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Chemical characterization of the essential oil of *Thymus vulgaris* and evaluation of its antifungal activity on the apple scab pathogen (*Venturia inaequalis* L)

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ABSTRACT

This study aims to investigate the chemical composition of essential oils (EO) extracted from the aerial part of *Thymus vulgaris* L. and to evaluate its antifungal activity against the apple scab pathogen (*Venturia ineaqualis* L). Chemical analysis of this EO revealed the presence of 30 compounds which represent for approximately 99.07 % of the total component. Linalool, is the most abundant constituent (36.41%), followed by Terpinen-4-ol (10.51%) and β-myrcene (7.5%). Other constituents were identified in varying amounts include para-Cymene (6.6 %); γ-Terpinene (6.11 %), and Carvacrol (0.75 %), with entirely absent of thymol compound. The evaluation of antifungal activity of thyme EO on the growth of *Venturia ineaqualis* mycelium was marked by a decrease in number of fungal filaments in the colony which was significantly influenced by increasing the doses. The EO concentration that had the minimal inhibitory effect was 300l/ml. *Thymus vulgaris* EO had a complete inhibitory effect on fungal growth at a dose of 600 l/ml, where no growth was observed. Despite the absence of thymol and the relatively low content of carvacrol, both of which are known to be highly toxic, thyme EO from the Seraidi region was very effective and revealed a very powerful activity against *V.ineaqualis,* which is likely due to the presence of high levels of monoterpenes (36.96%) and oxygenated monoterpenes (55.51%) associated with other synergistic components.
Keywords: Antifungal activity, Chemical composition EO, CMI, *Thymus vulgaris* L., *Venturia ineaqualis* L.

INTRODUCTION

The world's production of apples amounted to 64 million tonnes in 2016, China is the largest producer of apples in the world, and the United States of America comes second, accounting for half of the global total (planetoscope). Algeria is ranked 26th in the world, with 503,303 tonnes produced over an area of 40,553 hectares. Its products may be found everywhere in the national territory (Haffaf and Merzougui, 2014). The area planted to apples in Algeria has risen significantly since the establishment of the National Agricultural Development Program (PNDA). Apple yields are still weak and irregular when compared to other countries with a smaller area under this crop. The apple is susceptible to a variety of parasites that must be controlled in order to prevent the spread of disease. Apple scab, caused by the pathogen of *Venturia inaequalis* has remarkable symptoms that obvious on leaves and fruits. This disease is the most economically destructive cryptogamic disease worldwide and Algeria in particular (Parisi *et al*., 2004).

The different production regions in Algeria represent climatic conditions favorable to the development of scab. Therefore, the sanitary state of the orchard, its topography, the sensitivity of the cultivars, and the frequency of the periods of infection, combined with the influence of other factors make this disease the primary source of economic losses in orchards that requiring frequent application. As a result, numerous phytosanitary treatments are required to achieve a suitable commercial quality. One of the major challenges in arboriculture is to reduce the number of phytosanitary treatments used in commercial orchards that leads to lowering both production costs and negative environmental and human health implications (Mills & Laplante, 1951; Carisse *et al*., 2009).

Traditional chemical control is showing its limits and many arboriculturists are looking for new phytosanitary solutions. Consequently, the search for an alternative to chemical products through the use of natural substances is one of the solutions to limit the development of pathogens, among which are aromatic plant extracts and more specifically essential oils (PARISI *et al*., 2004).

Previous studies have already highlighted the effectiveness of using EOs as biopesticides and their microbial activity depends on the nature of its chemical compounds. The Mountain Savory essential oil, for example, which was studied as part of the Casdar HE project (2013-2016), shows a toxic effect on the apple scab pathogen (Mazoyer, 2016). This toxicity is linked to the main phenolic compounds (Carvacrol and thymol) present in this EO, each of which has a hydroxyl group that disrupts the integrity of the fungal plasma membrane (Ahmad *et al*., 2011). Similarly, *Syzygium aromaticum and Eucalyptus citriodora* EOs have been shown to be effective against apple scab with better efficacy than the commercial reference CuSO4 (Deweer *et al*., 2015; Muchembled *et al*., 2015) compared to *Origanum compactum*, *Saturejamontana and thymus vulgaris* EOs (thymol) which showed antifungal activity above the IC50 (Vital *et al*., 2018). However, thyme is known as an antimicrobial agent and its antifungal activity has been demonstrated by several authors. By El Ajjouri *et al*., (2008) on four lumber rot fungi, by Benhamou *et al*., 2012 on *Aspergillus niger*. Zahed *et al*., (2021) also demonstrated a larvicidal activity for this EO extracted from leaves harvested in the Mekhatria region (Ain Defla).

Researches have confirmed the antimicrobial properties of a range of plants on several plant pathogens, but the number of studies currently available on the

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fungicidal activity of essential oils on the genus *Venturia* is limited compared to other fungal species. Therefore, our study aims to explore the chemical composition of essential oils (EO) that extracted from the aerial part of thyme (*Thymus vulgaris* L.) and to evaluate its antifungal activity on the apple scab pathogen (*Venturia inaqualis* L.)

MATERIALS AND METHOD

Plant material

The plant material chosen in the present study is Thyme which identified as *Tymus vulgaris*, belonging to family Lamiaceae, which is a subshrub native to the western Mediterranean region including Algeria. The identification of this herb was carried out at the laboratory of malherbology at the Regional Station of Plant Protection in El-Tarf.

The aerial part (leaves and stems) of thyme was collected in November 2017 in the region of Seraidi (North of Annaba). The leaves and stems were thoroughly washed to remove dust and then air dried in the shade place for about 15 days and then stored in paper bags in a dry place at a rate of 100g per bag to be used for oil extraction.

Fungal material

The fungal pathogen (*Venturia ineaqualis*), which causes apple scab disease, was isolated from lesions developed on apple leaves collected from an orchard located in the commune of Ben M'hidi (El-Tarf). The identification of the fungal species, based on macroscopic observation (morphological aspect of mycelium, shape of ascospores formed, growth rate, mycelium colour) and the life cycle of fungal colony, was carried out.

Extraction of the essential oil

The extraction was carried out with a Clevenger type apparatus. Hydro-distillation consists of immersing 10 g of dried leaves in the shade in 100 ml of distilled water for 2 hours. After obtaining the essential oil, the oil was decanted from the aqueous layer, dried with anhydrous sodium sulphate (Na_2SO_4) and stored in sealed bottles at 4°C in a hermetically sealed bottle according to ISO 9235.

Determination of Kinetic performance

The recovered quantities of essential oil corresponding to 10 minute time intervals ranging from 0 to 90 minutes were measured to study the extraction kinetics of the essential oil from the dried aerial part of thyme. The yield, at each time interval, was calculated by the following formula (AFNOR, 1986):

$$RHE\ (\%) = M'/M \times 100 \qquad (1)$$

Where, RHE is the yield of essential oil from the dry areal parts and M' is the mass of essential oil (g), M is the mass of dry plant material (10 g).

Chemical characterisation of the essential oil of *Thymus vulgaris* by Gas Chromathography (GC)

Chromatographic analysis

The chromatographic analysis was carried out at the *Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques* (CRAPC-Centre for Scientific and Technical Research in Physical and Chemical Analysis), Algeria, on a Hewlett Packard electronically pressure regulated gas chromatograph (HP-5MS series), equipped with an HP-5 capillary column (30 m x 0.25 mm) with a film thickness of 0.25 µm, an FID detector set at 200 °C and fed with a H_2/Air gas mixture and a split-split less injector set at 275 °C. The injection mode is split (leakage ratio: 1/50). The gas used is pure helium with a flow rate of 0.5 $ml \cdot min^{-1}$. The column temperature is programmed from 50 to 250 °C at 4 °C· Min-1. The apparatus is controlled by a computer system of the HP Chem-Station type, which manages the operation of the apparatus and makes it possible to monitor the progress of chromatographic analyses (CRAPC, 2018).

Evaluation of the antifungal activity of the essential oil of Thyme

Isolation and identification of apple scab

Venturia ineaqualis was isolated from samples of young leaves showing symptoms of the disease. Samples of scabbed leaves were freshly cut with a scalpel, observed under a light microscope to confirm the presence of ascospores, then disinfected in 2% sodium hypochlorite for 10 minutes, followed by rinsing in 3 successive baths of sterile distilled water for 5 minutes each, and then dried between two sheets of sterile paper. The samples were then sliced into small pieces and plated on PDA medium. The plates were incubated at 18±2 °C for one week following the method of Ctifl, (2015).

The reference fungicide used

The score is the reference pesticide used in this experiment to compare its antifungal activity to that of thyme EO on the isolated fungal strain. It is a systemic fungicide, composed of 250 g of Difeno-conazole, used for the preventive and curative control of a wide spectrum of foliar diseases including apple scab. Score is rapidly diffused into the aerial parts of plants by translaminar action and local systemic action. It significantly blocks the development of mycelium in plant tissues and prevents the appearance of symptoms (Syngenta score data sheet, 2015).

Evaluation of antifungal activity of thyme essential oil on Venturia inaequalis

The antifungal activity of thyme essential oil was evaluated using the dilution technique in solid medium to determine the inhibition levels and in broth medium to determine the MIC and the MFC (Baba-Moussa, 1999; Batawila, 2002; Moulari, 2005).

a. Solid-state dilution technique

Each of the methanolic solutions (0.5ml) of the EO tested at different concentrations (100, 200, 300, 400, 500, 600 µg/ml) was added to 20 ml of a warm Sabouraud medium at 40 °C. After homogenisation, the mixture is poured into petri dishes. Inoculation was carried out by stabbing the medium and then the Petri-plates were incubated for 7 days at 27 °C. Mycelial growth was recorded daily. At the end, the diameters of the different colonies were measured to calculate the inhibition rate (I'%) (Kordali *et al*., 2003).

$$I'(\%) = 100 \times (dC - dE)/dC$$

Where, I'(%) is the inhibition rate expressed in percentage, dC is the diameter of colonies in the positive control' plates and dE is the diameter of colonies in the tested plates containing the essential oil. Each experiment was performed in triplicate with three separate trials for reliability of results. The efficacy of the EO on a given strain(s) can be assessed by expressing the proportion of those that showed an inhibition rate greater than or equal to 50%.

b. Liquid dilution technique

This technique consists of two steps: the first step to determine the MICs and the second step to determine the minimum fungicidal concentration (MFC) following the method of Rotimi *et al*., (1988).

Determination of MICs

The various solutions that had inhibition percentages greater than 50% are retained. A volume of 100 µl of these solutions is added to 900 µl of liquid Sabouraud medium containing the strain to be tested. The tubes thus prepared are incubated at 27 °C for 7 days. After incubation, the tubes in which no mould growth is noted are identified; MIC is the minimum concentration for which no mould growth is noted. For the determination of the MFC, after having identified the tubes in which no growth of "spores" is recorded. The experiment is sustained where 950µl of sterile liquid Sabouraud medium is introduced with 50µl of a determined test having presented a total inhibition. After 7 days of incubation, the subcultures in which there was no growth are noted; the MFCs are recorded.

Statistical analysis

The results are given as means ± SD. The bioassay data were statistically processed by analysis of variance (ANOVA) and Tukey's test using Minitab 16.1.1 software. All experiments were done in triplicates.

RESULTS AND DISCUSSION

The essential oil of *Thymus vulgaris* has been used since ancient times to achieve many beneficial roles for human health. In this part of the study, we focused on evaluation the inhibitory potential of thyme oil, as antifungal for the pathogen caused apple scab disease. The chemical composition of the essential oil was also explored.

Essential oil extraction kinetics and yield of essential oil

Extraction kinetics consists of determining the yield as a function of extraction time. The study revealed that the time needed to extract the maximum amount of oil is in a direct proportion with time of extraction. The extraction kinetics of the essential oil from the dry leaves of *Thymus vulgaris* (figure 1), indicates that the yield rapidly increased as a time function and then stabilised towards a plateau equivalent to 80 min.

The yield of *T. vulgaris* essential oils from the Seraidi region provided a rate of about 1.58%. These results are similar to the work of Djeddi *et al*., (2015) on the same studied species (*Thymus vulgaris*) collected in the same region with a yield equal to 1.55%.

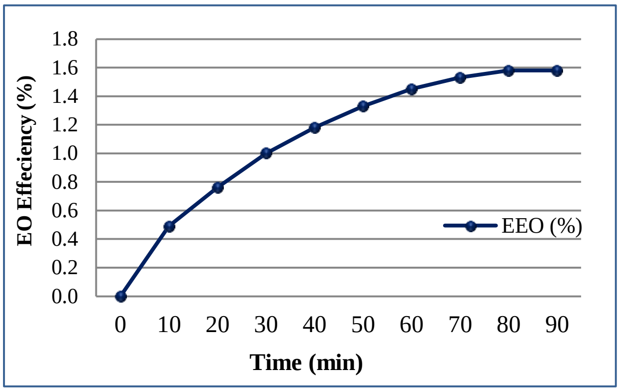

Figure (1): Extraction kinetics of thyme essential oil (EEO).

Additionally, the results of Bouguerra *et al*., (2017) on *T. vulgaris* collected in the region of Blida located in the north are relatively close to the thyme from the eastern region: Souk Ahras with a yield of 2%. In parallel, the results obtained by Yakhlef (2010) indicated that the dry leaves of thyme from the Batna region (eastern Algeria) yielded essential oil contents equivalent to 1.94%. In contrast, this rate is far from that found in thyme oil grown in the region of Tlemcen (North-West) which recorded a higher yield of around 4.2% (Abdelli *et al*., 2017). The difference in results could be referred to many factors including the geographical distribution of the plant species, the phenological stage, storage, and the mode of extraction.

The main compounds of the essential oil detected by Gas Chromatography

Chemical analysis of the essential oil using Gas Chromatography (GC) detected 30 compounds which represent about 99.07 % (Table 1, Figure 2). The essential oil of *T. vulgaris* are composed by linaool (36.41%) which presents the main major product, followed by Terpinen-4-ol (10.51%), β-Myrcene (7.5), respectively. Other constituents are identified at relatively medium levels which include: para-Cymene (6.6%) γ-terpinene (6.11%), α-thujene (4.28%), borneol (4.18%), Tricy-clene (3.31), β-pinene (2.48%), Limonene (2.45) α -Terpineol (2.05%) eucalyptol (1.72), β-caryophyllene (3.57%), α-Terpinene (3.1%) and β-pinene (2.48%). On the other hand, the chemical analysis also revealed a rather low content of carvacrol (0.75 %) and the total absence of thymol.

It is known that the genus *Thymus* comprises more than 300 species, 11 of which are located in Algeria

(Kaabouche, 2005). Within each species there is variability in its chemical profile which is due to the chemotype, which has made it possible to distinguish different essential oils of varying composition from the same species. *Thymus vulgaris* is the model with most marked by this diversity in essence. Several chemotypes have been characterised, based on the nature of the majority monoterpene including linalool; borneol; geraniol; sabinene hydrate; thymol; carvacrol, (Thompson *et al.*, 2003, Satyal *et al.*, 2016).

Several previous studies have recognised the quantitative and qualitative variability in the chemical composition of *Thymus vulgaris* from different regions in Algeria, such as Bougerra *et al*., (2017), who determined a yield of 1.58 % for thyme EO with an identical chemo-type as those identified in our study, which is characterised by a very high linalool content that exceeds 80 percent in the region of Tebessa (East of Algeria). In contrast, according to Stahl-Biskup and Sàez (2002), the chymo-type of Chlef is characterised by thymol as the primary product and low linalool levels (1.66 %).

Additionally, a study done by Benhamou *et al.*, (2012) concluded that the drying technique has a strong influence on the content of the major compound detected, while other investigation done by Svoboda and Hampson (1999) pointed out that the extraction procedures are a determining factor for the number of chemical compounds in EOs. Indeed, various factors, including edaphic, climatic, environmental, and genetic factors, are thought to have a role in thyme polymorphism (Bougerra et al., 2017).

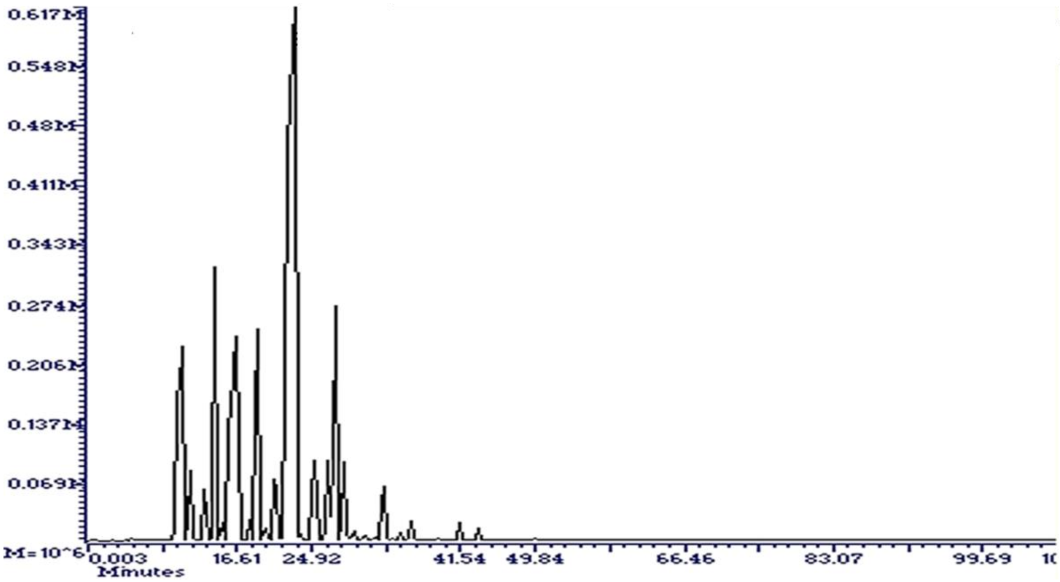

Figure (2): GPC chromatograms of *Thymus vulgaris* essential oil

Antifungal effect of Thyme essential oil on Venturia ineaqualis

Table 2 summarizes the results the antifungal effect of thyme EO on apple scab in which an inhibitory effect, of the applied doses on the growth of *Venturia ineaqualis*, was observed after 7 days of incubation wherea marked decrease in the colony diameter of the fungal colony. This decrease was calculated by light microscopy as a function of increasing EO concentrations and associated with a deformation of the filament structure. The 400µl/ml and 500µl/ml doses recorded a strong activity on the density of the scab mycelium which is manifested by a reduction in the number of filaments, 18 and 12 filaments respectively, compared to the control value (289.33 filaments). Finally, the 600µl/ml dose completely inhibited scab mycelial growth proving that this oil has the same antifungal activity compared to reference control.

Table 2-3, also showed that all doses injected, ranging from 100µl/ml to 600µl/ml, in the culture medium exerted a highly significant inhibitory effect ($p \leq 0.001$) on the tested strain. The doses of 100µl/ml and 200µl/ml showed a fairly close effect (36% and 67%) based on Tuckey test. In meantime, the doses of 300µl/ml and 400µl/ml exhibited the inhibitory activity with a higher rate of 77%. Meanwhile, the highest dose of 600µl/ml proved to be the most active dose towards the *Venturia ineaqualis*strain where it pronounced a fungicidal effect similar to that of the reference fungicide. This dose showed a complete inhibition of fungal growth during the time period of incubation (Figure 3).

Evaluation of MIC and MFC on liquid showed that the 300µl/ml dose recorded the minimum inhibitory concentration (MIC) and the highest dose 600µl/ml is the fungicidal dose where no growth was detected (MFC, Table 3). These results are consistent with those obtained on incorporated media where the 600µl dose proved to be the most toxic to the phytopathogenic fungus.

Table (1): The main compounds of the *Thymus vulgaris* essential oil detected by GPC at different retention time (RT).

No of compound recorded	Compounds detected	RT	Kovats retention index (KI)	Area %	Class of compounds detected*
1	Tricyclene	10.18	926	3,31	Bicyclic monoterpenoids
2	α-thujene	10.59	933	4,28	Bicyclic monoterpenoids
3	Camphene	11.44	947	1,34	Bicyclic monoterpenoids
4	Sabinene	12.99	972	1,02	Bicyclic monoterpenoids
5	β-Pinene	13.17	975	0,48	Bicyclic monoterpenoids
6	β-Myrcene	14.25	992	7,5	Acyclic monoterpenoids
7	α-Phellandrene	15	1004	0.28	Menthane monoterpenoids
8	α-Terpinene	15.88	1016	3.1	Menthane monoterpenes
9	para-Cymene	16.5	1025	6.6	Aromatic monoterpenoids
10	Limonene	16.76	1028	2.45	Menthane monoterpenoids
11	Eucalyptol	16.92	1031	1.72	Oxanes
12	(E)-β-Ocimene	18.14	1048	0.33	Acyclic monoterpenoids
13	γ-Terpinene	18.92	1059	6.11	Menthane monoterpenoids
14	cis-Sabinene hydrate	19.58	1068	0.22	Bicyclic monoterpenoids
15	cis-linalool oxide	19.92	1073	0.29	Tetrahydrofurans
16	Terpinolene	20.93	1087	1.26	Menthane monoterpenoids
17	Linalool	23.14	1117	36.41	Acyclic monoterpenoids
18	1-Octen-3-yl acetate	23.31	1120	0.1	Carboxylic acid esters
19	Camphor	25.19	1146	1.99	Terpenoid ketones
20	Hexyl isobutanoate	25.55	1151	0.68	Carboxylic acid esters
21	Borneol	26.78	1168	4.18	Bicyclic monoterpenoids
22	Terpinen-4-ol	27.74	1181	10.51	Menthane monoterpenoids
23	α –Terpineol	28.54	1192	2.05	Sesquiterpenoids
24	(2E)-Hexenyl butanoate	28.73	1195	0.1	Fatty acid esters
25	Verbenone	29.7	1210	0.16	Bicyclic monoterpenoids
26	Linalool acetate	32.96	1265	1.29	Acyclic monoterpenoids.
27	Bornylacetate	34.87	1297	0.1	Bicyclic monoterpenoids
28	Carvacrol	36	1312	0.74	Aromatic monoterpenoids
29	Geranyl acetate	41.43	1381	0.26	Acyclic monoterpenoids
30	(E)-Caryophyllene	43.5	1414	0.21	Bicyclic sesquiterpene
Total					**99.07%**

*Class of compounds detected and their percentage, Monoterpenes, 36.96%; Oxygenated monoterpenes, 55.51%; Sesquiterpenes, 0.21%; Other compounds, 6.39%.

Genus Thymus has a diverse range of therapeutic properties, the most notable of which is antimicrobial activity (Ourani et al., 2007; Yang and Clausen, 2007). This could be promising in the development of new biological pesticides, since recent studies have revealed that plant protection products have paradoxical effects.

In the present study, an antifungal activity was detected for all applied doses against the mycelial proliferation of *Venturia ineaqualis* where we observed a decrease in the number of filaments constituting the fungal colonies leading to an inhibition of the radial growth of the mycelium. This activity of thyme is probably linked to its chemical profile, in particular linalool, which exerted a remarkable action on the fungal strain tested. Linalool (3,7-dimethyl-1,6-octadiene-3-ol) is a primary volatile monoterpene alcohol found in a significant number of essential oils of aromatic plants such as *Lavandula angustifolia, Citrus bergamia*, *Ammi visnaga* and *Pistacia lentiscus* (Letizia *et al*., 2003; Satraniet *al*., 2004; D'Auria *et al*., 2005; Isham *et al*., 2013; Beldi *et al*., 2020). Similar studies of Franchomme (1981) and Kurita and Koike (1982) discovered the properties of monoterpenols, the major products of the oils studied. This is confirmed by the

Table (2): Effect of *Thymus vulgaris* essential oil on filament number and radial growth (RG) of *Venturia ineaqualis*

Treatments	**Incubation days**							**RG (%)**
	1	**2**	**3**	**4**	**5**	**6**	**7**	
Control (-)	95.67±4.51 ^{a}	181.67±3.06 ^{a}	300±2.65 ^{a}	408±3.00 ^{a}	411.33±3.06^{a}	301.33±4.16^{a}	289.3±3.51^{a}	100 ±0.00^{c}
Reference (+)	0.0±0.00 ^{b}	0±0.00 ^{g}	0.0±0.00 ^{e}	0.0±0.00 ^{f}	0.0±0.00 ^{f}	0.0±0.00^{f}	0.0±0.00^{f}	100±0.00 ^{c}
100	0.0±0.00 ^{b}	0±0.00 ^{g}	0.0±0.00^{e}	0.0±0.00 ^{f}	0.0±0.00 ^{f}	0.0±0.00^{f}	0.0±0.00^{f}	100±0.00 ^{c}
200	0.0±0.00^{b}	62.36±2.52 ^{d}	67.0±4.36 ^{c}	68±9.54^{c}	52.3±3.21^{c}	76.3±2.08^{c}	52±2.65^{c}	67.0± 8.00 ^{a}
300	0.0±0.00^{b}	85.67±3.51^{c}	45.0±3.00^{d}	49.3±2.52^{d}	34. 7±3.51^{d}	62. 7±3.06^{d}	34.0±3.61^{c}	77.0± 5.00 ^{ab}
400	0.0±0.00^{b}	48.33±3.06^{e}	38.33±2.08^{d}	27.3±3.06^{e}	38.0±3.00^{d}	44.7±1.52^{e}	18.3±3.51^{d}	77.0± 9.00^{ab}
500	0.0±0.00^{b}	29.64±3.06^{f}	23.0±0.00^{e}	18.0±2.65^{e}	17.0±1.52^{e}	15.0±3.00^{f}	12.0±4.58^{f}	88.0± 5.00^{b}
600	0.0±0.00^{b}	0.0±0.00^{g}	0.0±0.00^{f}	0.0±0.00 ^{f}	0.0±0.00^{f}	0.0±0.00^{f}	0.0±0.00^{f}	100 ±0.00^{c}

Table (3): MIC and MFC of *Thymus vulgaris* EO on the apple scab pathogen (*Venturia inaeqaulis*)

EO doses (µl/ml)	100	200	300	400	500	600
Venturia ineaqualis	+	+	MIC	-	-	MFC

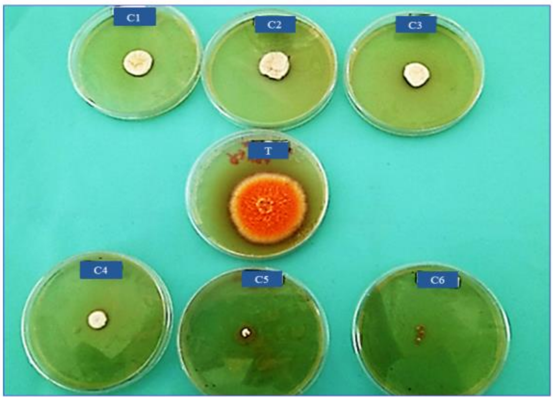

Figure (3): Antifungal Activity test showing the effect of different concentration of essential oil of T*hymus vulgaris* on the radial growth of apple scab. T= control; C1= 100, C2= 200, C3= 300, C4= 400, C5= 500, C6=600µg/ml**.**

work of Hitokoto *et al*., (1980) and Prabodh *et al*., (2016) who demonstrated the ability of coriander oil to inhibit the growth of several strains of *Aspergillus* due to its high linalool content. In another study, Isham *et al.,* (2013) illustrated that linalool has an antifungal effect on *Candida albicans* by inhibiting the formation of germ tubes and biofilm, both of which are involved in the infection process of apple scab. In confirmation to that study Hochbaum *et al*., (2018) reported antigerminative activity of thyme (*Thymol* chemotype) against apple scab.

In an attempted to clarify the mechanism of antifungal activity by linalool, Khan *et al*., (2010) and Zore *et al*., (2011) proved that linalool disrupts ergosterol biosynthesis by acting on membrane integrity and may also block the target strain's cell cycle. In the current study, the fungicidal activity was detected at dose of 600 µl/ml of *Thymus vulgaris* EO, where no growth was observed. This finding is promising as a large-scale manufacturing tool.

The activity of EO is often explained by the presence of the major products whereas it is preferable to emphasise the effect of the minority products, which may be effective by acting synergistically. According to the study done by Lahlou (2004), the use of the "totum" is more effective against the pathogen than the action of its major product. In addition, La Torre et al., (2016) recorded the applying of *Thymus vulgaris* EO against *Fusarium oxysporum* and *F. splycopersici* produced better results than the main product thymol. Furthermore, other constituents of thyme could contribute to this activity such as Terpènol-4-ol present in a significant percentage. Alama *et al*., (2017) found that *Malaleuca alternifolia* exhibited an inhibitory potential against *Candida albicans* probably due to Terpinol-4-ol.

Despite the absence of thymol and the rather low carvacrol content of 0.74%, thyme EO from the Seraidi region was very effective and revealed a very powerful activity against *Venturia ineaqualis.* Some authors have attributed the antimicrobial power of EO to the presence of oxygenated monterpenes in high levels (Marzoug *et al*., 2011). This result corroborates with our results where the percentage exceeds 55%. These oxygenated compounds can cause alterations in the walls of the target strains due to their high solubilisation (Griffin *et al*., 1999; Hammer *et al*., 2003).

CONCLUSION

This study highlighted the potential for using thyme essential oil (EO) to protect apple trees from the apple scab pathogen (*Venturia ineaqualis*). Evidently, the EO of *Thymus vulgaris* collected from Seraidi is characterized by high content of linalool and oxygenated monoterpenes, that giving it potent antifungal properties. However, the effectiveness of such molecules has been well demonstrated in the laboratory, it has yet to be proven in the field. To replace synthetic fungicides, it appears necessary to increase research efforts on this topic in order to propose EOs that retain their entire fungicidal efficacy on the field scale application to re-establish functional balances between the ecological compartments.

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التوصيف الكيميائي للزيت العطري المستخلص من نبات *Thymus vulgaris* وتقييم نشاطه المضاد للفطر المسبب لمرض جرب التفاح (*Venturia inaequalis* L)

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الملخص العربي

تهدف هذه الدراسة إلى دراسة التركيب الكيميائي للزيت الأساسي المستخرج من الجزء الهوائي من *Thymus vulgaris* L والذي يشمل الاوراق و السيقان. تم تقييم نشاطها البيولوجيي ضد فطر *Venturia ineaqualis* L والمسبب لجرب التفاح.كما تم دراسة تفصلية للمركبات الكميائية لهذا الزيت المستخرج. أظهرت نتائج التحليل الكيميائي لهذا الزيت وجود 30 مركبًا تمثل ما يقرب من 99.07 ٪ من إجمالي المكون.واثبتت النتائج ان مركب Linalool، هو المكون الأكثر تواجدا وكان تواجده بنسبة 36.41٪، وتلي ذلك مركب Terpinen-4-ol بنسبة 10.51 % ثم مركب β-myrcene بنسبة7.5%. وكانت المكونات الأخرى التي تم تحديدها بكميات متفاوتة تشمل: para-Cymene (6.6٪)؛ γ-Terpinene (6.11%)، و Carvacrol (0.75٪)، مع غياب كليا لمركب الثيمول. تميز تقييم النشاط المضاد للفطريات في الزعتر EO لنمو الخيوط لفطر *Venturia ineaqualis* وذلك بانخفاض واثباط نموعدد الخيوط الفطرية في المستعمرة والتي تأثرت بشكل كبير بزيادة الجرعات. كان تركيز EO الذي كان له الحد الأدنى من التأثير المثبط هو 300 ميكرولتر/مل. كما اثبت ان الزيت المستخرج له تأثير مثبط كامل على نمو الفطريات بجرعة 600 ميكرولتر/ مل، حيث ادي الي تثبيط كامل لنمو الفطر. على الرغم من عدم وجود thymol والمحتوى المنخفض نسبيًا من Carvacrol ، وكلاهما معروف بسميته العالية ، إلا أن الزعتر EO من منطقة السرايدي كان فعالًا للغاية وكشف عن نشاط قوي ضد فطر *V.ineaqualis* ، والذي من المحتمل أن يكون بسبب وجود المستويات العالية من (monoterpenes 36.96٪) و monoterpenes المؤكسج (55.51٪) المرتبطه بالمكونات التآزرية الأخرى.