the human diet and are not harmful to humans. The bioactive compounds from various such plants are extracted and can be used as an eco-friendly agent to fit the best as an alternative for synthetic pest control products (du Jardin, 2015; Singh Gurjar et al., 2012), in the form of fumigants, contact pesticides, and repellents too. (Nikolaou et al., 2021).

Among several pests, *Tribolium castaneum* has been a major problem in the storage of food grains over the years. This study was conducted to evaluate the response of two most commonly available plant species i.e., *Mentha x piperita* and *Cymbopogon* *citratus.* Both the selected plants had natural activities causing mortality and repellency against the *T.castaneum* making them suitable candidates for its control and management. Preliminary phytochemical screening of both the plants further confirmed the presence of a group of active constituents responsible for causing repellent and mortality action against the pest. However, the efficacy of these activities highly depends upon the extraction solvent used as the right choice of solvent is important for acquiring quality bioactive molecules (Abubakar & Haque, 2020b). Several studies reported the use of methanol solvent to attain maximum extraction yield and for maximum response against the target pest. Karunaratne evaluated methanol, ethanol, and acetone extracts of four plants to assess the repellent activity caused by them against *Callosobruchus maculatus.* Among all three solvent extracts, methanol extract showed the strongest and highest repellent effect against the target pest.

5.1. Toxicity Effect

Keeping under consideration the ever-rising concerns about the use of synthetic insecticides, present search work was conducted to evaluate the response of two plants extracts against *Tribolium castaneum* (Red flour beetle). The target insect population was exposed to three concentrations (5%, 10%, ad 15%) of plant extract peppermint and lemon grass. 2mL of each extract was applied on the filter paper and allowed to dry until the excess solvent evaporated. In each Petri plate, ten insects were released, and a small amount of diet was added to avoid death from starvation. Each concentration was repeated three times by using a completely randomized design. Their mortality data were collected after 6, 12, 18, 24, and 30 hours. Abbott's formula was then used to calculate the corrected mean mortality value.

The findings of our study revealed that methanolic extracts of both the plants had a significant impact on the mortality and repellency of *Tribolium castaneum* and it also increased with the increase in concentrations and exposure period. In addition, the results of the extracts were also equivalent to that of the positive control, which was the synthetic insecticide Permethrin in this study. This is because leaves of *Mentha x piperita* have different compositions of various constituents, among which they include 1.2-3.9 % (w/v) of the essential oils. The other classes of constituents present in the mint leaves are 52% of monoterpenes, 9% sesquiterpenes, 9% aromatic hydrocarbons, 9% aldehydes, 7% lactones, and 6% of alcohols. One of the major constituents of monoterpenes is menthol, which is around 35% to 60%. While other components of the monoterpenes include 0.1-6% limonene, 3-4% neomenthol, 2-5% isomenthone, 1-13% 1,8-cineole, 0.3-14% menthofuran, 0.7-23% menthyl acetate, and 2-44% menthone. (Eftekhari et al., 2021). Mint is known to exhibit an efficient response against various insects helping in their control and management and has been used as insecticide and repellent majorly in the form of essential oils and polar extracts in contact, fumigation, and repellency bioassays. (Brahmi et al., 2017).

The mint extracts have also been assessed against the two aphids namely *Rhopalosiphum padi* and *Sitobion avenae.* At all the concentrations for essential oils of both the *Mentha* species, the mortality rate increased with the time of exposure. These findings are also consistent with our obtained results on the mortality of *T.castaneum* at different concentrations and different exposure periods. The results partly contributed to the presence of constituents such as monoterpenes that have been well documented as repellents and insecticides for the stored-product insects. Some of the major components found in lemongrass extracts and essential oils include neral, citrate, geranyl acetate, and limonene (Lermen et al., 2015). In addition, terpenoids are the secondary metabolites that have major functions in the physiology of plants and their defense against pathogens and several insects. Due to the action of these active constituents, lemongrass may have a neurotoxic effect on *Tribolium castaneum*, causing its rapid lethality. According to (Plata-Rueda et al., 2020) lemongrass extracts that had Citral and geranyl acetate as major constituents induced the mortality of *Ulomoides dermestoides* between 24 and 48 hours of exposure time. It exerted a strong effect via the topical application with an LD_{50} of 5.17 μg insect^{-1} and LD_{90} = 19.1 μg insect^{-1}. The mortality caused by lemongrass oil here increased with the dosage and exposure period, which was also observed in other insect pests. (Hernández-Lambraño et al., 2014). Thus, lemongrass plant extracts do have the potential to control several stored-grain insects as a great alternative to chemical methods. In our results, figures 20 and 22 show the mean mortality effects of *Mentha x piperita* and *C.citratus* respectively at three different concentrations i.e., 5%, 10%, and 15% w.r.t. different time intervals. The maximum mortality that 1.5% of *Mentha x piperita* exerted after 30 hours of exposure was 76.67%, whereas that exerted by the same concentration of *C.citratus* was 86.67%. However, the highest concentration of both the extracts had a 100% effect against *Tribolium castaneum.*

5.2. Repellent Effect

The plant-based repellents have been widely used for generations now in traditional practice as a protective measure against insects. New natural products have been developed using the traditional repellent plants after a multitude of ethnobotanical studies. Most of the plants responsible for eliciting repellent response against different classes of pests is due to the presence of volatile components present in them. Such plants have been used throughout developing countries in the form of fumigants (Moore & Debboun, 2006). Various oil formulations were also prepared and applied to the skin to protect against nuisance mosquitoes. Essential oils extracted from the leaves of *Mentha x piperita* were proven to be effective against *T. castaneum* via fumigation, contact, and repellent activity bioassay. Twenty-one of the total chemical constituents were detected by the GCMS, out of which 97.5% constituted the total oil. The fumigation and toxicity posed by the oil against the target pest had LC_{50} = 18.1 mg/L air and LD_{50} = 2.9 μg/adult, respectively (Pang et al., 2020). These results of essential oils were also comparable to the positive control used in the study at the highest concentration evaluated in the experiment. The principal constituent to cause repellent action against the pest was menthol. The findings of the present research work were also in correspondence to the mentioned study as both the positive control and highest concentration (3.5%) of the mint extract showed equivalent percentage mean repellency responses i.e., 100 ± 0% after 75 mins of exposure. This can be due to the presence of a greater concentration of menthol, the presence of whose chemical group i.e., terpenoid was confirmed using the preliminary phytochemical screening tests as qualitative analysis.

The repellent activity of several other plant extracts including those of clove, mint, coriander, and neem was also investigated by (Ramsha et al., 2019). The extracts were used in multiple concentrations and the study proved that mint was also strongly active against red flour beetle and repelled 1.5% (both aqueous and ethanolic) of the extract repelled 100% of insects even at 1 hour of the treatment. Whereas, according to our findings, 1.5% of the mint extract applied caused 80% insect mortality after 75 mins of exposure. The difference in this regard can be due to distinct reasons such as the choice of solvent since it significantly affects the quality of extraction from the sample plants. In addition, it can also be due to different pest specie used, research methodology, laboratory conditions varietal and commodity variations.

In addition, this study indicated the significant effect of different concentrations of the extracts used and the response exerted by them at separate times of exposure. The highest percentage mean repellency caused by 1.5%, 2.5%, and 3.5% of mint extracts was 80 ± 0%, 87 ±11.5%, and 100 ± 0% respectively. The efficacy of mint extracts in the management of stored-grain insects has previously been also reported by (Khani & Asghari, 2012). The essential oil extracted from *Mentha longifolia* was tested for volatile toxicity against two stored grain insects i.e., *Tribolium castaneum* and *Callosobruchu maculatus F*. GCMS analysis of the essential oils confirmed the presence of major compounds including piperitenon (43.9%), tripal (14.3%), oxathiane (9.3%), piperiton oxide (5.9%), and d-limonene (4.3%). The results of the study suggested that mint species could be used as a potential control agent for the stored-product insect. Further studies also revealed the fumigant and repellent properties of essential oils from aerial parts of different *Mentha sp (Mentha pulegium* L.) against *Tribolium castaneum* where significant pest repellent activity was demonstrated with repellency effects of 80 and 60% at 0.31 µL/cm^2 and 0.078 µL/cm^2 dosage and exposure of 1 to 24 hours respectively (Salem et al., 2018).

The extracts of *Cymbopogon citratus* and its terpenoids have been proven to have the potential to control agricultural pests. The current research work assessed the effects of lemongrass extracts on the repellency of red flour beetle at different concentrations and exposure periods. The results revealed that with the increase in the concentration of the extract, the percentage mean repellency increased. Furthermore, the effect of repellency also varied with that of the exposure time. Maximum repellency shown by

1.5%, 2.5%, and 3.5% of lemongrass extracts at 75 mins of exposure were 66.7± 11.55%, 73.3 ± 23.09%, and 100 ± 0%. The repellency induced by 3.5% treatment of plant extract was similar to the results of a synthetic chemical (positive control) after 75 mins of exposure time. A study revealed the effects of native and exotic lemongrass against *Sitophilus zeamais,* commonly known as maize weevil. The findings revealed that the applied dose had a significant effect on the repellency action of the pest at all the times evaluated (Radünz et al., 2022).

Another study on the repellency of lemongrass oil revealed its effectiveness against mosquitoes and house flies. Lemongrass as an active substance was demonstrated for olfactory cells of *Stomoxys calcitrans*. It was further indicated that there was a significant increase in the electroantennogram response of the target fly with the increase in dosage of lemongrass oil applied. Larger number of flies were present in the untreated zone for a greater amount of time than that in the treated zone. As per the results, it was stated that lemongrass essential oil could also be used as an effective control agent against several classes of pests. (Baldacchino et al., 2013). Phytochemical identification of ethanol and hexane extracts of lemongrass also confirmed the presence of various chemical groups i.e., alkaloids, terpenes, flavonoids, saponins, terpenoids, and tannins. The repellent activity of the extract was evaluated proving terpenes as the major compounds to have a repellent effect against different species of mosquito vectors. (Adam et al., 2021).

The results of the current work also indicated that both the toxicity and repellent effects of the plant extracts were concentration and time dependent. As per the data obtained, *Mentha x piperita* proved to be a better repellent than *Cymbopogon citratus* exerting a percentage mean repellency of 80 ± 0%, 87 ±11.5%, and 100 ± 0% at 1.5%, 2.5%, and 3.5% concentration treatment. On the contrary, *Cymbopogon citratus* methanolic extract proved to be a better insecticide than that of *Mentha x piperita* because of its earlier response against *T. castaneum* at every concentration applied. 86.7 ± 5.77%, 96.7 ± 5.77%, and 100 ± 0% percentage mean mortalities were exerted by lemongrass at 5, 10 and 15% of concentration treatment. Lemongrass extracts pose a lethal effect on *T. castaneum* and the weight loss of infested feeds can also be reduced protecting the feedstuffs from damage by this insect. (Aboelhadid & Youssef, 2021). Furthermore, lemongrass also showed repellency against *Alphitobius diaperinus* (lesser mealworm) as per the work reported by (Francikowski et al., 2019). The interaction between the impact of repellent effects of plant extracts and target insects were also studied by the previous researchers. It was majorly related to the nervous system and two target sites including octopaminergic and acetylcholine posing effects on the receptors. (Jankowska et al., 2018). Due to these neurotoxic effects, they cause paralysis of the target insect or reduced motor activity overall. This presence of multitude of targets in the nervous system of insects allows the plant extracts and natural essential oils to be an effective and interesting candidate for bioinsecticides with lesser toxicity to human health and a cheaper approach of all.

CONCLUSION

The investigations of the study indicated *Mentha x piperita* and *Cymbopogon citratus* to possess effective botanical agents to serve as an alternative to synthetic control measures currently used against the *Tribolium castaneum.* All the assays conducted revealed the sensitivity of target insect against the sample plants, based on different concentrations and exposure periods. Even at lower concentrations, the plants were found to have a good impact on the insect. As per the data obtained, *Mentha x piperita* proved to be a better repellent than *Cymbopogon citratus* exerting a percentage mean repellency of 80 ± 0%, 87 ±11.5%, and 100 ± 0% at 1.5%, 2.5%, and 3.5% concentration treatment at 75 mins of exposure period. On the contrary, *Cymbopogon citratus* methanolic extract proved to be a better insecticide than that of *Mentha x piperita* because of its earlier response against *T.castaneum* at every concentration applied. 5%, 10% and 15% of concentration treatment of lemongrass exerted 86.7 ± 5.77%, 96.7 ± 5.77%, and 100 ± 0% percentage mean mortalities respectively after 30 hours of exposure. Additionally, the preliminary phytochemical screening as a qualitative identification of chemical groups also confirmed the presence of terpenoids present in both the sample plants, as they happen to be major constituents acting against *Tribolium castaneum.* The results of the study provide sufficient scientific support for preliminary screening bioassays using natural plant products in the form of extracts and essential oils as control agents rather than synthetic ones to minimize the resistance mechanism, pesticide intoxication, and environmental toxicity. These plants in the form of botanical insecticides can fit well in the Integrated Pest Management programs designed for the control of various pests causing losses in food commodities.

FUTURE PROSPECT

- Isolation and identification of the bioactive compounds from plant sources to develop a formulation for effective applications.

- Application methods can be rigorously evaluated on stored grains to enhance the efficacy of extracts for household products.

- Researchers can also venture into developing product formulations that could not only cause repellent effects but also generate antifungal and antimicrobial effects simultaneously to enhance the shelf life of food commodities that are otherwise damaged due to moisture and heat.

- Field trials of the extracts can also be carried out to check on-farm repellency or toxicity effects against several other pests.

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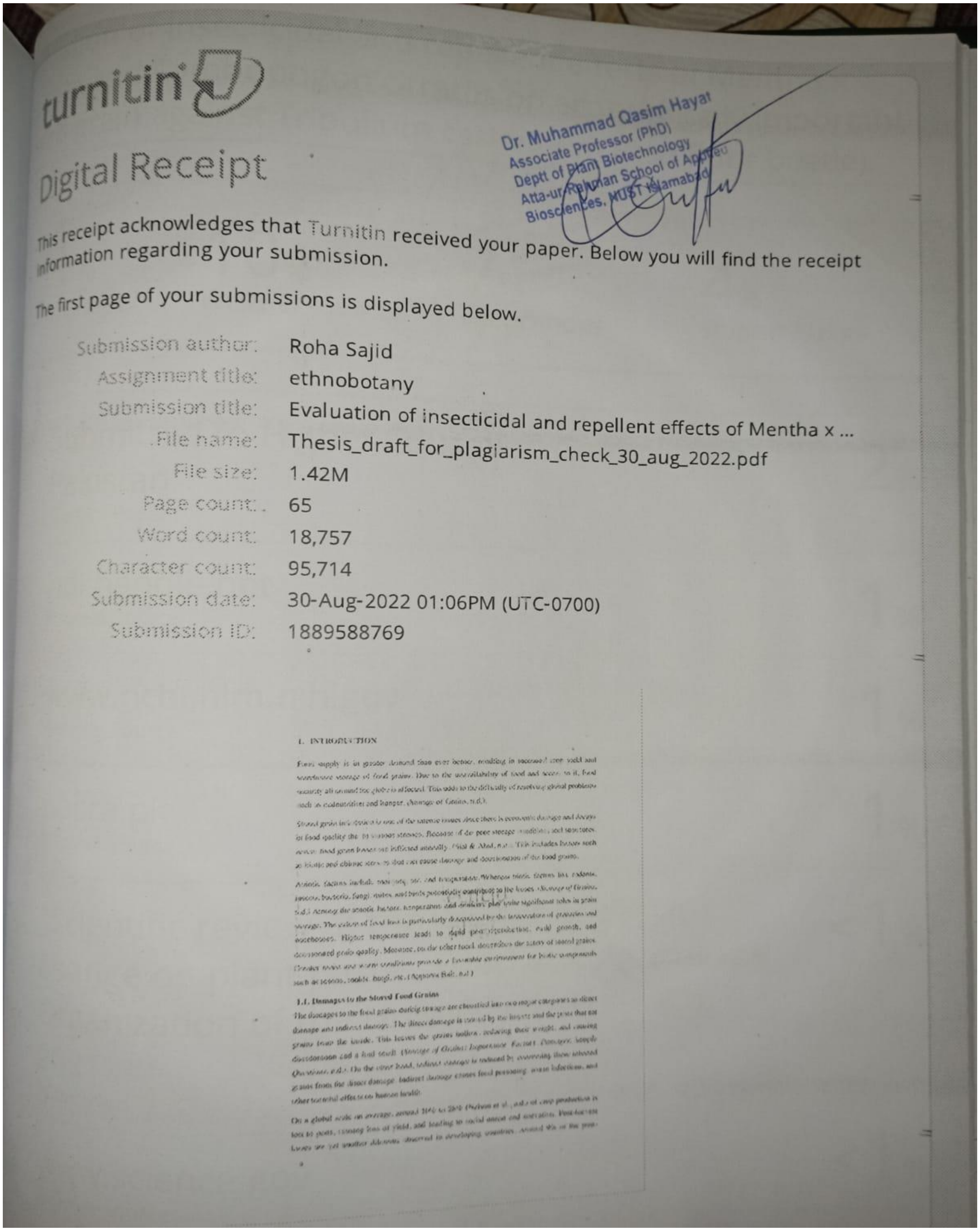

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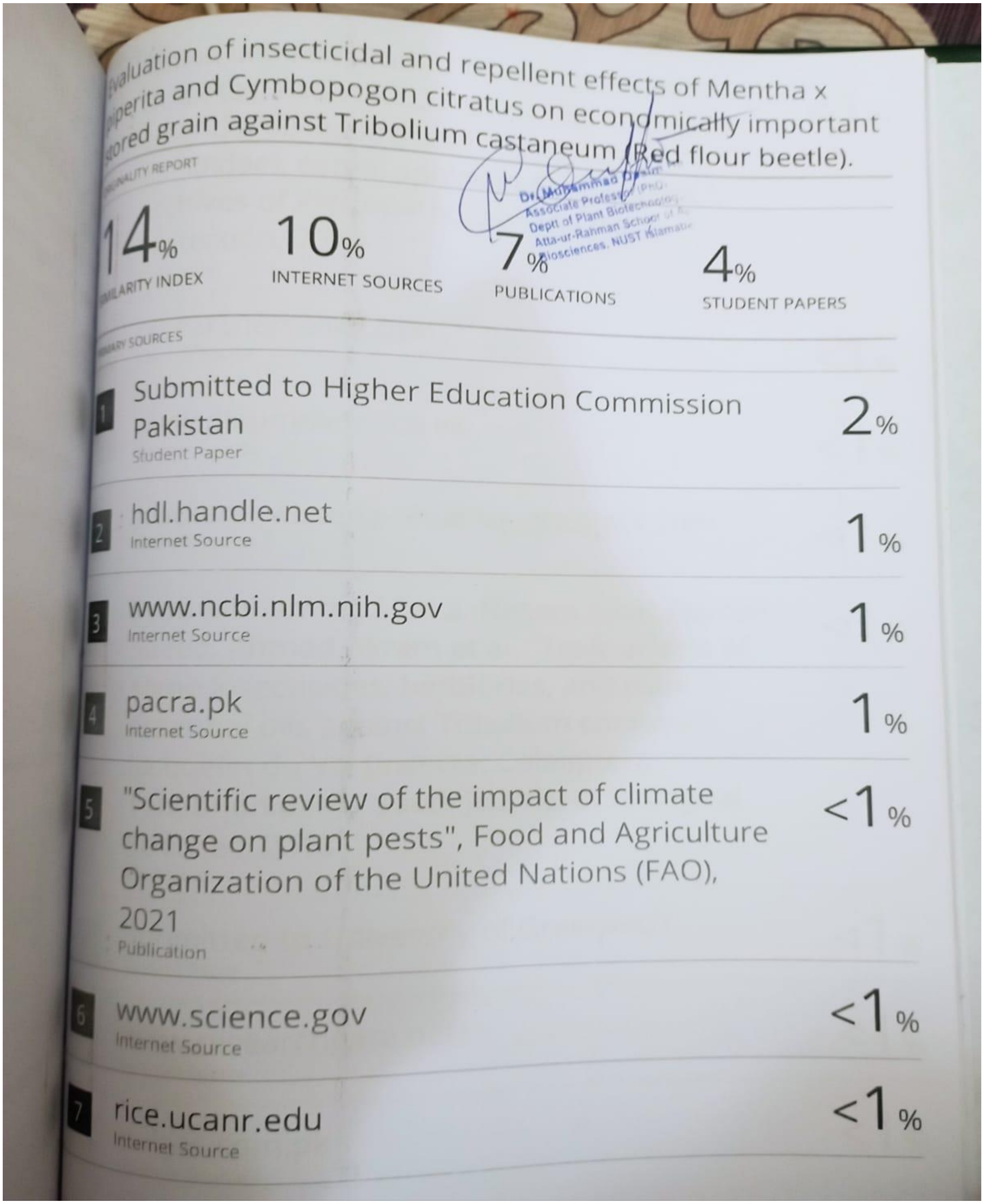
1. INTRODUCTION

1.1. Damages to the Stored Food Grains



Evaluation of insecticidal and repellent effects of Mentha x piperita and Cymbopogon citratus on economically important stored grain against Tribolium castaneum (Red flour beetle).

ORIGINALITY REPORT

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