The effect *Thymus vulgaris* L. volatile oil in some bioparameter for hairy grain beetle*Tragoderma granarium*(Everts.)

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Abstract

The present study was conducted to evaluate the effect of the volatile oil extract of thyme on the life of *Tragodermans granarium* (Coleoptera: Dermastidae), The essential oil had been extracted by steam distillation in a modified Clevenger-type apparatus from leaves of *Thymus vulgaris* camaldulensis and their chemical composition characterized by gas chromatography mass spectrometry *(GCMS)* .different concentrations of the volatile oil of thyme plant(10,000,5000,2500 ppm) were used to treat eggs, third larval stage and adults, in addition to test the concentrations in germination of wheat seeds. Results showed increase in the percentage of inhibition insect eggs, reached 93.3% at a concentration of 10000ppm, while the lowest percentage of inhibition was 80.0% at a concentration of 2500 ppm. As for larval mortality, the highest rate of larvae mortality was 87.7% at a concentration of 10000 ppm, while the lowest rate of mortality was 49.9% at a concentration of 2500 ppm. The results also showed the effect of the treatment of extract concentrations in relation to germination percentage , as the results showed significant differences, as the germination percentage reached (80.0,70.0,86.3)% in concentrations (10,000,5000,2500 ppm), respectively, compared to the control treatment, which reached to 86.6%. conclude that Thymus vulgaris oil used in the present work enhances the toxicity against the target pest and can be used as an alternative pesticide to the chemical pesticide and thus it constitutes an environmentally friendly and economically effective formulation for the management of stored grain without any toxic effect on plants as well as on farmers .

Key words: *Tragoderma granarium*, Mortality, volatile oil, wheat seeds.

Introduction:

The hairy grain beetle *Tragoderma granarium* (Evrst) (Colioptera:Dermestidae) is one of the most harmful pests and is one of the most 100 types of insect pests that attack stored products, especially wheat[17]. The infestation causes in the larvae stage, while adults do not feed[8]. in diapause the larvae of *T. granarium* are more tolerant to many chemical and non-chemical control methods[13] . The small larvae ones of this species were more large larvae susceptible than to contact insecticides [13] . The larvae *T. granarium* reported that diapausing and climated were tolerant to low and high temperatures that are usually lethal for other major storedproduct insects[21] . *T. granarium* one of stored product insect species that are resistant to pesticides[7] Despite the effectiveness of pesticides in eliminating pests, the continuous use has led to serious defects, including the emergence of resistance against the chemical pesticides used in the control in addition to the deadly effect of non-target organisms as well as the risks of environmental pollution resulting from their use[15] . Thyme plant known for its antiseptic and antifungal properties, It also contains other volatile oils such as carvacolo, geraneol and borneol and it is a rich source of flavonoid phenolic antioxidants In addition to it contains many important minerals, thyme leaves are excellent sources of potassium, calcium, iron, manganese, magnesium and selenium and it is a rich source of vitamins such as Vitamin A, which is vital for the maintenance of mucous membranes and skin and Vitamin C provides resistance against infectious diseases and

fights harmful pro-inflammatory free radicals[10], the aim of this study was to evaluate, gas chromatography analysis of thyme plant extract to know the active substances that the plant contains, estimate the most effective concentration in the mortality percentage of the insect stages, evaluating the effect of the concentrations used on the germination peracentage of wheat seeds.

Materials and methods

Preparation of Essential oil of thymus vulgaris

The volatile oils were extracted using the method of steam distillation by means of a cliffhanger device, where the water evaporated in a separate hermetically sealed container, and the rising steam was passed to the plant samples placed in a container at the bottom of the clavenger device, which was sealed with a quality opening that allowed the steam to escape after passing through the plant samples, as the steam was passed through the condenser and then collected The condensed liquid and the separation of the extracted essential oil are placed in a dark colored glass tube, sealed at a temperature of 5 °C [3].

Gas chromatography- mass spectrum analysis (GC-MS)

The active compounds in the essential oil of the thyme plant were diagnosed using, GC-MS QP2010 Ultra, SHIMADZU, JAPAN, the GC-MS device is equipped with a silica column (30m x 0.25mm, i.d.0.25µm). The operating conditions of the device were as follows: the use of helium as a carrier gas at a flow rate of 0.89ml / min, the temperature of the injector is 250c and the detector is 280c. The column temperature was fixed at 50C for 1 min, then the column temperature was raised from 50 c to 130c at a rate of c / min20, then the temperature was fixed for 1min, and then it was raised from 130c to 250c at a rate of c / min9, then it was fixed at 250 c for 8.33 so the total duration of the thermal program is 27.66min. The volume of sample injected with the device was 1µl of oil [6]

Insect breeding:

The hairy grain beetle *T. granarium* was obtained from Department of Plant Protection / storage Insect Laboratory, University of Baghdad. Many farms have prepared for the laboratory insect breeding by using wheat grains free of infection after putting it in the refrigerator at -18 degree for two week to ensure to get rid of any infection with stored pests, the grains were placed in plastic boxes 27 cm high and width diameter 9 cm, then ten pairs of adults were added, and then covered with muslin cloth cover the upper part with a dull cloth and the cap was tied with a rubber band to ensure that the adults did not exist, Then boxes were placed in the incubator at a temperature of 33 ± 1C° and a humidity of 65 ± 5 in order to ensure that all stages of the insect were obtained[18] to obtain the eggs of the hairy grain beetle, ten pairs of adults in age (1-2) days were placed for one day inside a lantern that was covered on the top of its lantern with a muslin cloth dull cloth Fixed with a rubber band and the base of the bottle was placed in a petri dish, 9 cm in diameter and 1.5 cm in depth, and the effect of the concentrations of the volatile oils of thyme plant in the stages of insect (eggs, larva, adults) petridish containing insect eggs (10 per dish) were treated with three replicates and three concentrations (10,000,5000,2500) ppm with the control, in which the dishes were treated with distilled water only and the dishes were left in the incubator with a degree of 33 ± 1C° and a humidity of 60 ± 5, and the hatching rate was calculated after one week of treatment, as same as the treatment was conducted for the larvae, but the mortality percentage were calculated after (1,2,3) days. As for the adults, the petridish containing 10 adults (per dish) were treated with three concentrations in three replicates, in addition to a control treatment that was treated with just distilled water and the mortality percentage were calculated after one week of the

treatments, and all results were corrected according to [2]

$$\text{Mortality Rate} = \frac{\textit{Death rate per treatment}\,\% - \textit{death rate in control treatments}\%}{100 - \textit{Death rate in control treatments}} \times 100$$

The effect of thyme volatile oil on wheat seeds

wheat seeds were treated with volatile oil extract of thyme plant at a concentration (10,000,5000,250) ppm and with three replicates in addition a control in which treated just with distilled water, Sterile Petri dishes were prepared containing filter paper saturated with water, and placed ten seeds on each dish, after one week of treatment the percentage of germination was calculated, as according to the following equation [9] .

$$\textbf{Germination percentage} = \frac{\textit{The number of emergence seeds}}{\textit{The total number of seeds}} \times 100$$

Statistical analysis

Statistical Analysis System -[22] was used in analyzing the data to study the effect of different factors on the studied traits according to a complete random design (CRD) in two directions and one direction according to the trait. The significant differences between the averages were compared with the Least Significant Difference-(LSD).

Results:

The results in Table (1) showed that the active substances separated by the GC-MS method of thyme oil, which amounted to 31 chemical compounds, The highest percentage was of Linalool was 20.78%.it is one of the active substances of the thyme plant, followed by the compound Limonene at an average of 12.59, while the percentage of Isothymol and Thymol were (11.80 and 11.04) respectively, while the lowest percentage was for the compound 4-isopropyl phenyl ester by 0.01 followed by the compounds Isoaromadendrene epoxide and 4-Octene at a percentage of (0.03 0.0.05), respectively.

Table (1): Chemical composting of thyme essential oil of the thyme plant**.**

Peak	Component	Formula	Relative concentration (%)%
1	7- methylbicyclo	C_9H_{16}	0.76
2	3-Methyl-5-heptanone	$C_8H_{16}O$	0.45
3	gamma Terpineol	$C_{10}H_{18}O$	0.34
4	o-Cymene	$C_{10}H_{14}$	3.24
5	Cineole	$C_{10}H_{18}O$	1.14
6	Crithmene	$C_{10}H_{16}$	2.77
7	alpha-Methyl-alpha	$C_{10}H_{18}O_2$	0.20
8	Terpinolene	$C_{10}H_{16}$	0.40
9	Linalool	$C_{10}H_{18}O$	20.78
10	Methyl sulfoxide	C_2H_6OS	0.13
11	1-Allylimidazole	C_6H_8N2	0.37
12	L-terpin	$C_{10}H_{18}O$	1.35
13	Thymol methyl ether	$C_{11}H_{16}O$	1.44
14	Isothymol	$C_{10}H_{14}O$	11.80
15	3,5-Heptadiena	$C_{10}H_{14}O$	11.04

16	**Carvacrol**	**$C_{10}H_{14}O$**	**0.13**
17	**Titramethylphenol**	**$C_{10}H_{14}O$**	**3.85**
18	**Caryophyllene**	**$C_{15}H_{24}$**	**2.55**
19	**Limonene**	**$C_{10}H_{16}$**	**12.59**
20	**Isocaryophillene**	**$C_{15}H_{24}$**	**5.68**
21	**Naphthalene**	**$C_{15}H_{24}$**	**0.59**
22	**1-Pentene**	**$C_{12}H_{20}$**	**0.31**
23	**Caryophyllene oxide**	**$C_{15}H_{24}O$**	**3.95**
24	**Isoaromadendrene epoxide**	**$C_{15}H_{24}O$**	**0.03**
25	**4-Octene**	**$C_{12}H_{24}$**	**0.05**
26	**4-isopropylphenyl ester**	**$C_{15}H_{21}BrO_2$**	**0.01**
27	**Sulfurous acid, 2-ethylhexyl hexyl ester**	**$C_{14}H_{30}O_3S$**	**8.01**
28	**Butane, 1-bromo-2-methyl**	**$C_5H_{11}Br$**	**0.22**
29	**Hexahydrofarnesyl acetone**	**$C_{18}H_{36}O$**	**0.40**
30	**3-Benzylsulfonyl-2,6,6- trimethylbicyclo (3.1.1)heptanes**	**$C_{17}H_{24}O_2S$**	**0.19**
31	**Tridecyl bromide**	**$C_{11}H_{23}Br$**	**0.47**

The results in Table (2) indicated that the effect of the volatile extract concentrations of the thyme plant on the inhibition rate of eggs of the *Trogoderma granarium* insect. The results showed the effect of the concentrations treatments, the highest inhibition rate of eggs was 93.3% at the concentration of 10000ppm, while the lowest inhibition rate was 80.0% at the concentration of 2500 ppm, the results of the statistical analysis indicated the significant differences between the concentration at *P* (0.05).

Table 2: The effect of the volatile extract concentrations of thyme on the egg inhibition rate of *Trogoderma granarium* beetle .

Concentration (ppm)	**The egg mortality rate %**
10000	**93.3**
5000	**83.3**
2500	**80.0**
Control	**0.0**
Average	**85.5**
LSD 0.05	*** 7.921**
***P≤0.05**	

The results in Table (3) indicated the effect of the volatile extract concentrations of the thyme plant on the mortality percentage of the larvae of the *T. granarium* beetle .The results showed the effect of the used concentrations, as the highest percentage of larvae mortality was 87.7% at 10000ppm, while the lowest mortality percentage was **41.1**% at 2500 ppm. As for the time factor, the highest mortality percentage was 76.6% on the third day after the treatments, while the lowest rate for the time factor was 47.7% on the first day after treatments, As the results of the statistical analysis indicated the significant differences in the results between the concentration at (P≤0.05), The majority of the results of the

analysis indicated the significance of the existing differences. As for the interaction effect, the highest percentage of depreciation was 93.3% at a concentration of 10000 ppm on the second and third days, while the lowest percentage was 20.0% at a concentration of 2500 ppm on the first day after the treatment.

Table 3: The effect of the volatile extract concentrations of thyme on the mortality rate larvae of the *Trogoderma granarium* of the insect.

Average	the mortality rate larvae %			concentrations(ppm)
	third day	second day	First day	
87.7	93.3	93.3	76.6	10000
67.7	83.3	73.3	46.6	5000
41.1	53.3	50.0	20.0	2500
0.0	0.0	0.0	0.0	Control
---	76.6	72.2	47.7	Average
Concentrations: * 7.02 ،Time: * 6.44 ،interaction:* 13.26				LSD 0.05
*P≤0.05				

The results in Table (4) indicated the effect of the volatile extract concentrations of the thyme plant on the mortality of adults of the *T. granarium* beetle. As the results of the table showed the effect of the concentrations used, as the highest percentage of adult mortality was 83.3% at the concentration of 10000ppm, while the lowest mortality percentage was 72.0% at the concentration of 2500 ppm. The results of the statistical analysis indicated the significance of the differences in the results between the concentration of 10000 and 2500 ppm at (P≤0.05).

Table 4: Effect of volatile extract concentrations of thyme on the adult mortality rate of *Trogoderma granarium*.

Concentrations(ppm)	The adult mortality rate %
10000	83.3
5000	76.6
2500	72.0
Control	0.0
Average	77.3
LSD 0.05	* 7.419
*P≤0.05	

showed the results in Table (5) the effect of the volatile extract concentrations of the thyme plant on the germination percentage of wheat seeds, where the percentage of seed germination was not significantly affected by the extract treatment, The highest percentage of wheat seed germination was 86.3% at a concentration of 2500 ppm, while the germination percentage reached (70.0 and 80.0) for the concentrations 10,000 and

5000ppm, respectively, compared to the control treatment, which amounted to 86.6%. The results of the statistical analysis indicated the significant differences in the results at the level of ($P \leq 0.05$).

Table 5: The effect of the volatile extract concentrations of thyme on germination percentage of wheat seeds

Concentrations(ppm)	germination percentage %
10000	70.0
5000	80.0
2500	86.3
Control	86.6
Average	80.72
)0.05 (LSD	* 5.634
) *P≤0.05.(	

Discussions

Thyme is a common herb and is used as a flavoring for many foods, as it plays an important role in global cuisine, and it has been proven in several studies that its essential oil contains anti-inflammatory properties[11] . The thyme oil sample, more than half of its contenting was linalool (56.5%), it is soluble in water at a concentration of 1.59 mg/mL at 25 °C[19] Referred to his study [20] thyme oil contains nearly half of its thymol content (46%), followed by fewer amounts of γ-terpinene, carvacrol, and p-Cymene, Which could explain the effectiveness of aqueous extract of thyme plant in the current study in addition to other studies.

The highest percentage in inhibition the eggs of cowpea beetle *Callosobruchus maculatus* (60.2%, 52.0%) for hot water extract of dill and hot pepper at the unit weight of 5 g, and the results of the statistical analysis indicated that there were significant differences between the treatments [4] . The reason for the failure of the eggs to hatching may be due to the formation of a sticky insulating layer due to the crude extract of the terpenes around the egg, which caused the embryo to suffocate, meaning that its effect Physicist [12]

The mortality of the larval stage of the insect may be due to its sensitivity to toxic substances present in the plant or to poisoning the cells of the middle gastrointestinal tract responsible for absorbing food and thus reducing its efficiency in the process of digesting and converting food, The highest mortality rate of the second larval life of the khabra insect was 83.33% when the aqueous propolis extract of aqueous propolis was treated at a concentration of 50 mg. ML^{-1}, while the mortality rate for the fifth stage was 66.67% at the same concentration, as for adults, the mortality rate was 83.33% at the same concentration, as for the alcoholic extract, the decay rate of the second larval stage was 83.33% when treated with a concentration of 3 mg. Ml^{-1}, while the mortality rate of the fifth larval stage was 70.00% at the same concentration, as for adults, it reached 63.33 at a concentration of 3.0 mg. Ml^{-1} [5] . The cause of mortality may be due to the effect of the extract through contact with the surface of the insect's body or entering it through the respiratory openings, in addition to the toxic effect of alkaloid compounds and volatile oils fumes that cause physiological effects in insects [14]. Another study showed that most of the effective compounds and essential oils extracted from

plants and used as insecticides are monoterpenoids, which are typically volatile and fat-soluble compounds that can quickly penetrate in the body of insects to interfere with the physiological functions in the insect's body [16] .

refer the study conducted by [1] confirmed the germination percentage was (96.7, 86.7, 86.7, 76.7) for ginger, Cinamome, turmeric, and lemon peel powders, respectively, at a unit of weight of 5 g, compared to the control treatment, which amounted to 96.7%, the reason for this may be due to the lack of sensitivity of wheat seeds to the extract or the presence of chemical compounds inside the seeds that reduce the effectiveness of these extracts. While another study referred to by [4] confirmed the effect of the interaction of the quantity and type of the powder of *Anethum graveolens* and Capsicum annum on the germination percentage of cowpea seeds Vigna unguicalata . As the germination percentage were (96.0, 86.0)% for dill and hot Pepper powder, respectively, compared to the control treatment, which reached to (97.0)%.

Ethical approval

No human participant or animals were included in this study or any of its protocols .thus, informed consents are not applicable.

Conclusion:

Thymus vulgaris oil used in the present work enhances the toxicity against the target pest and can be used as an alternative pesticide to the chemical pesticide and thus it constitutes an environmentally friendly and economically effective formulation for the management of stored grain pests.These results of this study confirm the possibility of using this type of extracts to treat the seeds and protect them from reservoir infection and use them as seeds for cultivation.

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