T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCE AND TECHNOLOGIES

EFFECT OF PEPPERMINT LEAF EXTRACT AND PEPPERMINT OIL ON FLOUR BEETLE *Tribolium castaneum* (COLEOPTERA: TENEBRIONIDAE)

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M. Sc. Thesis

April 2024
KAYSERİ

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SCIENTIFIC ETHICS CONFORMITY

I, Zaman Abdulkadhim Alshaibani, hereby declare that all information in this thesis has been composed and presented in accordance with the academic rules and ethical guidelines. I also declare that, as required by these rules and conduct, I have fully and accurately cited and referenced all materials and results that are not original to this work.

Zaman Abdulkadhim Neamah ALSHAIBANI

The MSc thesis entitled **"Effect of Peppermint Leaf Extract and Peppermint Oil on Flour Beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae)"** was prepared in accordance with Postgraduate Thesis Proposal and Thesis Writing Directions of Erciyes University.

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M.Sc. Thesis, February 2024
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ABSTRACT

The red flour beetle (*Tribolium castaneum*) has a particular importance as a negative factor affecting both the quality and quantity of stored grains and food products. In the present study, to investigate the possibility of controlling this pest using environmentally friendly natural materials, a series of laboratory experiments were conducted to evaluate the efficiency of peppermint oil and alcoholic (methanolic) peppermint extract against the different stages of the red flour beetle. The results of this study showed that the peppermint (*Mentha piperita*) oil treatments at all concentrations used (8, 10 or 12%) were significantly effective resulting in remarkable individual mortality of the T. castaneum. The mortality range changed from 36 to 80% in third larva instar and from 40 to 94% in adult stages by spray method. The treatment of the peppermint methanol leaf extract at the highest concentration (13000 ppm) led to larva mortality up to 87% after 7 days of treatment. Peppermint oil showed insecticidal effect against the third instar larvae of the red flour beetle, especially where sprayed at the highest concentration of 12%. Hoever the lower concentrations of 8% and 10% of the oil did not differ much from the control. The results of this study showed that the larvae mortality increased as the mint oil inncreases in the feed provided to the insect, especially at highest concentration of 12%, which recorded a mortality rate of 82% in 3rd instar larvae and 95% adults mortality. This study confirmed the posobility of using mint essential oil at relatively low concentrations as environmentally friendly poroduct in controlling thered flour beetle *T. castaneum*.

Keywords: Essential oil, Peppermint, *Mentha piperita*, Methanolic extract, *Tribolium castaneum*, Red flour beetle

NANE YAPRAĞI EKSTRESİ VE NANE YAĞININ KIRMIZI UN BÖCEĞİ ÜZERİNDEKİ ETKİS *Tribolium castaneum* (COLEOPTERA: TENEBRIONIDAE)

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ÖZET

Kırmızı un böceği (*Tribolium castaneum*), depolanan tahılların ve gıda ürünlerinin hem kalitesini hem de miktarını etkileyen olumsuz bir faktör olarak dikkate değer bir öneme sahiptir. Bu çalışmada, kırmızı un böceği zararlısını çevre dostu doğal materyaller kullanarak kontrol etme olasılığını araştırmak amacıyla, nane yağı ve alkollü (metanolik) nane ekstraktının kırmızısı un böceğinin farklı aşamalarına karşı etkinliğini değerlendirmek üzere bir dizi laboratuvar deneyi yapılmıştır. Bu çalışmanın sonuçlarına göre, %8, 10 ve 12'lik konsantrasyonlardaki nane (*Mentha piperita*) yağının uygulamalarının, tüm dozlarda kırmızı un böceği mortalitesi üzerine önemli bir etkiye sahip olduğu tespit edilmiştir. Sprey yöntemiyle yapılan uygulama sonucu ölüm oranı üçüncü larva döneminde %36'dan %80'e, ergin evrede ise %40'tan %94'e çıkmıştır. Nane yaprağı metanol ekstraktının en yüksek konsantrasyonda (13000 ppm) uygulanması, 7 günlük uygulama sonrası larva ölümlerinin %87'ye varan oranda artmasına neden olmuştur. Nane yağı, özellikle en yüksek konsantrasyonda (%12) uygulandığında, kırmızı un böceğinin üçüncü dönem larvalarına karşı önemli insektisidal etki göstermiştir. Ancak, nane uçucu yağının %8 ve %10'luk daha düşük konsantrasyonları kontrole göre önemli bir farklıkık ortaya çıkarmamıştır. Bu çalışmanın sonuçları, kırmızı un böceğine verilen yemde nane yağı arttıkça larva ölüm oranının arttığını, özellikle %12'lik en yüksek konsantrasyonda, 3. dönem larvalarda %82 ve yetişkinlerde %95 ölüm oranı kaydettiğini gösterdi. Bu çalışma, kırmızı un böceği *T. castaneum*'un kontrolünde çevre dostu bir ürün olarak nane esansiyel yağının nispeten düşük konsantrasyonlarda kullanılmasının mümkün olabileceğini ortaya koymuştur.

Anahtar Kelimeler: Esansiyel yağı, Nane, Metanolik Ekstraktlar, *Tribolium castaneum,* Kırmızı un böceği

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LIST OF ABBREVIATIONS

Abbriviate	Complete term
°C	Celsius
µg	Micro-gram
µl	Micro-liter
ANOVA	Analysis of variance
ClO_2	Chloride Di-oxide
cm	Centimeter
Con.	Concentration
g	gram
g/mol (W:M)	Gram/mole (weight:mole)
GC-MS	Gas chromatography–mass spectrometry
Kg	Kilogram
L	Liter
LD_{50}	Lethal dose
LSD	Least significant difference
Meth	Methanol
min	Minuit
ml	Mil-liter
ng/mg	Nano-gram/milgram
P	Propability
Ppm	Part-per milion
R.t	Repetition
RCD	Randomized Complete Design

CHAPTER 1

INTRODUCTION

1.1. Introduction

Agricultural pests pose a significant challenge to the global agricultural sector, causing substantial losses to crops and reducing their productivity. Among these pests, the red flour beetle (*Tribolium castaneum*) which has a particular importance as a negative factor affecting both the quality and quantity of stored agricultural products. Additionally, it leads to the deterioration of grains and various agricultural commoditie (Martelli et al., 2009). Belonging to the Tenebrionidae family of darkling beetles (Coleoptera), this insect diminishes the quality of agricultural products and undermines food safety (Uritu et al., 2018). In recent years, research efforts have increasingly developed into exploring sustainable alternatives to conventional chemical pesticides for pest management. A promising avenue in this regard involves the utilization of aromatic plant extracts, owing to their potential to provide both a natural and effective solution as alternatives to traditional chemical pesticides (Ziaee, 2014).

1.2 Aim of Study

This study aims to evaluate the effect of mint leaf extract and mint oil on the red flour beetle and explore their potential as a biological solution for controlling this pest. These objectives will be achieved through a series of laboratory and field experiments, including assessing the influence of these extracts on the growth, development, survival and deterrence of the target insects.

The increasing reliance on chemical pesticides for pest control highlights the need to explore natural and sustainable alternatives. In this context, aromatic plant extracts represent a promising source for their use as ingredients in agricultural pest control

(Amiri et al. 2016; Kennedy et al. 2018). This is due to its ability to provide a variety of effective compounds that contribute to reducing the proliferation of pests and their negative impact on crops and agricultural stocks (Mahendran & Rahman, 2020).

The properties of mint leaves and their oil combine an attractive aroma with potential effects on insects. Natural peppermint compounds have the ability to affect the sensing and communication systems of insects (Mishra et al., 2012). which may lead to reduced mating and egg reduction and thus limit the reproduction of this pest (Regnault-Roger, Vincent, and Arnason 2012; Lu et al.", 2012). In addition, it is believed that some compounds in mint may affect the hormonal and developmental systems of insects, enhancing the chances of controlling their populations (Kumar et al., 2011).

1.3. Scientific and Practical Importance

This study is believed to be of significance in terms of searching for a sustainable biological solution to control the red rusty flour beetle, which is based on the use of natural plant extracts. If the effectiveness and safety of these extracts is confirmed, this may offer a more environmentally friendly alternative that does not cause the accumulation of chemical residues in crops and agricultural products.

1.4. Theoretical Framework and Background

The red flour beetle, *Tribolium castaneum*, is one of the most well-known agricultural pests in the field of food and grain storage (Pinto et al., 2020). It feeds on a variety of foodstuffs, such as grains, ceareals, pulses, and other products. Its presence can lead to significant economic losses, spoilage of stored goods, and a reduction in product quality and storability.Several methods have been developed in controlling this pest, including the use of traditional chemical pesticides (Aktar et al., 2009). However, concerns about the environmental and health impacts of these pesticides, as well as the potential for the development of pesticides resistance, have reasonably increased the importance of seeking safe and effective alternatives to control this pest (Stejskal et al., 2015).

1.5. The Role of Mint in Insect Control

Mint leaves and its oil are innovative options that can play a significant role in agricultural insect control. Mint (Mentha) is an herbaceous plant that contains active

compounds with insect-repelling properties (Padín et al. 2013;A Khan et al. 2015). Compounds such as menthol, methyl acetate, and menthone have biological effects that can affect the nervous system of insects, potentially inhibiting their growth, development, and, consequently, reducing their harmful effects on agricultural storage facilities (Kumar et al., 2011).

CHAPTER 2

LITERATURE REVIEW

2.1. The Value of Flour to the Economy

Flour is one of the most important foods, if not the most important at all, in the warm region of the Earth, and this is evident from the increasing demand for it in areas with high population density in this region and other regions of the world (Elbrense et al., 2022). Even beyond that, flour is the main food source for many developing countries in the world, which remain in constant demand for this product from other countries, even with the expansion of wheat cultivation in these countries (Linsley, 1944). The 2022-2023 statistics indicate that the per capita consumption rate of wheat, often calculated on the basis of flour consumption, is 64.7 kg/year. The same statistics indicate that global wheat production amounts to 781.31 million tons, with China leading the first place in global production, with the total global consumption rate of wheat reaching 781.31 million tons. 792.69 million tons. On the other hand, the per capita consumption rate in the United States of wheat products is higher than the average, which is 79 kg per capita annually, and this is approximately 15 kg higher than the global per capita consumption rate (Mahendran & Rahman, 2020).

The world is also witnessing an increasing demand for wheat and flour products, mainly in most parts of the world, even in those areas where wheat cannot be grown. Therefore, it can be said that the context and level of global production of wheat and flour is not necessarily proportional to the level of consumption and its distribution (Elbrense et al., 2022).

2.2. Pests Affecting Flour

The presence of insect residues in grains leads to a high percentage of toxic urea in the fine grains, which may cause changes in the components of the grain. This affects the incubation of industrial processing and leads to difficulties in grinding, impedes aeration and moisture absorption, raises the water content of the grain, and reduces the effect of powders added to control pests (Kennedy et al., 2018). On the other hand, the marketing value of stored products decreases as a result of insect infestation, as well as product damage due to high humidity resulting from insect activity, which exposes the product to fungal infection and then spoilage (Kumar et al., 2011). Losses caused by insects in stored materials have been estimated at approximately 5-10% of global production (Kumar et al., 2011). This percentage varies depending on countries, from 0.5 to 3% in developed countries and 45 to 50% in developing countries (Ziaee, 2014). Sometimes, the presence of insect pests of stored materials, including rice weevils, corn weevils, red and similar rusty flour beetles, and other insects, is considered the determining factor for the survival of the crop with good economic quality (Wagan et al., 2022). since in these cases the specific damage to the stored material is more severe than the damage caused by quantitative loss (Kumar et al., 2011).

2.3. The Rust-red flour beetle *Trogoderma castaneum*

The red flour beetle lives on grains and their products, as well as seeds, dried vegetables, and tobacco. It spreads in mills and imparts a pungent odor to the materials it infects. The infestation also causes a decrease in the viscosity of the dough and makes it unfit for baking. The insect feeds on flour, bran, and other grain products such as grits, semolina, bulgur, and others. Although insects have jaws that cannot break intact grains, we find them in whole grains accompanied by protozoal storage insects that can break grains with their strong jaws to feed on them. The latter allows pests to spread throughout the world, like many warehouse pests, which spread with food trade to many parts of the world. (Suzuki, 1980).

2.3.1. Economic Importance

The red flour beetle, *T. castaneum*, is one of the most dangerous insect pests that infect stored grain products, causing widespread contamination and loss of grain weight as a

result of the insect's feeding (Amoura et al., 2021). It also causes economic losses as a result of the insect's high ability to reproduce, its adaptation to different storage conditions, and its wide global spread (Pavela, 2015). It is one of the secondary warehouse insects that spread in most regions of the world, especially warm and dry areas.

T. castaneum is a serious pest of food stored in stores and warehouses (Golob, 1997). as it feeds on many types of foods, but it is considered one of the most important pests of flour and stored grain products, and it is a pest of other stored products such as beans, peas, dried fruits, milk, and mixtures. Ready-made cakes, spices, medicines, chocolate, cocoa, oilseeds, dried flowers, herbs, etc. (Hossain et al., 2010). This insect is considered a globally widespread pest, along with the similar flour beetle *T. confusum* and the long-headed beetle *Latheticus oryzae* (Halliday et al., 2015). In general, losses affecting stored grain products are mainly caused by two species of beetles, *T.castaneum* and *Rhyzopertha dominica*, which pose a threat to farmers (Chimayo & Abdullahi, 2011).

The economic damage caused by insects to stored materials includes changing the nature of the materials to something unacceptable, losing the weight of the product, and contaminating the product with insect residues and various chemical secretions (Isman, 2000). These secretions have an unacceptable smell and are due to the secretion of benzoquinones compounds secreted by the abdominal gland, and these compounds are considered carcinogenic substances and cause a number of allergic diseases in humans (Lu et al., 2012).

(Chimayo and Abdullahi, 2011) stated that 20% of crops are destroyed by pests in the post-harvest period, and this percentage increases in third world countries, sometimes reaching 80%. It was found that flour resulting from grinding wheat grains infected with the flour beetle for 8 weeks at a rate of 50 insects per kilogram had its natural, doughy, nutritional and health properties deteriorated significantly (Gabarty & El Nour, 2016). also confirmed that human consuming flour of infected grains infected with the flour beetle leads to a decrease in the body's immunity and a decrease in the blood enzymes GSH_PX and GSH_R, in addition to a decrease in blood plasma vitamins, which are E, C, and A. It was also found that flour beetles Red and similar ones are infected with a type of tapeworm, *Hymenolepis diminuta*. It is known that this parasite infects mice and

rats, in addition to the transmission of these parasites to humans when they are fed flour infected with such insects (Yan et al., 1998).

2.3.2. Description and Life Cycle

The adult insect is 3-4 mm long, with a flat, elongated body of a reddish-brown color. The last terminal pieces of the antenna are larger than the rest of the basal pieces. Its chest has curved sides, and the edges of the head capsule are not raised. The distance between the compound eyes is less than the diameter of the eyes, and adult insects are distinguished by their ability to fly (Fthenakis & Kim, 2011).

The insect breeds on broken grains, grain fragments, high-moisture pulp, flour, etc. Females lay 300-400 eggs in their lives. The eggs may reach 1,000 eggs in highly fertile females. Eggs are laid at a rate of 12 eggs daily in flour and other foods over a period of five to eight months. The eggs are cylindrical in shape and white in color. The female secretes a sticky substance on the eggs to which flour and other materials stick easily. The eggs hatch within 5-12 days into miniature white larvae with a dull brown head that are cylindrical in shape. The lifespan of the larvae ranges from 22 to more than 100 days, and the pupal cycle lasts about 8 days. The pupa is free, naked, and white in color, then turns brown after that. Then the adult comes out. The average life cycle is 7 to 12 weeks, and adults may live for three years. The insect has six generations per year, which prefers to live at a temperature of 30°C, which is optimal for development and reproduction. Growth and reproduction stop at a temperature below 18°C, and the temperature range in which the insect lives ranges from 22 to 40°C (Kumar et al., 2011).

2.4. Storage Pests Control

2.4.1. Use of Chemical Pesticides

Chemical pesticides are most often the quickest option to reduce insect damage in general, including warehouse insects, despite the negative effects associated with the use of the pesticide (Aktar et al., 2009). Different chemical preparations were used to reduce insect damage to stored materials. Methyl bromide pesticide has been used to combat warehouse insects, but fumigation with this substance may not be effective against insects that reside deep in grain piles (Azab et al., 2017). Phosphine was also used to control types of stored pests of Coleoptera, including *T. castaneum*, *O. Surinamensis*, *S.*

granaries, *R. dominica*, and *C. Ferrugineus*. As *O. surinamensis* was the most sensitive insect at distances of 5, 10, and 15 meters from the fumigation spot, as they noticed, the further the distance from the smoking spot with phosphine, the less the effect, and over time this leads to an increase in individuals resistant to the pesticide (Stejskal et al., 2015).

Many pyrethroid pesticides, such as Ficam 5% emulsion concentrate, Trust 20% wettable powder, and Decis 20% emulsion concentrate, have also been used as protectants of stored wheat grains against adults of the red flour beetles T.*castaneum* and *T.confusum* and the long-headed beetle (*Latheticus oryzae*). Ficam was the most toxic among the three types of pesticides against adults of the red flour beetle and the long-headed beetle, followed by Decis, while the toxicity of Decis against similar flour beetle adults was the highest, followed by Ficam and Trust (Al-Rahimy et al., 2019).

In a study conducted to test the effect of the pesticide Alfacypermethrin at three concentrations of 0.5, 1, and 1.5% on the mortality of *T. castaneum* adults, the results showed that a concentration of 1.5% led to a mortality rate of 90-100%, compared to a mortality rate of 20-80% at a concentration of 0.5% , as well as pesticide residues in stored products (Hajlaoui et al., 2009); Uritu et al., 2018).

In general, this led to the emergence of high levels of resistance in various insects to phosphine resistance, which has become a serious problem globally (Parrey et al., 2023a). The highest case of resistance was recorded in the insect *T. castaneum*, which reached a thousand times, which threatens the use of phosphine in fumigation (Siddeeg et al., 2018).

Chemical insecticides have been used effectively to control *T. castaneum*, including organophosphorus pesticides and pyrethroids to control larvae and adults (Parrey et al., 2023a). To reduce the use of insect growth regulators, such as Methoprene, which has shown effectiveness in resisting *T. castaneum* insect larvae through its effect on the morphogenesis process (Stejskal et al., 2015). In a study (Lu et al., 2012), it was shown that repeated treatments with the growth regulator Methoprene and Synergized pyrethin were more effective than the effect of Synergized pyrethin alone in controlling *T. castaneum*. It was also found that the use of chlorine oxide gas (ClO^2) at a concentration

of 200 ppm led to complete mortality of adults and last instar larvae during an exposure period of 24 hours (Kim et al., 2015)

2.4.2. Use of Plant Extracts

Natural plant extracts are characterized by the simplicity of their chemical composition, consisting only of carbon, hydrogen, oxygen, and nitrogen. They do not have a long residual effect and therefore do not affect the vitality of exposed organisms. These quickly deteriorate and break down in the environment because they are quickly affected by weather factors, especially heat and light, so they are transformed during metabolic processes into less or non-toxic substances (source). Most of the natural plant poisons are neurocontact poisons, and they also have a smoky effect through their vapors, as most of their molecules have a high degree of volatility, especially when the temperature rises. Some plant extracts have anti-nutritional, hormone-like, or attractive and repellent effects. Among these substances are alkaloids, alkaloids, phenolic compounds, some uncommon amino acids, flavonoid tannins, cyanogenic glycosides, terpenes, and diterpenes (Uritu et al., 2018). Plant extracts were used, and their effects were tested in many studies against various warehouse insects, including the rust-red flour beetle. Different concentrations of alcoholic and aqueous extracts of *Thymus vugaris*, *Artemisia herba*, and *Mentha piperita* were used against the larvae and adults of the Rust-red flour beetle. The effect was observed after 24 and 48 hours of treatment and compared with diazinon pesticide. The synergistic effect of the extracts on the toxicity of diazinon pesticide was also studied at different mixing ratios (Biosci et al., 2013). In a study conducted by (Najafabadi et al., 2014), they tested the effect of ethyl alcohol extract of three plants: *Azadirachta indica*, *Mentha longifolia*, and *Datura stramonium*, against the insects *Oryzaphilus surinamensis* and *T. castaneum*. The results showed that all extracts had a repellent and lethal effect on the two insects compared to the control treatment under mixing ratios of 10 mg/g with grains, to death rates that reached 4.01±48.30, which is the highest for the M. longifolia extract, while the death rates reached 3.21±35.26 and 2.33±25.6%. For Datura and Neem extracts, respectively, when treating *O. surinamensis*, while the same extracts affected *T. castaneum* with mortality rates reaching 2.31±21.42, 1.54±16.66, and 1.89±15.95% for Datura, Neem, and Mint extracts, respectively. It was also found that the methyl alcohol extract of the leaves of *Clerodendron inerme* (L.), *Rhyzopertha dominica*, and Callosobruchus chinensis had a repellent effect against *T.*

castaneum. The most influential was *C. chinensis*, followed by *R. dominica*, then *Clerodendron inerme*. The methanolic extract of the plant leaves was more effective than the commercial preparation Teflubenzuron (No:SZBB291XV) and the rest of the solvent extracts (Guruprasad & Pasha, 2015). Pyrethrin I and II compounds extracted from the white flowers of the *Chrysanthemum cineraiaefolium* plant were also used as a pesticide to kill the red flour beetle during different periods and using several concentrations, as the death rate increased as the concentration of the pyrethrin compound and the time of exposure increased (Al-Rahimy et al., 2019). In another study, powders of four plants: *Borago officinaalis*, *Mentha piperta*, *Anise officinalis anisum*, and *Eucalyptus glublus* were used against *T. castaneum*. The powders showed a repellent effect on the insect, and mint powder was the most effective in repelling it, followed by star anise powder. Then eucalyptus, while borage powder was the least effective (Ferman, 2009). The effect of powders of four plants: *Cinnamomum zeylancium*, ginger *Zingiber officiale*, nutmeg *Myristica fragranse*, and coriander *Coriadrum sativum* against the red rusty flour beetle was also studied at concentrations of 0, 0.5, 1, 2, and 4%. The results after seven days of treatment showed that the mortality rates were 5.050%, 4.350%, 4.350% and 1.483% for powders of nutmeg, ginger, coriander and cinnamon plants, respectively (Al-Hadidi, 2014).Extracts of crude alkaloid compounds from the leaves of the *Callistemon rugolosus* plant were also used to destroy the first and last larval instars and adults of the red rusty flour beetle. The results showed that the concentration of 20 mg/ml was most effective in the first larval instar, as the mortality rate reached 30% compared to with 8.5%, the concentrations 15 and 20 affected the adults, as the mortality rate reached 11 and 12%, respectively, compared to 0.2% in the control treatment(Guruprasad & Pasha, 2015). The repellent effect of some compounds extracted from the Murraga alata plant against *T. castaneum* was also tested, where a 100% repellent effect was recorded for some of the compounds identified in the plant, which are coumarins, meranzin, phebalosin, and murracarpin , and also found an anti-feeding effect for some compounds extracted from the *Glycosmis lucida* plant that contain sulfur in their composition, including Methylgerambullin, Methylisogerambullone, and N-{2-[4-(6,7-dihydroxy-3,7-dimethyl-oct). -2-enyloxy)-phenyl]-ethyl}-3-methanesufinyl-N-methyl-acrylamide(Chimayo & Abdullahi, 2011).

2.5. *Mentha piperita*

Mint is a well-known plant with oval leaves arranged on the plant stem in the form of criss-crossing pairs. It has bright green leaves and small purple flowers. The original homeland of the plant is Europe, and it is considered one of the plants that spread widely in different regions of the world. It is considered one of the most widespread garden plants, as is the mint plant (Mamadalieva et al., 2020). It has multiple uses: It is used for food and as a repellent for most types of insects and some other pests because of the compounds it contains, which have either a lethal or repellent effect. Mint contains many substances, the most important of which are volatile oils, carvone, and flavonoids, including thimonin. Thymonin and also derivatives of caffeic acid, caffeic acid, which contains rosmarinic acid in the essential oil (Uritu et al., 2018). It is rich in vitamins, especially vitamin B complex and A, and contains a high percentage of calcium, magnesium, sulfur, potassium, sodium, iron, as well as tannic acid. They also occupy monoterpenes. The main ingredients in the essential oil of all types of peppermint (Salehi et al., 2018).

Peppermint has anti-colic, antispasmodic, and carminative effects. It stimulates the digestion process, relieves nausea, and reduces stomach disorders (Singh & Pandey, 2018). However, concentrated peppermint extract is fortifying, and some research indicates the possibility of its use (Hussain et al., 2021). To treat diarrhea, dermatitis, cramps, and muscle pain, as well as to treat asthma, bronchitis, sinusitis, and influenza, it is an anti-toxin. It protects against fungi and protects against infections of the bone marrow, stomach, and intestines resulting from exposure to radiation (Shaikh, 2014) There are no known health risks for mint if consumed in moderate quantities, but some scientific research and studies have proven that some medicinal plants, including mint, have harmful effects on the reproductive system and thus affect the fertility of both males and females (Kalemba & Synowiec, 2020). Therefore, it is recommended to reduce its intake in large quantities due to its effects on sexual fertility.

2.5.1. General Uses of Peppermint

Pliny the Great, 79 AD, was an ancient Roman author, natural philosopher, and commander in the naval and military forces. He wrote the Naturalis Historia (Book of Natural History), mentioned on how the Greeks and Romans crowned themselves with

peppermint by using it in their feasts and decorating their tables with its special sprays (Uritu et al., 2018). Their food is flavored with peppermint, such as food sauces and wines (Anwar et al., 2019). Mint is the oldest and most popular flavor used in desserts and is often used in tea and as a flavoring in ice cream, candy, gum, and toothpaste. Peppermint is also used in some shampoos, soaps, and skin care products. Menthol activates TRPM8 cold-sensitive receptors in the skin and mucous tissues, which are the main source of the cool mint sensation resulting from topical application of peppermint oil (Cohen et al., 2020). Peppermint flowers also produce large quantities of mint nectar and honey, while the rest of the parts of the plant from which the nectar is extracted are widely used as animal fodder.

2.5.2. The Most Important Uses of Peppermint Oil

Peppermint oil or extract of active peppermint substances is used in the manufacture of some medications to relieve joint pain, rheumatic infections, and medications for high blood pressure and atherosclerosis. It has also been found that it is possible to prepare foods for treating atherosclerosis and angina pectoris (Mahendran & Rahman, 2020). Mint can also be used to treat asthma, sinusitis, relieve cough and sore throat, and it has been found to play a role in affecting some types of cancer (Kandoudi et al., 2023). Some studies indicate the use of peppermint tea to treat heart palpitations, help reduce headaches and migraines, and facilitate sleep (Anwar et al., 2019). Some studies also indicate the use of mint leaf extract as a gas repellent in cases of bloating and colic (Elbrense et al., 2022). Peppermint oil is used in the manufacture of toothpastes and as an antiseptic and flavoring for mouthwashes and gums. It is also used in the food and cosmetic industries and is also used as a perfume for many pharmaceutical preparations (Mahendran & Rahman, 2020). Peppermint oil is considered a local antiseptic and a mild anesthetic, especially in the case of tonsillitis (Elbrense et al., 2022) and (Mahendran & Rahman, 2020). It is used to treat digestive system disorders, including diarrhea, colic, loss of appetite and acidity, and is widely used for cholera patients (Elbrense et al., 2022) and (Mahendran & Rahman, 2020).Improving the appearance of the skin, as it helps soothe burns, rashes, and other skin irritations (Kandoudi et al., 2023). Peppermint oil has also been found to help relax muscles, treat colds and influenza (Mahendran & Rahman, 2020), and can help as a stimulant for retained menstruation, and relieve cramps accompanying menstruation ((Cohen et al., 2020). It is also believed to play a role in

stimulating brain function and can increase the ability to concentrate by strengthening memory centers in the brain (Ajayi & Olonisakin, 2011).

2.5.3. The Use in Insect Pest Management

It was reported that mint plant extract *Mentha piperita* was the second-best treatment, leading to mortality in juveniles with a rate higher than 50% in *T. castaneum* juveniles and a lower rate in *T. granarium* juveniles, compared to higher levels of mortality using *R. Officinalis*. In the same study peppermint extract also recorded a similar effect on adult females with a mortality rate higher than 70%, but its effect was much less on *T. granarium* adults, with a significant higher effect than *Thymus vulgaris* and *Melissa officinalis*, but a lower effect than *R. officinalis* (Parrey et al. 2023).

CHAPTER 3

MATERIALS AND METHODS

3.1. Laboratory Experiments

3.1.1. Collecting, raising and diagnosing insects

Adults of the red rusty flour beetle *T. castaneum* were taken from samples of infested flour collected from local retail markets. The insect was reared and propagated on flour under natural storage conditions (approximately 27°C). Then, 50 pairs (males and females) of the insect were isolated, distributed into 2-liter glass breeding bottles, covered with pieces of milling cloth, and tied with rubber bands. The insects were fed on wheat flour free of insect infestation. The bottles were then placed in the incubator at a temperature of 26±2°C and a relative humidity of 70±4%. The colony was constantly renewed with careful renewal every 2-4 weeks to obtain different larval stages and adults for subsequent experiments. The insect was identified as the rusty-red flour beetle *T. castaneum* with the assistance of the Natural History Museum and Research Center/University of Baghdad.

3.1.2. Isolating the Insect at Different life Stages

The first larval instar was collected by placing pairs of adults in a plastic dish while monitoring the first instar larvae due to the difficulty of obtaining eggs. Then the successive moults and the size of the larvae were observed. On this basis, the different larval stages were isolated until the pupal stage and then the adults. To control the movement of the adults during the examination, they were stopped. They were moved for a short period by anesthetizing them by placing them under freezing for one minute (Arakane et al., 2004).

3.1.3. Plant Leaf Extract (*M. piperita* L.)

Collecting and identifying plant samples: Plant samples were collected on 3/1/2016 from one of the orchards of the city of Kufa, Al-Alwa area. The plants were identified in the laboratories of the Department of Horticulture/College of Agriculture/University of Kufa to confirm the type of plant: the local mint *Mentha pirperita* L. The leaves were washed and then air-dried at laboratory temperature away from sunlight on sheets of newspaper, taking care to turn them constantly. When they were completely dry, the dry leaves were ground with an electric grinder and turned into to a fine powder, put it in plastic containers and store it in the refrigerator until use.

3.2. Experiment General Procedure

3.2.1. Insect Rearing

Insect culture of adult *T. castaneum* insects used in this experiment was collected from a culture kept at the Department of Plant Protection, Faculty of Agriculture, University of Kufa, Iraq. The culture was maintained on organic whole flour containing 5% brewer's yeast in a dark incubator at 26 ± 2 º C and 70 ± 4 ºC % relative humidity (Figur1). The flour was replaced every 2–4 weeks. Adults were starved for 24 hours before the start of the experiments.

3.2.2. Plant Extracts and the Insecticide Used

Fresh mint plants were collected from a farm located in the south of Najaf province/Iraq and then washed with running tap water. After washing the mint leaves, they were left in the laboratory for seven days until completely dry. The dried leaves were cut, ground by hand, and stored in black polythene bags.

3.2.3. Methanol Leaf Extract of Mint *M. piperita*

100 g of the acquired powder was extracted by adding 0.5 L of 70% methanol (MeOH) for 48 hours at room temperature. The methanol was then evaporated using a rotary evaporator at 30–40 °C to obtain the crude extract.

3.2.4. Essential oil of Mint *M. piperita*

100 g of dried plant material and 500 mL of water were subjected to hydrodistillation for 4 h by using the Clevenger apparatus. It was then separated into the Mint essential oil and kept in a refrigerator at 4 °C for further analysis. In order to compare the effect of selected plant extracts and essential oils with conventional insecticides as ctr+, a Deltamethrin (Decis® 2.5EC) was chosen.

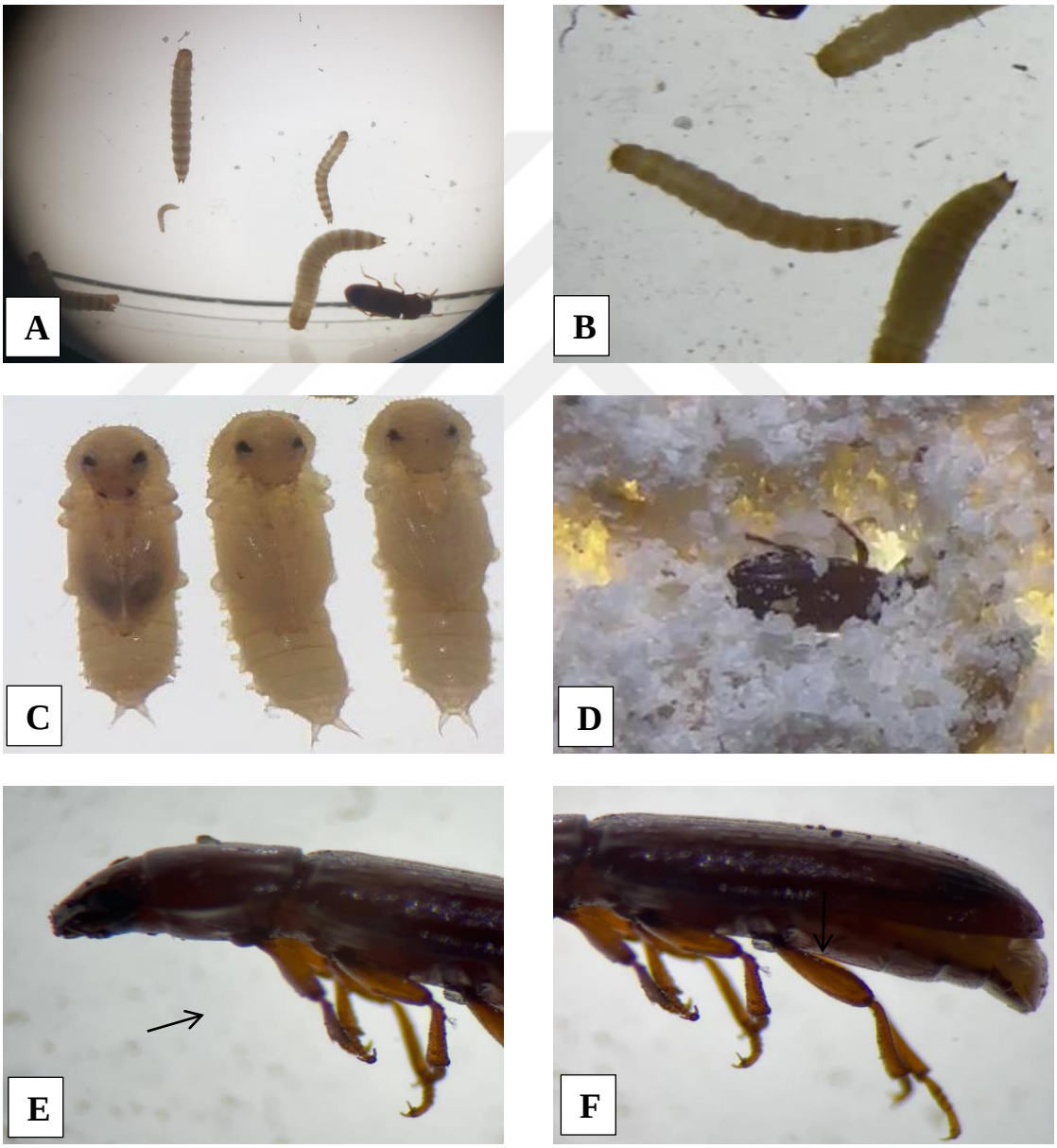


Figure 1. Different life stages of the red flour beetle *T. castanem* rared in the laboratory; A) Insect larvae at different instar ages, B) The 3rd instar larvae, C) Developped insect's nymphs, D) Adult insect digging and feeding in the flour, E and F) Adult insect's anterior

3.2.5. GC–MS Analysis

The essential oils and methanolic extracts of Mint were analyzed by using gas chromatography with Mass spectrometry (GC–MS) to identify the components based on the comparison of their relative retention index and compared to the standards of mass spectra.

3.2.6. Bioassays

a. Spray method

Adults, pupae and 3rd instar larvae were sprayed with 3 mL of the toxic solution by using a hand sprayer after calibration to make a homogenous coverage. Insects were left for 15 min and then transferred to Petri dishes containing white flour supplemented with brewer`s yeast at 30°C. Five biological replicates were used. Each replicate consisted of 10 insects. Survival was monitored on a daily basis for 7 days. In order to obtain 1% solutions treatments, 0.5 ml. of tested materials and 1 ml of soap liquid were put in a glass flask and filled to 50 ml

b. Delivery of tested materials (TM) by feeding

The extracts and essential oils were delivered via flour disks prepared as described by (Xie et al., 1996). 10µl of flour suspension (TM, 5% brewer`s yeast) was prepared in flat bottom wells of a 96-well microtiter plate and allowed to dry out at room temperature. Next, individuals were added to each well. Five biological replicates each consisting of 10 insects for the survival experiments and 5 larvae for each replicate were fed for 72h on flour disks at a range of concentrations (250, 500,1000, and 2000 ng/mg diet) in addition to ctr+ and ctr-. Five replicates are used for each treatment which lasts for 7 days for adults and 21 days for immature insects.



Figure 2. The red flour beetle *T. castaneum* adults distributed according the experimental treatments

Repellent/attractive effect on the red flour beetle *T. castaneum* adults

The attractive and repellent effect was tested with different concentrations of peppermint oil or the methanol extract of mint leaves using a Chemotropometer (Figure 3-1). The device is a wooden box with dimensions of 100 x 30 x 30 cm with a movable lid. It contains two opposite openings through which a glass tube with a length of 140 cm and a diameter of 3 cm passes. In the middle of the tube is an opening for inserting insects. The tube is graduated to centimetres. The test was carried out. I placed a piece of cotton at the entrance to the opening of the tube containing the concentration of the extract or oil to be tested, and in the other hole I placed a piece of cotton containing only sterile distilled water. I inserted ten adults for each replicate and three replicates for each concentration. The lid of the box was closed to prevent light from reaching the adults and I was left. The adults were allowed to move for 15 minutes, then the box was opened and the numbers of adults moving towards or away from the piece of cotton treated with the concentrations

of the extract or oil were recorded. The rate of attraction and repulsion was calculated according to the equations:

Atraction (%)=(No.insects directed to substance)/(Total No.of insects) x100

Repellenec (%)=(No.insects opposit to substance)/(Total No.of insects) x100



Figure 3. Chemotropism device used in testing the repulsive and attractive properties of mint essential oil and mint methanol leaf extract

3.3. Experimental Design and Data Analysis

All experiments were repeated twice, and data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). Waller-Duncan's multiple range test was used for means separation wherever appropriate. The experiment was conducted according to Completely Randomized Design RCD with five replications. Means were compared for the least significant difference LSD with multiple comparison using Duncan's multiple range test at 95% of confidence ($P \leq 0.05$). and posttirior body parts, respectively.

CHAPTER 4

RESULTS

4.1. Effect of Spraying With Peppermint Oil on Flour Beetle Larvae

The results (Figure 4) indicate that peppermint oil showed a clear antibiotic effect against the third instar larvae of the red flour beetle, especially at the highest concentration of 12%, while the lower concentrations of 8%-10% of the oil were in most cases less or equal to the effect of spraying with the control treatment (water and soap). It is noted from observing the figure that the higher concentration led to a mortality rate of more than 80% from the first day, compared to a smaller number of deaths that did not exceed 10% in 10% oil spraying treatments, which generally recorded relatively higher mortality rates or close to those recorded when using oil at a higher concentration. 8%.

4.2. Effect of Spraying With Mint Oil on Flour Beetle Adults

As for the effect of spraying with peppermint oil on insect adults (Figure 5), the results showed that the effect of the oil was increasing on insect mortality with increasing concentration used, as the concentrations differed significantly ($P \leq 0.05$). The concentration of 8% generally recorded the lowest mortality rate from the first day to the seventh day, which ranged from 35% -to 42%, while the 10% concentration led to a significantly higher mortality rate than the previous concentration, as it recorded a mortality rate that ranged from 68% -to 75%. This is compared to the higher concentration (12%), which recorded a mortality rate in insect adults on the first day, which exceeded 85% and ended on the seventh day with a percentage of 94%, which differed significantly ($P \leq 0.05$) from the effect of the two lower concentrations of 10% -8% of the mint oil (Figure 5)

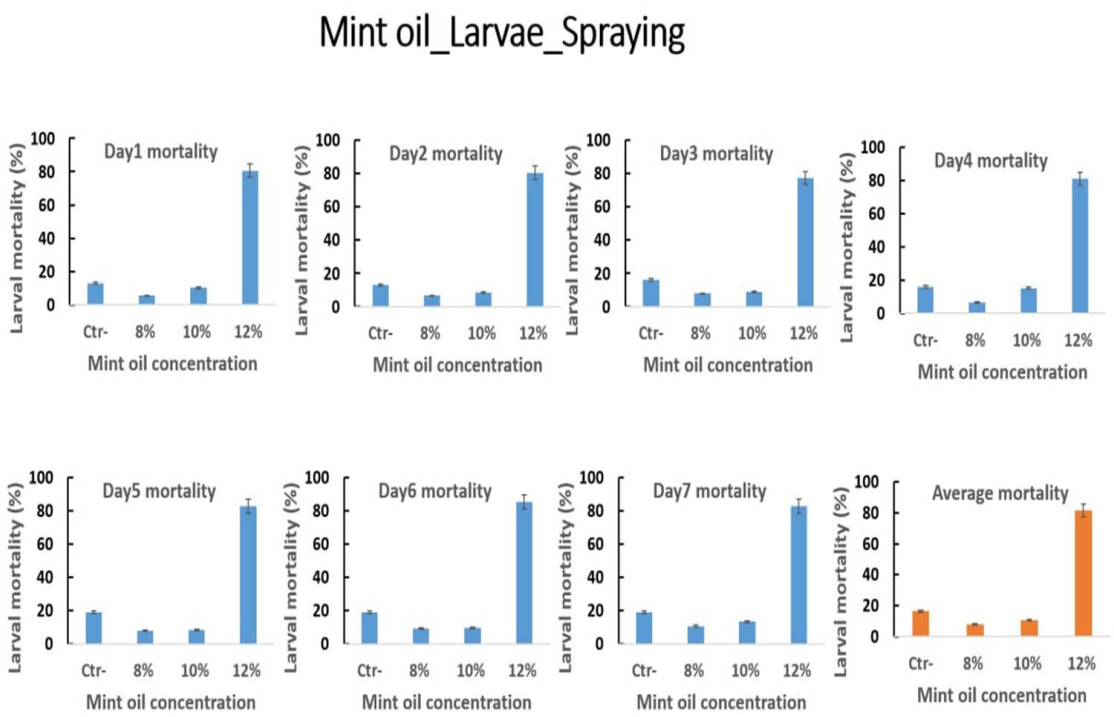


Figure 4. Effect of spraying with mint oil at different concentrations on the red flour beetle larvae

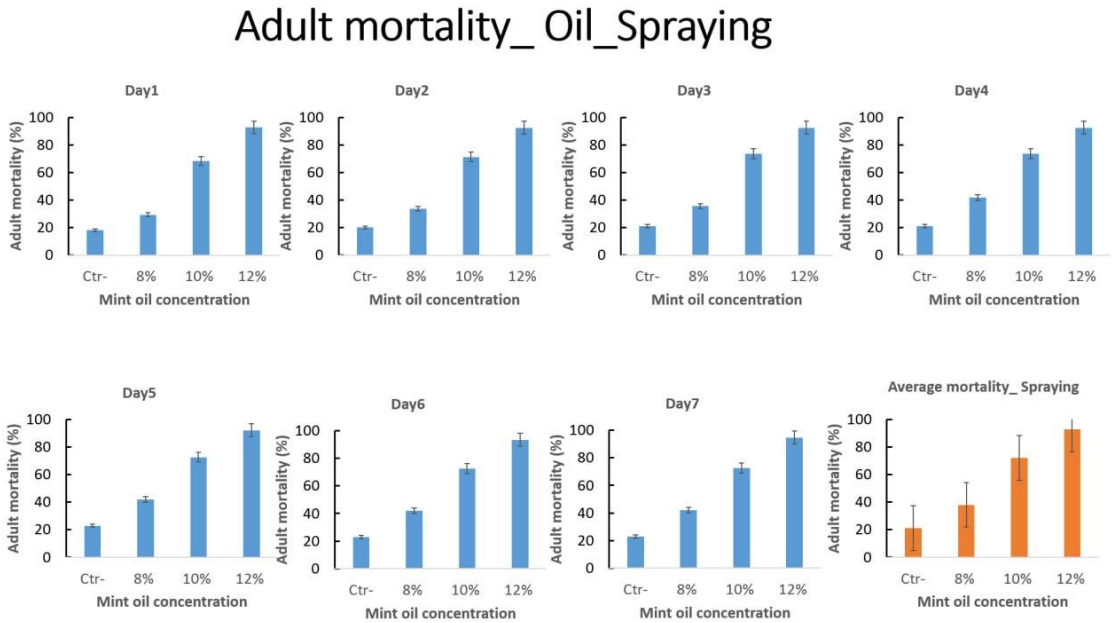


Figure 5. Effect of spraying with mint oil at different concentrations on the red flour beetle adults

4.3. Effect of Feeding Tablets Treated with Mint oil on Flour Beetle Larvae

The results show that the concentration of oil in the insect's food had a significant effect in reducing the number of larvae, especially in the treatment with the highest concentration of 12%, which recorded a mortality rate of 82%, which differed significantly from the two lower concentrations of 10% - 8%, as it was noted (Figure 6) that the last two concentrations were sometimes even less effective with an effect less or equal to the control treatment (soap and water). In general, the mortality rate at the 10% mint oil was slightly higher and sometimes even equal to that at the 8% concentration, as the mortality rate at these two concentrations started from 3% and did not exceed 18%.

4.4. Effect of Feeding on Mint oil-treated Tablets on the Red Flour Beetle Adults

It was found (Figure 7) that the effect of feeding food treated with peppermint oil at different concentrations on Noah was similar to the effect of spraying oil on flour beetle adults, as the mortality rate of adult insects increased with an increase in the concentration of oil in the food. The lowest mint oil concentration (8%) recorded mortality rates that ranged from 28% on the first day to 40 % on the seventh day, with a significant difference from the higher concentration (10%), which recorded a mortality rate higher than 60% from the first day, which increased to more than 75% after seven days. While the higher concentration of 12% also differed significantly from the two lower concentrations, recording percent mortality higher than 85% compared to more than 95% on the seventh day (Figure 7).

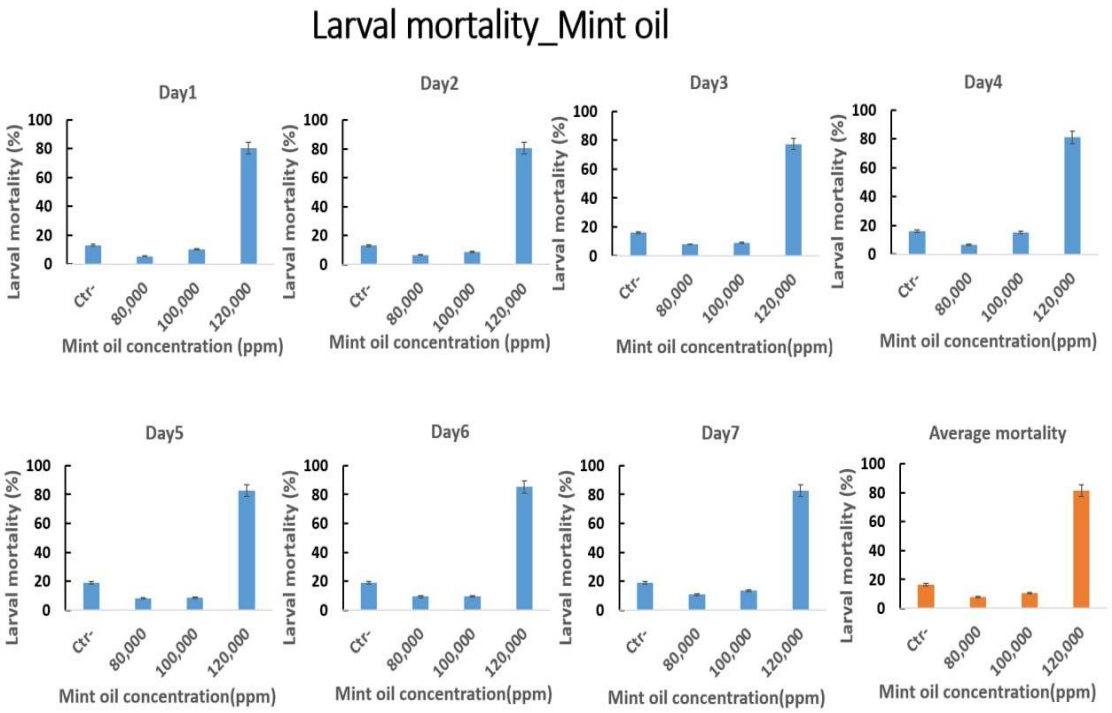


Figure 6. Effect of feeding on mint oil-treated tablets on the red flour beetle 3rd instar larvae

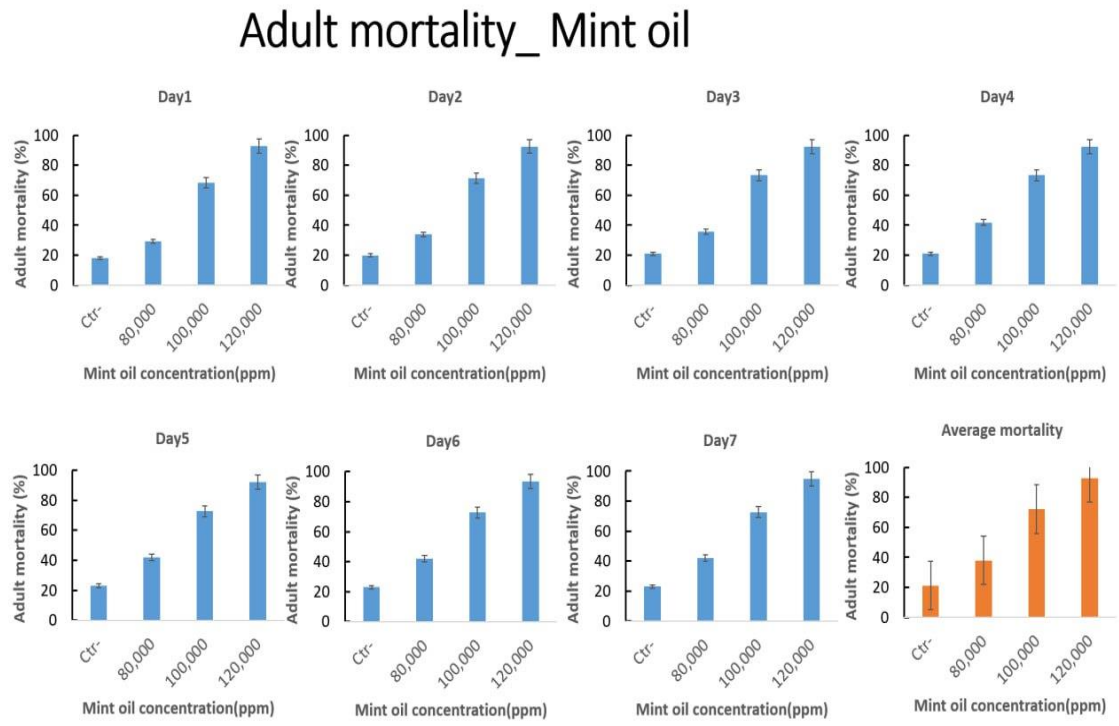


Figure 7. Effect of feeding on mint oil-treated tablets on the red flour beetle adults

4.5. Effect of Spraying With Methanol Mint Extract on the Red Flour Beetle Adults

It is noted from the results (Figure 8) that the concentrations of the mint extract were close in their influence and with slight differences in the percentage of insect adults' mortality. In general, the lowest concentration (10000ppm) recorded mortality rates ranged from 75% -to 82% after seven days of treatment, with a significant difference from the control treatment sprayed with soap and water, which recorded a natural mortality rate that did not exceed 15% after seven days of treatment. The death rate increased to approximately 80% on the first day, with two concentrations 13.333ppm and 20000ppm which were close in their effect and mortality rate, with slight differences in most of the treatment days. Where, it was found that the average mortality in the concentration 13.3333ppm was sometimes increased on the percent mortality recorded in the treatment with the highest extract concentration (20000ppm). Generally, the highest focus has led to higher mandatory deaths than those registered in the less than 100,000PM concentration.

4.6. Peppermint Oil Repellent/Attractive Effect on the Red Flour Beetle Adults

In testing the repellent/attractive effect of peppermint oil on adult insects of the red rusty flour beetle, the results generally showed that peppermint oil showed a repellent effect towards the insect, especially at high concentrations. The repulsion rate of peppermint oil at the lowest concentration of 8% differed with significant differences from the attraction rate after 15 minutes of treatment (However, the results showed that sometimes the repellent/attractive effect of peppermint oil was not clear in the repulsion/attraction test, which did not differ between them at the concentration of 10% (Figure 9). On the other hand, the 12% concentration treatment recorded high significant differences between the rate of attraction/repulsion of the insect adults. Where the largest number was recorded in the direction opposite to the oil treatment with a percentage of expulsion (Figure 9).

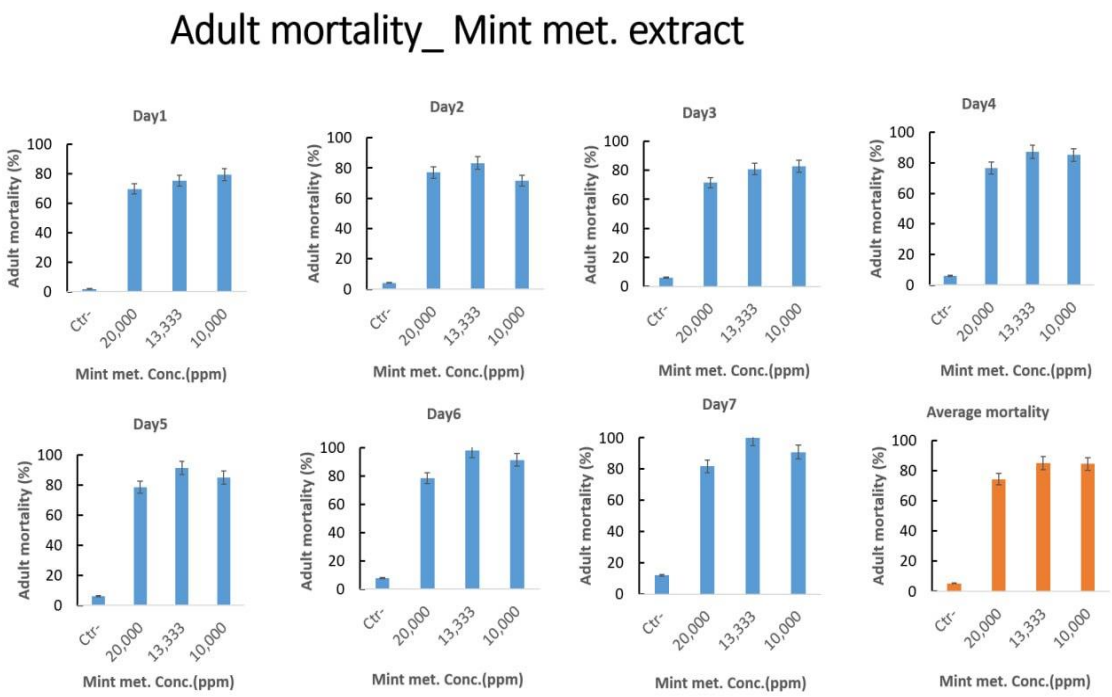


Figure 8. Effect of spraying with methanol mint extract on the red flour beetle adults

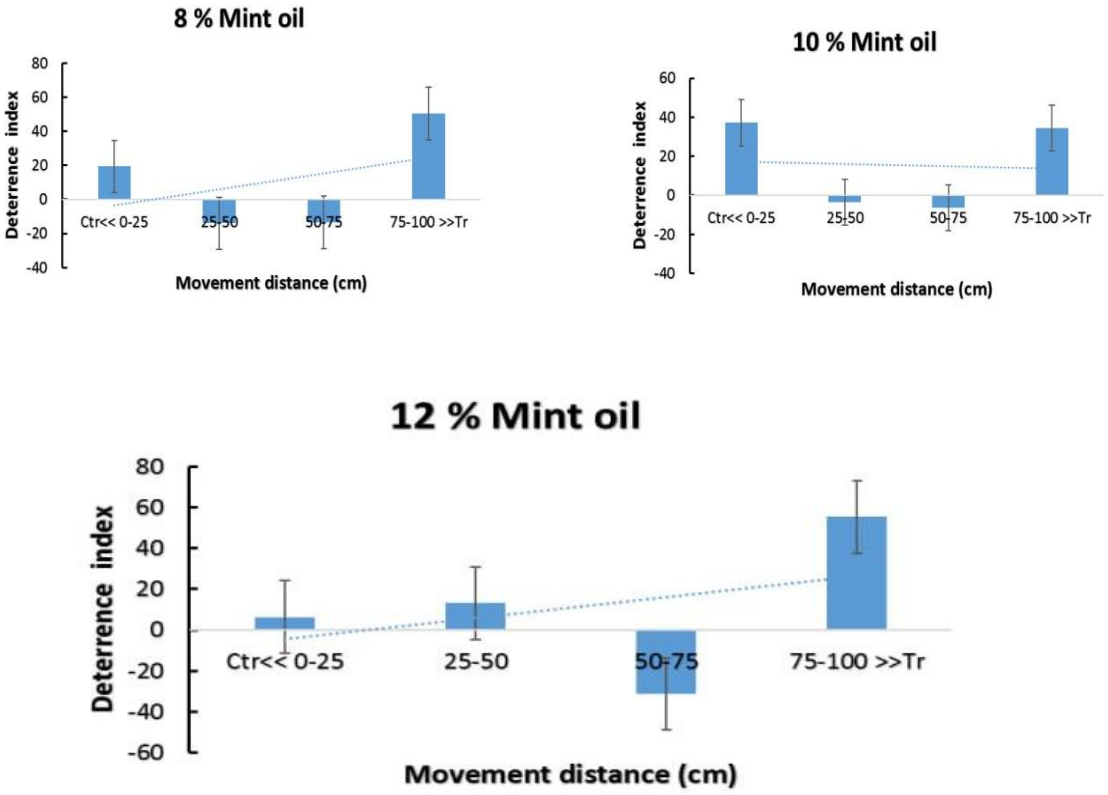


Figure 9. Peppermint oil repellent/attractive effect on the red flour beetle *T. castaneum* adults

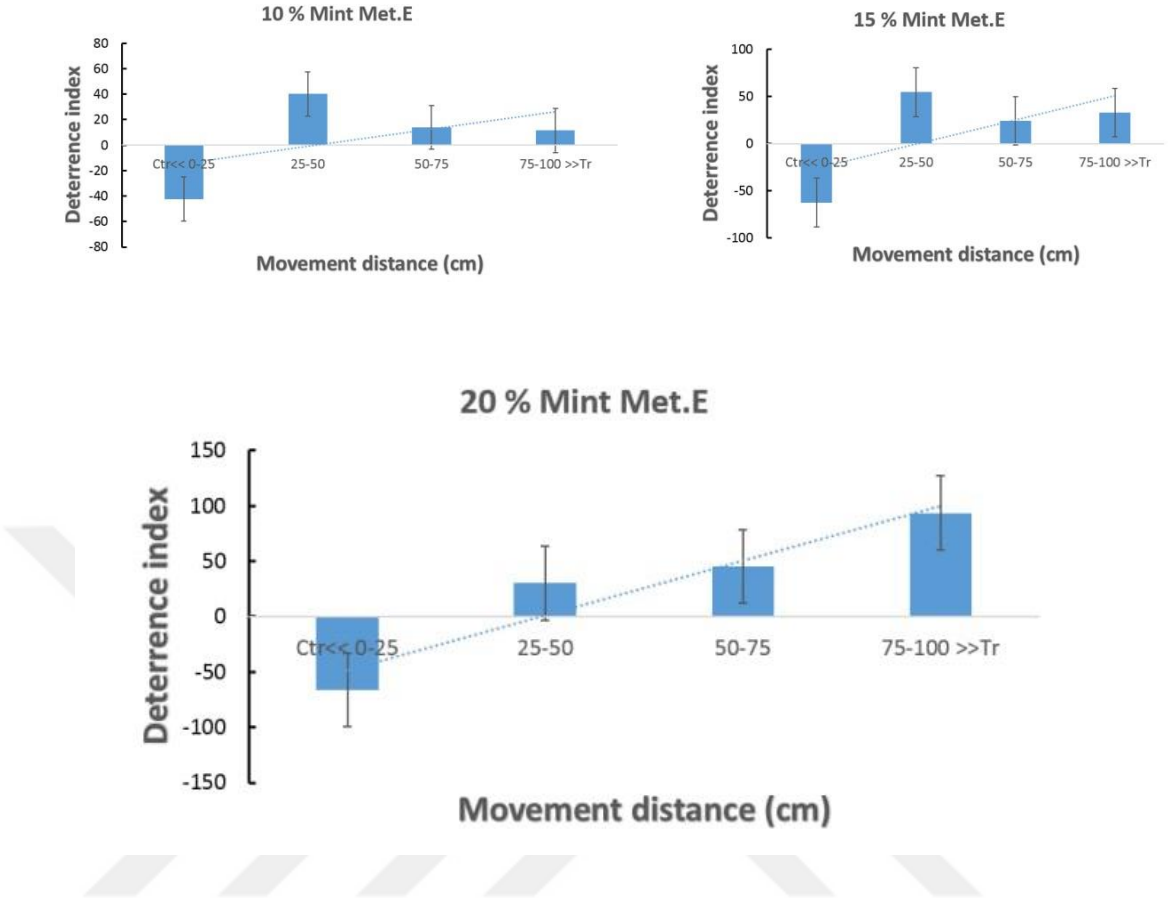


Figure 10. Peppermint *M. piperita* leaf methanol extract repellent/attractive effect on the red flour beetle *T. castaneum* adults

The results showed that the effect of the methanolic mint leaf extract was somewhat similar in its repellent effect recorded in peppermint oil on the insect, especially at the higher concentration of the extract (15%). It was found at a low concentration of the extract that the distribution of insects at a distance of 10-50 cm did not differ between them, while the insect concentration was large towards the control (Figure10), the repellency rate also increased at the distance closest to the control 25-50 cm, and a significantly low repellency rate was recorded in The distances are 50 - 100 cm, although the repulsion towards the treatment was mathematically higher than the average distance of 50 - 75 cm (Figure10). However, it was found that the repellence to the adult insects was significant at the higher concentration of the 20% extract, which recorded a significant repellency at the end tube spot compared to all other distances (0 - 75 cm) of the scale (Figure10).).

4.7. GC-MS Analysis

4.7.1. GC-MS Analysis of Peppermint Leaf Methanol Extract

The results of the GC-MS analysis of alcoholic mint extract (Table1) showed that there are ten active substances that recorded the highest estimated peaks for the compounds: Oxime-, methoxy-pheny ($C_8H_9NO_2$) with an area of 45.02 and a retention time of 4.169 min, Cyclotrisiloxane, hexamethyl ($C_6H_{18}O_3S$) with an area of 34.67 and a retention time of 6.566 min. Cyclotetrasiloxane, octamethyl ($C_8H_{24}O_4Si_4$) B with an area of 3.56 and a retention time of 9.249, Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) with an area of 1.72 and a retention time of 21.125 min, and Tetraacetyl-d-xylonic nitrile $C_{14}H_{17}NO_9$) with an area of 2.04 and a retention time of 21.713 min (Table1).

4.7.2. GC-MS Analysis of The Actice Compounds in Peppermint Essential Oil

On the other hand, the results of the GC-MS analysis of peppermint oil showed that 15 compounds of active substances were recorded (Table2). The most abendence of which recorded the highest estimated peaks were: 5-methyl-2-(1-methyl ethyl-trans) ($C_{10}H_{20}O$) with an area of 23.23 and retention time of 4.124 min, Cyclohexanol ($C_{20}H_{26}O$, and $C_{20}H_{26}O$) with an area of 47.50 and a retention time of 8.739 min, Menthyl acetate ($C_{11}H_{22}O$) with an area of 11.59 and a retention time of 10.678 min,

Table 1. GC-MS analysis of the actice compounds inpeppermint leaf GC-MS analysis of peppermint leaf methanol extract.

No.	R.t (min)	Compounds	Molecular formula	M.W (g/mole)	Area %
1	3.710	Glycine-N,N-bis(methylenephosphonic acid) 113289 002439-99-8	$C_4H_{11}NO_8P_2$	263.08g/mol	1.84
2	4.169	Oxime-, methoxy-phenyl-_ 24837 1000222-86-6	$C_8H_9NO_2$	151.16 g/mol	45.02
3	4.731	11H-Dibenzo[b,e][1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino) propyl]- 128388 013450-70-9	$C_{13}H_{10}N_2O$	210.23 g/mol	2.88
4	6.566	Cyclotrisiloxane, hexamethyl- 79619 000541-05-9	$C_6H18O_3Si_3$	222.46 g/mol	34.67
5	6.809	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl- 129526 055682-87-6	$C_{21}H_{30}$	282.4629 g/mol	4.83
6	7.423	2-Aminononadecane 130243 031604-55-4	$C_{19}H_{41}N$	283.5 g/mol	1.92
7	9.249	Cyclotetrasiloxane, octamethyl- 141484 000556-67-2	$C_8H_{24}O_4Si_4$	296.61 g/mol	3.56
8	10.141	Arsenous acid, tris(trimethylsilyl) ester 178799 055429-29-3	$C_9H_{27}AsO_3Si_3$	342.49 g/mol	1.53
9	21.125	Hexadecanoic acid, methyl ester 119400 000112-39-0	$C_{17}H_{34}O_2$	270.5 g/mol	1.72
10	21.713	Tetraacetyl-d-xylonic nitrile 178849 1000130-04-4	$C_{14}H_{17}NO_9$	343.29 g/mol	2.04

beta.-ylangene ($C_{15}H_{24}$) with an area of 3.61 and a retention time of 13.101 min, and Tetraacetyl-d-Caryophyllene oxide ($C_{15}H_{24}$ O) with an area of 0.34 and 16,052 min retention time (Table2).

Table 2. GC-MS analysis of the actice compounds inpeppermint essencial oil

No.	R.t (min)	Compounds	Molecular formula	M.W (g/mole)	Area %
1	5.623	D-Limonene 15682 005989-27-5	$C_{10}H_{16}$	136.23 g/mol	1.12
2	6.999	Cyclohexanol, 5-methyl-2-(1-methy lethyl)-, (1.alpha.,2.alpha.,5.alph a.)- 28381 000491-02-1	$C_{10}H_{20}O$	156.26 g/mol	0.36
3	7.440	Butanal, 3-hydroxy- 2033 000107-89-1	$C_4H_8O_2$	88.11 g/mol	0.30
4	8.124	Cyclohexanone, 5-methyl-2-(1-methy lethyl)-, trans- 26912 000089-80-5	$C_{10}H_{18}O$	154.25 g/mol	23.23
5	8.349	Cyclohexanone, 5-methyl-2-(1-methy lethyl)-, trans- 26912 000089-80-5	$C_{10}H_{18}O$	154.25 g/mol	5.50
6	8.739	Cyclohexanol, 5-methyl-2-(1-methyl ethyl)- 28342 001490-04-6 Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.) 28387 015356-70-4	$C_{20}H_{26}O$ $C_{10}H_{20}O$	282.4g/mol 156.26 g/mol	47.50
7	10.046	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)- 25293 000089-81-6	$C_{10}H_{18}O$	154.25 g/mol	0.43
8	10.357	1-Decanol 29593 000112-30-1	$C_{10}H_{22}O$	158.28 g/mol	1.54
9	10.678	Menthyl acetate 59498 000089-48-5	$C_{12}H_{22}O_2$	198.30 g/mol	11.59
10	11.456	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethyl idene)-64474 003242-08-8	$C_{15}H_{24}$	204.35 g/mol	0.17
11	12.495	(-)-.beta.-Bourbonene 64360 005208-59-3	$C_{15}H_{24}$	204.35g/mol	1.25
12	12.824	photocitral B 25079 006040-45-5	$C_{10}H_{16}O$	152.23 g/mol	0.35
13	13.110	.beta.-ylangene 64294 1000374-19-1	$C_{15}H_{24}$	204.35 g/mol	3.61

Table 2. GC-MS analysis of the actice compounds inpeppermint essencial oil (continued)

No.	R.t (min)	Compounds	Molecular formula	M.W (g/mole)	Area %
14	13.309	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- 64463 023986-74-5	$C_{15}H_{24}$	204.35 g/mol	0.94
15	13.560	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- 64463 023986-74-5	$C_{15}H_{24}$	204.35 g/mol	0.27
16	13.707	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]- 64579 017334-55-3	$C_{15}H_{24}$	204.35 g/mol	0.62
17	14.140	Spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]- 64504 024048-44-0	$C_{15}H_{24}$	204.35 g/mol	0.25
18	14.572	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- 64463 023986-74-5	$C_{15}H_{24}$	204.35 g/mol	0.38
19	16.052	Caryophyllene oxide 77539 001139-30-6	$C_{15}H_{24}O$	220.35 g/mol	0.34
20	21.116	Hexadecanoic acid, methyl ester 119400 000112-39-0	$C_{17}H_{34}O_2$	270.5 g/mol	0.26

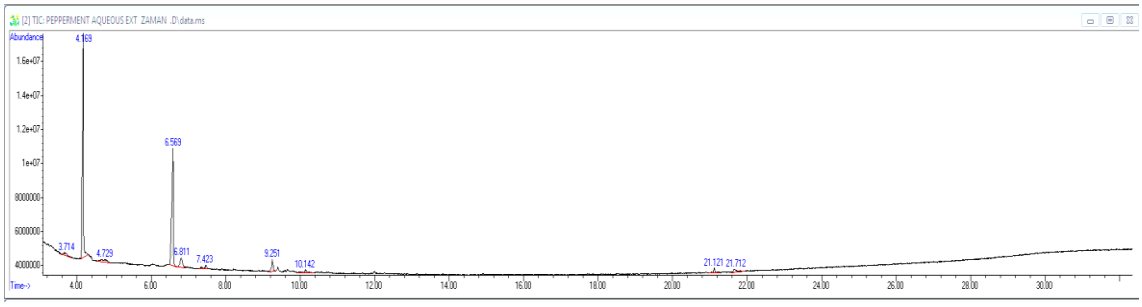


*Figure 11.*GC-MS chromatogram analysis (MassHunter) of peppermint *M. piperita* mathonol extract

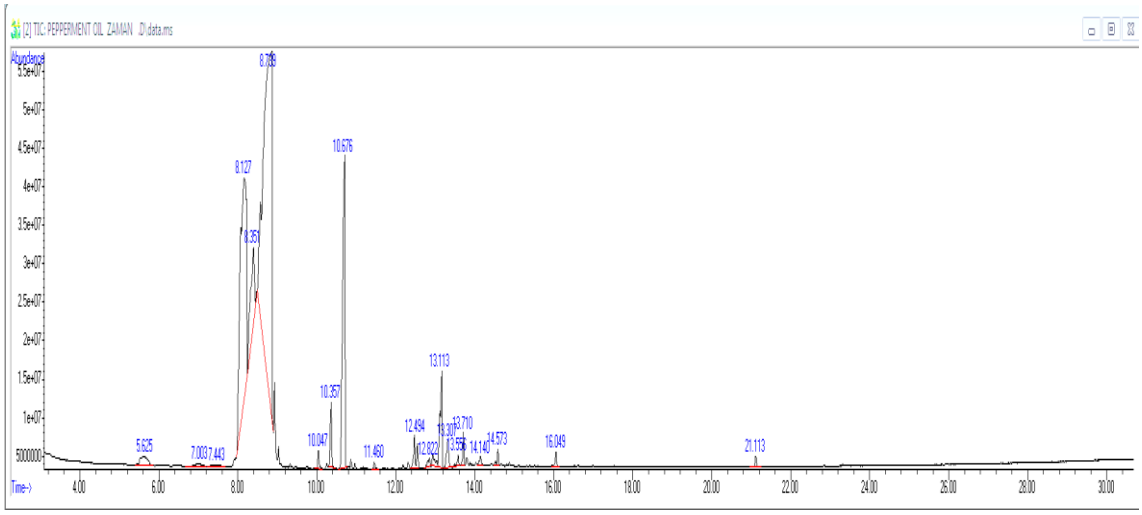


Figure 12. GC-MS chromatogram analysis (MassHunter) of peppermint *M. piperita* essencial oil

CHAPTER 5

DISCUSSION

5.1. Discussion

The results obtained in this study agreed with previous results in testing the efficacy of plant extracts from four plants: *Melissa officinalis*, *Thymus vulgaris*, *Mentha piperita*, and *Rosmarium officinalis*. Mint was the second-best treatment, leading to mortality in juveniles with a rate higher than 50% in *T. castaneum* juveniles and a lower rate in *T. Granarium* juveniles, compared to higher levels of mortality using *R. officinalis*. Peppermint extract also recorded a similar effect on adult females with a mortality rate higher than 70%, but its effect was very less on *T. granarium* adults, with a higher effect than *Thymus vulgaris* and *Melissa officinalis*, but a lower effect than *Rosmarium officinalis* (Panezai et al., 2019).Damage to food products and stored grains can be considered an ongoing problem in all regions of the world and is more serious in developing countries (Stejskal et al., 2015). In general, stored grains are often affected by insect infestations that accompany crops stored in large warehouses or retail stores. The damage resulting from stored grain pests is estimated at approximately 10-40%, not only through the consumption of stored grains and materials, but also in reducing the quality of the stored product and making it unfit for human consumption or a significant reduction in its marketing value (Paine & Lieutier, 2016). The anti-inflammatory effect of peppermint oil on warehouse insects is often due to the nature of the oily fats, which interfere with their content of active substances to influence basic metabolism and physiological-biochemical processes, which may affect the behavior of the treated insects (Linsley, 1944). In general, the anti-insect effect of peppermint oil has been the subject of many studies, most of which were directed towards combating insects. and Coleoptera insects (Kumar et al., 2011). On the other hand, although vegetable oils are

environmentally friendly and harmless to humans, the treatment of stored food products and grains must be accompanied by controls and consideration to remove the effect of the oil to a level that does not affect the stored food material and does not reduce the commercial properties of the material (Golob, 1997) .Sometimes, the presence of insect pests of stored materials, including rice weevils, corn weevils, red and similar rusty flour beetles, and other insects, is considered the determining factor for the survival of the crop with good economic quality (Keskin & Ozkaya, 2013), since in these cases the specific damage to the stored material is more severe than the damage caused by quantitative loss (Kumar et al., 2011). Natural peppermint compounds have the ability to affect the sensing and communication systems of insects (Mishra et al., 2012), which may lead to reduced mating and egg reduction and thus limit the reproduction of this pest (Regnault-Roger et al., 2012). It also reported that some compounds in mint may affect the hormonal and developmental systems of insects, enhancing the chances of controlling their (Kumar et al., 2011). The study of Lee et al., (2002) indicated the effectiveness of *M. piperita* oil when used in relatively low concentrations (LD50-25.8µl/l) against the red rusty flour beetle *T. castaneum*. This confirmed previous results in the study (Khani et al., 2012) When using it on *S. Oryzae* languages. Where it was found that mint oil used by evaporation or contact differed in its impact on the main warehouses insects *T. castaneum, Lasiooderma Serricorne* and *Liposclis Bostrychophiva* where a high mortality rate with low concentrations was recorded on *T. castaneum* vaporization was LD50 18.1 MG/L /L (Pang et al., 2020). Mint is known for its active compounds such as menthol, methyl acetate, and menthone, possessing antibacterial (Fazal et al., 2023), antifungal (Abdelli et al., 2016), and insect-repellent properties (Kumar et al., 2011). The results of the study implicitly agreed on the effect of the Mentha SPP. *T. castaneum*, which was found to be relatively less sensitive compared to the *C. maculatus* insect, which showed a higher allergy to vegetarian oil (Kalaf, n.d.). Similar results recorded in the effect of methanolic mint extract of mint pepperclecomers, which recorded a death rate of 70% in insect food treatment after a period of 7 days of treatment in laboratory plastic vessels (Saljoqi et al., 2006). Studies indicated that even by using evaporation on warehouse insects using vegetable oil, the percentage of insect mortality depends on the type of insect and the type of vegetable oil used. Where it was found in the vital test that T µL/L in a row (Negahban et al., 2007)

5.2. Conclusion

The main results of this study can be summurized as followes:

- The study included evaluating the effectiveness of peppermint oil and peppermint leaf extract (methanol) against different stages of the red rust flour beetle.
- The study showed that treatments of *Mentha piperita* oil at all concentrations under study, when used as a spray on the insect or digestive in the insect's food, were significantly effective in causing high mortality rate in *T. castaneum* larvae and adults.
- In general, spraying peppermint oil on insects was sometimes more effective than digestive effect by treating insect feed with oil.
- On the other hand, it was found that spraying with methanol mint leaf extract also led to a clear mortality rate in insect larvae and adults, but with less efficiency than mint essential oil treatments.
- The results of ths study also showed that peppermint oil and methanol extract of mint leaves showed a moderate to high repellent effect on insect adults, which was fluctuating depending on the concentration used and period post treatment.

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YAYINLAR

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