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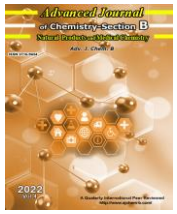

Original Research Article

Evaluation of Chemical Compound of Thyme Essential Oil and Repelling and Lethality Effect of Thyme Plant Essential Oil on Rice Weevil

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ABSTRACT

The use of plants, especially medicinal plants, for treating diseases and controlling pests biologically has increased significantly in recent years. Wild Mountain Thyme is one of these plants widely used in Iran, especially Khoy, Iran. Concerning the importance of this plant in the traditional medicine of the region and the need for controlling grain pests, we decided to study the plant's chemical compounds, the antibacterial effects of its essential oil, and its repelling and lethality effect. Wild Mountain Thyme was collected from the southeast of Khoy in the spring and identified in the Institute of Forests and Pastures. The plant's essential oil was extracted by distillation with water using a Clevenger machine. The resulting oil had a yellow color and a pungent smell, and its percentage was 1.64% compared to the dry weight of the plant. A total of 37 chemical compound was identified and isolated using a gas-mass chromatography device, the main of which was carvacrol (36.8%), thymol (36.8%), gamma-terpinene (10.6%), and paracymene (8.5%). The microbial test of the essential oil of the Thymus Fedtschenkoi plant was performed on three Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. anthracis*) and three Gram-negative bacteria (*P. aeruginosa*, *Sa. paratyphi*, and *E. coli*). Thymus Fedtschenkoi plant essential oil was effective on all Gram- and Gram-negative bacteria except for *Pseudomonas aeruginosa*. In addition, plant essential oil's repellent and lethal effect on rice weevil were evaluated in three repetitions with different concentrations. The highest removal rate was at the highest concentration of 25 µl and 72 hours. The lethal effect of the plant on the weevil was at a maximum concentration of 20 µl and duration of 72 hours. LC50 and LC90 of essential plant oil were calculated and determined based on probit analysis software. Based on the results, the plant essential oil can have a good effect in controlling the rice weevil pest both in the field and warehouse

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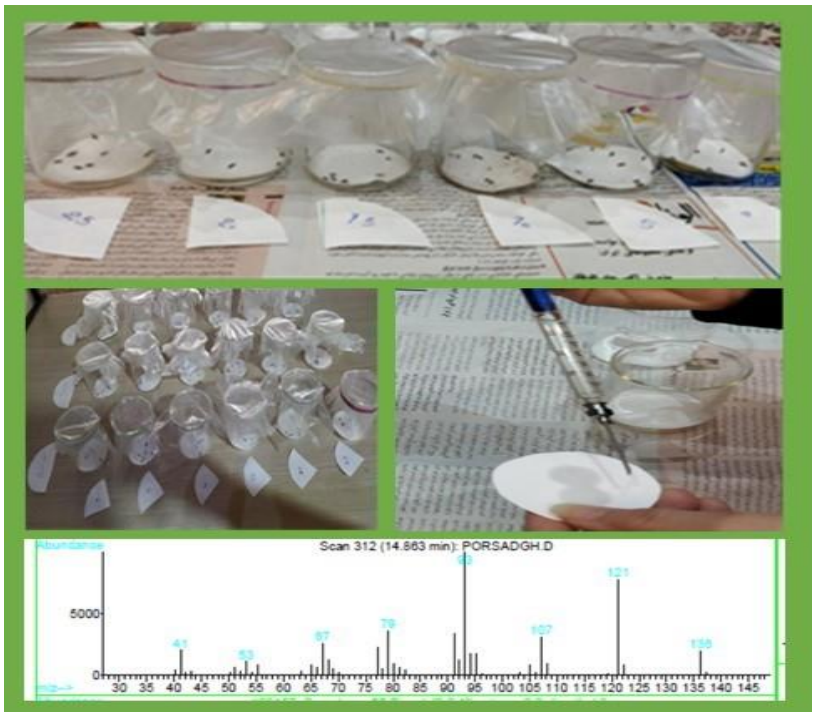
GRAPHICAL ABSTRACT



Introduction

A great deal of research led man to realize that he could only find peace and health by returning to the lap of nature and plants since plants are always by his side and respond to his physical and spiritual needs. On the other hand, humans get food, medicine, and many necessities from plants. Therefore, investigating plants and their position in different dimensions of human life significantly affects human attitude towards these noble beings of creation to create interaction and balance in human's relationship with them and pay more attention to non-material aspects, leading to man the truth of the role of plants in the spiritual life of man [1].

The thyme plant is one of the valuable plants that humanity has utilized for various treatments and non-therapeutic uses. Thyme is a plant from the mint family, which has a bushy structure and a straight, herbaceous, or woody stem with a height of 10 to 30 cm, and the branched stems of this plant are covered with white hairs. Thyme leaves have almost no petioles or very short petioles with gray leaves and oval shapes. Wild mountain thyme is a plant that grows naturally in fields and semi-arid or moderately hot areas at high temperatures and in places with intense and direct sunlight. The amount of water and soil and the light that shines on this plant directly change the growth of mountain thyme colonies (dry weight of roots and shoots). According to research, the light conditions and soil moisture were investigated on the growth rate of thyme, and the humidity does not affect the excellent growth of thyme as much as the light intensity. Thyme contains 0.8 to 2.6 essential oils, most of which are phenols, monoterpene hydrocarbons, and alcohols. Thymol is the main component of phenolic compounds in the thyme plant, and essential oil is the practical substance of thyme. Thyme essential oil is a dark yellow or reddish-brown liquid with a strong pleasant smell and a sharp, stable, and cooling taste, extracted from

the distillation of the leaves and flowering branches.

Aside from its use in food products, thyme leaf essential oil is used in pharmaceuticals, health, and cosmetics. Thyme oil has properties such as antispasmodic, windbreaker, antifungal, antibacterial, disinfectant, anthelmintic, anti-rheumatism, expectorant, antioxidant, natural food preservative, and anti-aging. Thyme essential oil is one of the ten famous essential oils with a special place in the world trade. A large amount of grain is destroyed every year by pests. Contrary to what is thought, storage products do not enclose only a few types of grains or products, but all the food that is somehow kept in warehouses, silos, and houses is attacked by pests [2].

Research conducted on antimicrobial properties

Amin Khani *et al.* (2018) conducted a study titled antimicrobial activity and chemical composition of essential oil extracted from the stem of leaves and flowers of mountain thyme. The wild mountain thyme plant is used as a medicinal plant to treat colds, respiratory infections, and bacterial infections, as well as food flavoring and preservatives in Iran. In this study, the essential oil of the plant's aerial parts of the flower stems and leaves was extracted using water distillation, and their chemical compounds, along with their antibacterial properties, were studied. According to the results, there was a significant difference in the chemical composition of the essential oil obtained from different parts of the plant, showing similar and significant antibacterial effects against Gram-positive and Gram-negative bacteria [3].

Momiwand *et al.* (2019) researched the antimicrobial activity and chemical composition of mountain thyme essential oil in different phenological stages. Changes in the chemical composition and biological activity of medicinal plant essential oils were observed due to the growth stage of the collected plant parts. In this study, the chemical composition and antimicrobial activity of the essential oils of aerial parts were investigated in different stages of growth. Both species' highest amount of essential oil was observed in the entire flowering stage. Hence, all essential oils had high antibacterial and antifungal activity against the tested microorganisms. Generally, this study highlighted the importance of selecting the harvesting time to achieve the optimal quality and quantity of essential oil along with antimicrobial activity used in the pharmaceutical and food industries [4].

Mosahafi *et al.* studied mountain thyme essential oil's antibacterial and antioxidant effects and extracted it *in vitro*. According to the findings, lipid peroxidation is a multi-step process in aerobic cells and leads to the interactions between molecular oxygen and unsaturated fatty acid. Free radicals are well-known factors that significantly affect lipid peroxidation and cause the destruction of food, creating the basis for the growth of microorganisms and cancer development. Antioxidant compounds prevent the production and activity of free radicals. Mosahafi pointed out the significant germicidal ability of thyme essential oil against eight tested bacteria. The results about the bactericidal effects of this essential oil were described as that the essential oil showed a more vital bactericidal ability compared to the methanolic extract. The reason can be due to several compounds with more polarity or heat resistance, including flavonoids and polyphenolic compounds. In addition, the presence of rosmarinic acid in the methanol extract of the plant is probably the cause of its antibacterial effects. In the present study, different methods were used to investigate the antimicrobial effect of thyme essential oil. This penetration is not done well because of the penetration necessity of the essential oil into the agar mesh tissue to affect the bacteria. This is especially likely for essential oils that have an oily

structure. Therefore, the standard microplate dilution method should be used after performing the disk diffusion method. This method determines the effective concentration of essential oil to inhibit the growth of bacteria (MIC). Sometimes, the results of this method need to confirm the results of microplate dilution due to the inaccuracy of the disk diffusion test. Moreover, the appropriate gel of essential oil to kill microbes (MBC) is prepared by culturing a small volume of the contents of the wells with an essential oil concentration higher than MIC in the agar culture medium. A small number of colonies growing in a concentration can be ignored in this method since the concentration is assumed to be lethal [5]. Jebelli explored the effect of Ajwain and thyme essential oils alone and together on some bacteria causing food spoilage (*Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*). Jebelli sed the standard microplate dilution method and calculated the MIC value of mountain thyme essential oil to be 250 ppm for Bacillus cereus bacteria and 500 ppm for other bacteria [6]. Motevassel *et al.* (2014) examined the bactericidal effect of wild mountain thyme on methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Staphylococcus saprophyticus*. The MIC values for the mentioned strains were reported as 32, 16, 16, 16, and 8 mg/mL, respectively, by the microplate dilution method. This study proved that the alcoholic extract of thyme could destroy 62.6% of methicillin-resistant *Staphylococcus aureus* MRSA bacteria. The minimum MBC bactericidal concentration of methicillin-sensitive *staph aureus*, *staph aureus ATCC25923*, *staph epidermidis*, and *staph saprophyticus* was reported as 256, 512, 256, and 512 mg/mL, respectively. In this study, more research was suggested due to the bactericidal effect of small amounts of thyme extract on the tested bacteria to use this extract in skin bandages with the purpose of bactericidal. The results showed the germicidal power of thyme essential oil on rice weevil. In addition, the non-allergenic nature of this essential oil was also proven in the study. The results of the present study indicated that thyme essential oil is an excellent candidate to replace other substances in removing rice weevil [7].

What is the difference between essential oil and extract?

1. Essential oil

According to the definition of plant extract and essence, we realized the difference between extract and essence. Despite the differences between essential oils and herbal extracts, there is no evidence to suggest that essential oils or extracts are appropriate. Since essential oil and extract have different properties, it cannot be mentioned that the mere presence of one can cause the other to have a higher quality or effectiveness over the other. Depending on what kind of therapeutic properties we want to use and in which one the desired substance is found, the use of extract and essence will differ depending on each person [8].

2. Extraction

According to the definition of plant extract and essential oil, it can be concluded that extract is a more general compound of essential oil, one of its components is essential oil. In other words, essential plant oil is a part of plant extract. Another difference between extracts and essential oils is that extracts have more effectiveness than essential oils. In addition, the preparation method of plant extracts differs from herbal essence. The extract separation and extraction mechanism are based on the solubility mechanism, while the process of separating essential oil is done by the distillation method through volatilization. Therefore, the main difference between essential oil and plant extract is its separation method through two different

mechanisms, based on which essential oil is no longer an extract [9].

Fighting warehouse pests with plant essences and natural toxins of wheat, barley, and rice is a strategy for warehouses attacked by pests such as wheat borer and flour bugs. The emergence of resistance to some toxins, especially malathion and festoxin, in most warehouse pests, and the complications caused by the accumulation of these chemical toxins in agricultural products and various diseases and poisonings have caused the replacement of natural toxins and biological methods of pest control. Cereals and legumes are one of the humans' most important food needs, and after harvesting, these products are kept in warehouses until consumption. Concerning the importance of legumes and grains in the human diet, experts have always made great efforts to protect these products from pests and plant pathogens during their growth and development in the field and storage. However, grains and legumes are still attacked by different pests and diseases, of which pest insects play the most prominent role. Every year, 10 to 25% of stored products are lost due to the attack of pests worldwide [10].

Synthetic toxins, which are used to protect stored products from insect damage, have led to an increase in the resistance of pest insects to insecticides. Today, the demand for plant insecticides is increasing due to the low residual risk of toxins on stored products. The damage of storage pests in countries without advanced storage technology is between 10 and 40% of the product. In Iran's rural areas, the damage caused by warehouse pests has been estimated at up to 80% due to the traditional warehouses. Tribolium confusum is one of the important pests of storage materials, which causes significant damage to the product due to its feeding, and due to the rapid increase in population, it contaminates the stored product with its feces and larval shells and severely reduces its quality. Every year, 10 to 20% of agricultural products in Iranian warehouses are lost to pests and other harmful factors. For example, the activity of the flour bug in warehouses and the rapid increase in its population causes the product to be mixed with feces, larval shells, and pupae in a short time and reduces its quality. Pests in storage are controlled mainly by using pesticides and gaseous chemical compounds. Repeated use of these substances over successive decades has destructed natural enemies, disrupted biological control, affected non-target organisms, caused environmental pollution, endangered human health, spread pests, and emerged resistance to target pests. First and foremost, warehouse pest control starts with keeping the human environment healthy even before any activity is economical. Spermatophytes have secondary metabolites, which play a vital role in plant ecological relationships, especially the interaction between plants and insects, and sometimes cause plant resistance to insects. An important part of these compounds are terpenoids, which are present in plant essential oil, are less dangerous for mammals, and seem to be a suitable substitute for chemical toxins to control warehouse pests. Various studies have shown that the essential oils of several plants have significant insecticidal effects [11].

Materials produced by plants are one of the potential sources of producing new pesticides. Essential oils extracted from plants usually break down in nature sooner. Therefore, they are less toxic for humans and other mammals, and also leave less harmful environmental effects.

Many plants and their secondary metabolites have physiological and behavioral effects on pest insects, including toxicity, repulsion, attraction, and anti-nutrition.

Influential factors in the quality of plant essential oils

The ingredients of essential oils change under different conditions. Therefore, it is only possible to comment on the substances in the essential oil

of a plant by considering specific environmental conditions. Essential oils have a direct relationship with the biosynthesis, metabolism, and biological activities of the plant, which are subject to the climatic conditions of the plant's environment [12].

Several factors affect the quality of essential oils, among which can be mentioned:

Weather: Weather is one of the influential factors in the compounds in a plant. Plants rich in effective substances are consistently grown in warm weather, and the quality of effective plant substances usually decreases in cold weather. The plant will always have the most effective substances and the best compounds in its natural growth and development conditions. Even the height above sea level prominently affects a plant's number of effective substances. There is a height at which each plant species has the maximum activity. Some plant species make more secretory glands and secrete essential oil when cultivated near light, which can influence their chemical composition. The role of the plant breed should also be considered, and the collection time significantly affects the active ingredients of the plant. In many cases, at least in green parts of plants, the formation of effective substances is very high during a plant's most active growth period. Even the hours of the day affect the number of plant compounds [13].

Chemical properties of essential oil

Essential oils are made of a series of complex compounds that differ from each other in terms of chemical composition. Various organic compounds such as hydrocarbons, alcohols, ketones, aldehydes, ethers, and esters, oxides are found in the volatile oil. Generally, 500 chemical compounds have been made, such as bitter almond oil, benzaldehyde, and methyl salicylate. The smell and taste of essential oils are related to their oxygenated compounds (such as alcohols, aldehydes, acetones, and others). Terpenes make up most of the structure of essential oils [14].

The word "essence" in the Persian language has equivalents such as "perfume," "extract," and "essence."

Application of essential oil

Essential oils, derived from essential oils, have many uses outside of aromatherapy. Edible essential oils are used differently depending on the food industry.

- Topical use of essential oil makes the oil absorb into the skin. Some ways to use essential oils on the skin include:
- Massaging the desired areas
- Odor distribution in diffusers
- Using as a personal perfume
- Many people use them to scent their homes or freshen things like laundry.
- Utilizing as a natural fragrance in home cosmetics and quality natural products.

Essential oils can be a safe and environmentally friendly alternative to man-made mosquito repellents. However, results regarding their effectiveness have been different. Studies have shown that some oils, such as citronella, may repel certain mosquitoes for about 2 hours. The use time in combination with vanillin may increase by up to 3 hours.

Furthermore, some essential oils can be used industrially to extend the shelf-life of foods due to their properties.

The extracting method of essential oils from medicinal plants

Clevenger apparatus and method of extracting essential oils from medicinal plants

The essential oil extraction device for the laboratory is for personal items and small amounts of plants called "Clevenger," which is sold ready-made in laboratory supply stores. The Clevenger device consists of a balloon and an exothermic tank. It is standard and has one size that can be installed for half to two-liter balloons. The appropriate electric oven is selected

according to the plant and balloon size. The place of installation of the device should be near the electrical outlet, water tap, and water drain.

Using the Clevenger apparatus

First, we crushed powder a certain amount of the plant (depending on the type of balloon, up to 100 g of dry plants or 200 g of fresh plants) and poured it into the balloon. Then, at least half of the balloon was filled up with distilled water. Then, the Clevenger device was installed on the balloon and fixed it with a clip. Thereafter, the rubber tube of the device was connected to the water and the oven was turned on. After some times, the water was boiled. We adjusted the temperature of the device so that the boiling of water in the balloon was balanced. The essential oil was evaporated due to the water heat and moved to the cooler (refrigerant) along with the water vapor. Water vapor and essential oil were boiled in the cooler (refrigerant) and collected in the collection pipe (vertical) [14].

Disadvantages of chemical pesticides

Chemical pesticides, including pesticides, fungicides, and herbicides, are substances used to prevent, destroy, or repel pests, including harmful insects and mollusca, plant diseases, and weeds. The active substances are diverse and classified based on these substances such as organochlorine (organochlorine), phosphorus (organophosphorus), or carbamates. Pesticides such as these penetrate and pollute the environment, but compared to the natural pesticides and have a smaller share. This group consists of substances that are of plant origin. Chemical toxins used in agriculture are the ones that are harmful to both the environment and human health if not used correctly (13).

Today, the risk of these substances' pollution of wetlands, rivers, and seas is strongly felt due to the indiscriminate use of fertilizers and chemical toxins. The number of aquatic animals in rivers and wetlands has decreased alarmingly due to the presence of nitrates caused using toxins and chemical fertilizers. Toxins enter the atmosphere either through evaporation or directly through the air. Surface water, industrial and urban effluents, and direct application of pesticides to control aquatic pests are among the ways toxins enter water sources. The soil is also contaminated through plants treated with toxins, rain, burying empty containers of toxins, and their direct use on the soil. Chemical toxins affect human health in two ways: First, the harmful effects of chemical toxins on users' health, and second, the effects of the residues of these substances in food products on the consumers' health. The health of agricultural products is essential because of their relationship with human health.

Scattered actions have been taken in measuring toxins, which are not regular and should be planned in this field. Many toxins enter the country, which are not tested, and no measures have been taken to know their effects on human health. Sometimes body absorbs toxin, or the toxin remains only on the skin without being absorbed. Absorption of pesticides in the body can cause problems for human health, including eye irritation, respiratory problems, and acute poisoning that may lead to death. Leaving toxins on the skin instead of absorbing them can result in skin allergies (15).

The body can become indirectly contaminated with chemical toxins when eating foods that have been permeated with pesticides, which can increase the number of toxic substances in the body (cumulative property). The human body has several regulatory systems that make all the body's parts show a specific reaction in response to external stimuli. This type of regulation is known as self-sustainability and occurs for all physical processes without awareness. Unusual symptoms and illness appear when the body cannot adjust due to the environmental conditions (the extreme heat or cold) or internal conditions (disease or poisoning). Therefore, the

symptoms of pesticide poisoning are often like different types of disease complications. For example, headache, fever, nausea, vomiting, and diarrhea are common disease symptoms in different conditions [15].

Chemical toxins cause a change in the speed of various body activities, which may be manifested as an increase (increase in heart rate and sweating) or a decrease in them (complete cessation of breathing). For example, people exposed to the insecticide malathion experience profuse sweating. The long-term consumption of food contaminated with pesticides may lead to illness. Humans tend to be more aware of diseases that show apparent symptoms, while some of our worst enemies sneak up on us slowly and unknowingly. As toxins enter the body in small amounts and over time, the symptoms of contamination with toxins in food are often quite like disease complications. For example, headache, fever, nausea, vomiting, and diarrhea are non-specific general symptoms of the disease in different conditions. Chemical poisoning by pesticides is similar to symptoms caused by microorganisms such as bacteria, viruses, and fungi [16].

Pesticides cause abortion, lack of intellectual development, destructive structural effects in the body at birth, and defects in body functions and tissues. In addition, pesticides have destructive and highly toxic effects on reproductive organs, interfering with hormonal actions, sterility of men and women, and irregular menstrual periods in women. Political-economic solutions should be provided to reduce the consumption of chemical toxins to achieve sustainable development in agriculture and realize the goals and policies foreseen for sustainable agriculture and preserve human health and the environment. The reducing policies of chemical toxin consumption in the country include removing the subsidy of chemical toxins, developing biological control methods and standards for the optimal use of toxins, presenting the maximum permissible limit of residual toxins, and removing high-risk toxins from the list of consumed toxins.

Method

First, the lids of the watch glass containers were seperated by heating them to place on the container through a net. Then, we divide the filter paper into two halves and place it inside the watch glass. After that, one side of the filter paper was dipped with (essential oil + acetone) concentrations of (0, 5, 10, 15, 20, and 25 µl) with a Hamilton syringe inside the glass cap for hours, and then acetone was added to the other side of the filter paper. Next, we ten rice weevils were placed in part containing essential oil and the net was immediately put on the watch glass, and thereby the effect of plant removal was checked during the prescribed times.[45] This experiment was performed in three repetitions, and the results are presented in Table 1.

This experiment was performed in 3 repetitions, and its results are listed in the following tables.

0.1 cc of the desired essential oil was taken and dissolved in 4 cc of acetone. We take 1 cc from it, which is equivalent to 25 µl.

Table 1. Determining the concentrations used in the experiment

Concentration/µl	Acetone + essential oil	Acetone
25	1	0
20	8/0	2/0
15	6/0	4/0
10	4/0	6/0
5	2/0	8/0
0	0	1

The removal test was performed in three repetitions at the following concentrations, and it was observed that the essential oil tested at a concentration of 25 and a time of 72 hours had the most significant effect on weevils (Figure 1, 2 and 3).

The results of lethal effect of plant essential oil on rice weevil

According to tests and investigations conducted on rice weevils, the death rate of these insects also increased with time and increased concentration of wild mountain thyme essential oil. The highest percentage of casualties was 100% on the third day and at the highest concentration of 25 microliters [16].

Figure 1. Repetition in lethality testing

Figure 2. Pouring plant essential on rice weevil

Figure 3. Repetition in lethality effect of the test

Figure 4 Pouring rice weevil on the essential oil of the thyme plant

Wild mountain thyme essential oil is one of the famous essential oils and has a special place in the world trade (Figure 4). Furthermore, the wild mountain thyme plant is abundant and has various uses in industries and food. Various works have been done by other researchers on the plant in Iran. According to Table 2 among the identified compounds, the most important and highest number of compounds was carvacrol and thymol. The compounds identified in this study were carvacrol (36.85) and thymol (36.85), among the compounds identified in all studies. Then, paracymene compounds (8.5) and gamma-terpinene (10.60) were among our identified compounds, which is consistent with many samples reported in our research.

Table 2. Comparison of chemical compounds with reported compounds

Studies	The main ingredients of wild mountain thyme essential oil
Aminkhani	Carvacrol 91/14-Osmin 7/91-Thymol 1/06-Simen 4/70-Terpene 0/87
Sefidkon and Asgari	Carvacrol 4/41-Thymol 19/50- Gamma-terpinene 10/03-Paracymen 10/3
Roustaeian *et al.*	Carvacrol 2/14- thymol 38-1.8 cineole 2/13
Rasouli *et al.*	Carvacrol 11/65- Paracymene 17/74- Gamma-terpinene 6/50
Nikvar *et al.*	Carvacrol 33.9- Thymol 6.38- Paracymene 3.7- Gamma-terpinene 5.2
Mohammadi	Cavacrol 4/52- thymol 4/10- gamma-terpinene 10/12
Present research	Carvacrol 85/36 - Thymol 85/36 - Para Simon 5/8 – Gamma-terpinens 60/10

The repellency and lethal effect of thyme plant essential oil

The removal percentage was calculated using the following equation after counting the number of insects in the control half:

$$PR = [(NC - NT)/(NC + NT) \times 100])]$$

Where, PR is the percentage of repellency, NC shows the number of insects in the control half, and NT donates the diseased area. Positive values indicate the repelling effect, and negative values reveal the absorption effect of the essential oil. Then, the standard deviation (criterion) was calculated using Excel software (18-20).

As presented in Table 3, the removal effect was the highest at the concentration of 25.

Table 3. The repelling effect of the essential oil of wild mountain thyme on rice weevil

Essential oil concentration	The first repetition			The second repetition			The third repetition		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0	0	0	0	0	0	0	0	0	0
5	0	10	20	20	20	0	0	0	20
10	20	0	20	0	20	20	20	40	40
15	0	20	40	20	20	40	20	40	40
20	20	40	60	40	40	60	40	60	60
25	40	60	80	60	60	80	60	80	100

Table 4. The repelling effect of wild mountain thyme plant essence on rice weevil based on standard deviation

The concentration of the essential oil	24 hours	48 hours	72 hours
5	57/0 ± 47	81/0 ± 50	**81/0 ± 50**
10	81/0 ± 50	81/0 ± 60	**47/0 ± 63**
15	47/0 ± 57	47/0 ± 63	**0 ± 70**
20	47/0 ± 67	47/0 ± 77	**0 ± 80**
25	80±0	100±0	**100±0**
0	**-**	**-**	**-**

The amount of plant essential oil concentration in microliters (µlit) Standard Deviation = ± (standard deviation)

The lethality effect was calculated using the repetitions of the first 1 hour, the first 24 hours, 48 and 72 hours, and their average using Excel software. Then, the standard deviation was calculated in Excel software, and the plant's LC50 and LC90 (lethal concentration) were calculated using probit analysis. The lethality effect was the longest at 20 hours and the shortest at the 25th concentration. The most lethal effect was at the concentration of 48 and 72 hours (Table 4 & 5).

Table 5. The lethal effect of essential oil of wild mountain thyme plant on rice weevil based on standard deviation

Essential oil concentration amount*	1 hour	24 hours	48 hours	72 hours
0	0	0	0	**0**
5	47/0 ± 43**	47/0 ± 53	47/0 ± 67	**47/0 ± 76**
10	47/0 ± 53	47/0 ± 63	47/0 ± 73	**47/0 ± 83**
15	47/0 ± 63	47/0 ± 73	0 ± 80	**0 ± 90**
20	47/0 ± 73	47/0 ± 77	47/0 ± 87	**0 ± 100**
25	0 ± 80	47/0 ± 87	0 ± 90	**0 ± 100**
LC_{50}***	215/7	917/4	425/2	**223/2**
LC_{90}***	243/61	962/43	205/30	**220/12**

* The amount of plant essential oil concentration in microliters (µlit)

**± standard deviation

*** Lethal concentration LC50 and LC90

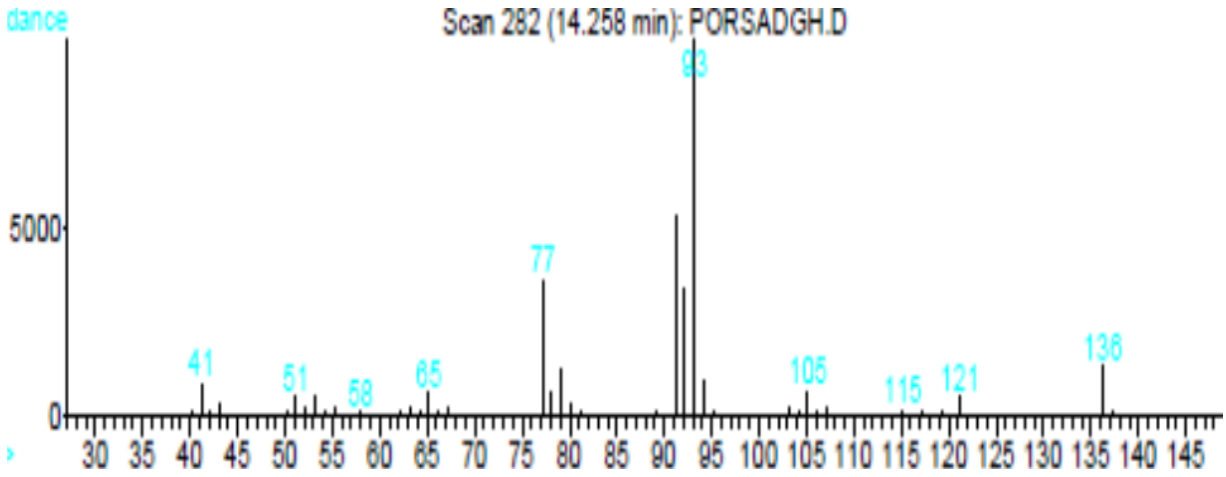
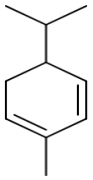


Figure 5. The GC-MS chromatogram of α-Phellandrene



Chemical Formula: $C_{10}H_{16}$
Exact Mass: 136.13
Molecular Weight: 136.23
m/z: 136.13 (100.0%), 137.13 (11.0%)
Elemental Analysis: C, 88.16; H, 11.84

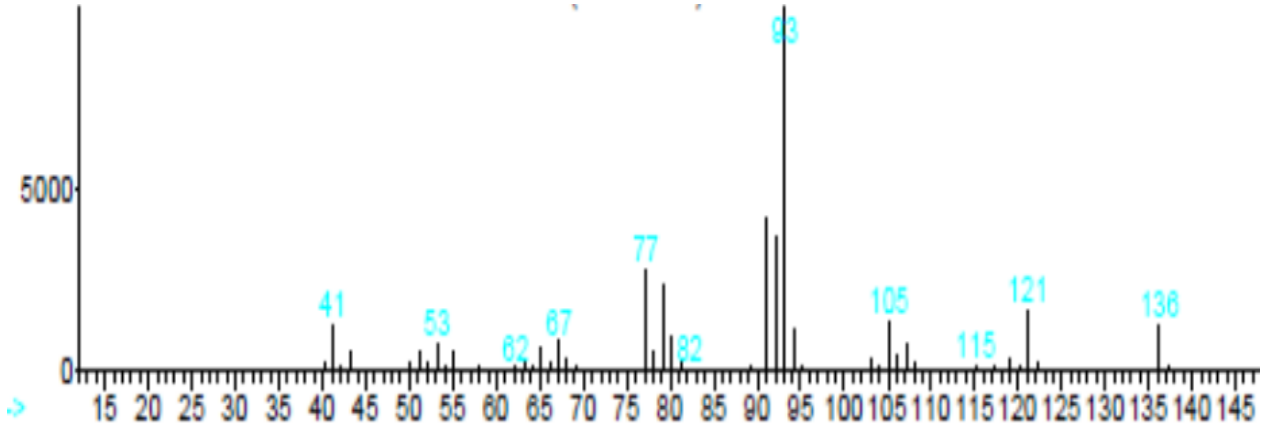
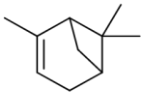


Figure 6. The GC-MS chromatogram of α-Pinene



Chemical Formula: $C_{10}H_{16}$
Exact Mass: 136.13
Molecular Weight: 136.23
m/z: 136.13 (100.0%), 137.13 (11.0%)
Elemental Analysis: C, 88.16; H, 11.84

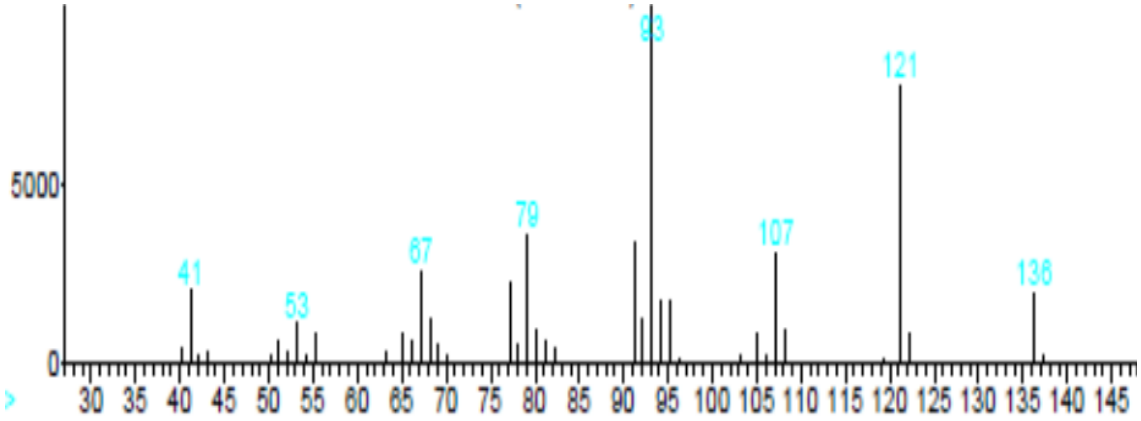
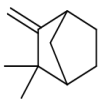


Figure 7. The GC-MS chromatogram of Camphene



Chemical Formula: $C_{10}H_{16}$
Exact Mass: 136.13
Molecular Weight: 136.23
m/z: 136.13 (100.0%), 137.13 (11.0%)
Elemental Analysis: C, 88.16; H, 11.84

Conclusion

In this study, the essential oil of the wild mountain thyme plant was extracted by water distillation and using a Clevenger device after collecting and drying. The essential oil components were identified and separated using a GC/MS device. A total of 37 compounds were identified, and carvacrol 54%, thymol 10%, and gamma-terpinene 7% were the most important compounds. In recent years, the use of plant essential oils derived from aromatic plants as low-risk insecticides has increased significantly with the effects of repelling, insecticidal, and reducing the growth of the immature stages of various insects. Because plant essential oils cannot be used purely in greenhouses and farms, commercial formulations should be prepared from these compounds. Rice weevil, which is one of the important pests of crops, was calculated using the repellency and lethality of the plant essential oil formulation in five concentrations (5, 17, 15, 20, and 21) (percentage of repellence). The removal percentage during three repetitions was significantly higher at the highest concentration of 25 µl than at other concentrations.

Furthermore, the lethal effect of plant essential oil, LC50, and LC90 on the plant was calculated after 1 hour, 24 hours, 48 hours, and 72 hours. The three repetitions showed that the insecticidal property of the plant essential oil had the longest time in concentration 20 and the lowest time in concentration 25. Therefore, the wild mountain thyme plant had good repelling and was lethal, and its essential oil can be used as a repellent and insecticide in grain warehouses. Planting this plant around fields is used as a pest repellent. The microbial test of the essential oil of the Thymus Fetschenko plant was performed on three Gram (+), (*S. aureus*), (*E. faecalis*), and (*B. anthracis*) bacteria and three Gram (-) bacteria, (*E. Coli*), (*Salmonella*), and (*P. aeruginosa*). The essential oil of thymus Fetsenchkoi plant was effective on all Gram-positive and Gram-negative bacteria except *Pseudomonas aeruginosa*.

Suggestions

1. The desired essential oil should be extracted and compared with the plant collected from different regions for one year.
2. The environmental effect on the amount and type of secondary metabolisms should be investigated.
3. The effect of different stresses on the plant's essential oil should be assessed.
4. The type of other compounds present in this plant extract cannot be determined by extracting essential oils.
5. Negotiating with pharmaceutical companies to prepare antibacterial solutions is necessary because the essential plant influences all warm (+) bacteria and many warm (-) bacteria.

References:

[1] R. Hak, N. Zagh. The effect of flowers and plants on the human state of mind. *The 8th Conference of New Researches in Science and Technology*., (2015) 1-12.

[2] A.H. Sharifi. Secrets of medicinal plants. Hafez Navin Publications. 2003.

[3] Y. Chi. Medical vocabulary and medicinal plants of Iran. Tehran University Publications. 1991.

[4] Y. Chi. Iranian Medical Vocabulary and Medicinal Plants, University of Tehran Publications. 137

[5] M.A. Bakht, A. Hosseini, F. Mouloud, The necessity of standardizing medicinal plant extracts in research and how to do it. *Razi Journal of Medical Sciences*., (2015) 10-23.

[6] R. Mehboodi, Extraction, isolation, and identification of wheat flower plant and investigation of their cytotoxic effects. *Master's thesis, Yasouj University*., (2013).

[7] S. Rudy, Studying the essential plant components and investigating the essential

oil's antimicrobial properties. Master thesis of Payam Noor University. (2009).

[8] MA. Bakht, Classification of medicinal plants, Taimurzadeh Publishing Cultural Institute, Tabib Publication. (1999).

[9] R.M. Beygi, Approaches to producing and processing of medicinal plants, Tehran publishing house. (1995) 2.

[10] H.T. Khani, K. Tat, P. Han. A review of various extraction methods in plant extracts. *The 4th International Conference on Recent Innovations in Chemistry and Chemical Engineering*. 2017.

[11] A. Zargari, Medicinal Plants. *Tehran University Publications*. (2019).

[12] T. Robiston. Plant chemistry, translated by Hamad Izaddoost, Tehran University Press. 1985.

[13] M. Naqvi. Fighting warehouse pests using plant essences and natural toxins. *New technologies in the food industry*., (2016)7.

[14] M. Gholami, A. Nasser. Investigating the antifungal properties and identifying the chemical compounds of the essential oil of the mountain thyme plant (Thymus kotscuyanus Boiss. & Hohen.). *Iranian Journal of plant physiology and biochemistry*., 1 (2016) 52-62.

[15] A. Delazar, M. Bahmani, H.H. Shoar, A. Tabatabaei-Raisi, S. Asnaashari, L. Nahar, *et al*. Effect of altitude, temperature, and soil on essential oil production in Thymus fedtschenkoi flowers in Osko and surrounding areas in Iran. *Journal of Essential Oil Bearing Plants*., 14 (2011) 23-9.

[16] S. Oryzae, Effects of six plant extracts on rice weevil Sitophilus oryzae L. in the stored wheat grains. *Journal of Agricultural and Biological Science*., 1 (2006).

[17] S.I. Kim, J.Y. Roh, D.H. Kim, H.S. Lee, Y.J. Ahn. Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. *Journal of Stored products research*.; 39 (2003) 293-303.

[18] M.B. Devi, N.V. Devi, S.N. Singh, The effects of six botanical plant powder extracts on controlling rice weevil, Sitophilusoryzae L. in stored rice grains. *International Journal of Agriculture Innovations & Research*., 2 (2014) 683-6.

[19] S. Sighamony, I. Anees, T. Chandrakala, Z. Osmani, Efficacy of certain indigenous plant products as grain protectants against Sitophilus oryzae (L.) and Rhyzopertha dominica (F.). *Journal of Stored Products Research*., 22 (1986) 21-3.

[20] J. Karungi, E. Adipala, S. Kyamanywa, M. Ogenga-Latigo, N. Oyobo, L. Jackai, Pest management in cowpea. Part 2. Integrating planting time, plant density, and insecticide application for management of cowpea field insect pests in eastern Uganda. *Crop Protection*., 19 (2000) 237-45.

[21] E. Lucas, J. Riudavets, Biological and mechanical control of Sitophilus oryzae (Coleoptera: Curculionidae) in rice. *Journal of Stored Products Research*., 38 (2002) 293-304.

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