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Determination of total phenolic content, antioxidant activity and antifungal effects of *Thymus vulgaris*, *Trachyspermum ammi* and *Trigonella foenum-graecum* extracts on growth of *Fusarium solani*

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Abstract: Ajowan, thyme and fenugreek are spice and aromatic crops with a number of medicinal properties which are known as important sources of essential phytochemicals. The purpose of this study was to investigate the antioxidant capacity, total phenolic content and antifungal activities of these plant extracts on growth of *Fusarium solani*, an important plant pathogen, soil saprophyte and one of the causal agents of fusariosis in human and animals. Their total antioxidant activity was measured using DPPH radical scavenging assay and their antimicrobial activity was determined through poison food assay at two concentrations (1000 and 1500 ppm) and spore germination assay *in vitro*. All methanolic extracts showed high antioxidant activity which among them methanolic extract of thyme demonstrated higher antioxidant potential with a low IC_{50} (16.50 mg ascorbic acid/g). Also, the highest phenolic content (70.55 mg GAE g^{-1}) was observed in methanolic extract of thyme. The highest and lowest amount of thymol was determined in methanolic extract of thyme and aqueous extract of ajowan. Methanolic extracts of thyme leaves and ajowan seeds at concentration of 1500 ppm were potentially effective against *F. solani* over the control treatments by 90.33% and 85.73%, respectively. Followed by hydro-ethanolic and aqueous extracts exhibited a lesser percentage of inhibition. The MIC value for methanolic extract of thyme and ajowan was 3.75 mg/ml followed by hydro-ethanolic and aqueous extracts, respectively. The amount of calculated MFC was ranging from 7.5 to 30 mg/ml for thyme methanolic and fenugreek aqueous extracts, respectively.

Key words: Antioxidant; Antifungal activity; Total phenolics; Thymol, *F. solani.*

Introduction

Over the years, a few species of *Fusarium* such as *F. verticillioides*, *F. solani* complex, *F. oxysporum* and *F. proliferatum* are being mentioned in relation to severe damage to agriculture crops (1). These species are the causative agents of superficial and systemic infections in humans and animals through inhalation of air-borne conidia and cuts in the skin (2). In addition, they are responsible for the production of harmful secondary metabolites known as mycotoxins which have negative impacts on the agricultural industry as well as being the main factor for mycotoxicosis in humans and animals (3–5).

Fusarium solani (Mart.) Sacc. is a complex species currently estimated to contain at least 60 phylogenetically distinct species known as the "*Fusarium solani* species complex" (FSSC) (6,7). They can cause various diseases such as crown rot, head blight, scab and wilt in plant hosts (8,9). In addition, they also have reported as opportunistic infections of humans and other animals, causing systemic infections with a high mortality rate and localized infections in the skin, nail or eye in healthy humans (10,11). Infection caused by FSSC isolates which is called fusariosis is the second major cause of fungal infection after *Aspergillosis* in immunocompromised patients (12).

Despite the undesirable effects of chemicals compounds, they are applied for disease management in agriculture. In recent years, because of some serious problems and threats against the effective use of chemical compounds such as the emergence of resistant strains the potential of natural compounds and biological agents for control of plant diseases has been considered (13,14). Thus, there is a need to search for an alternative, safe and natural methods approach with the minimal environmental impact and danger to consumers to disease control (15–17).

Traditional Persian Medicine (TPM) is one of the richest branches of alternative medicine with thousands of years of background having a good potential source to find new approaches for the prevention and treatment of many conditions (18). From a traditional Persian scholar viewpoint, many plants have antiseptic properties (19). Plant extracts consist in a complex mixture of several compounds such as phenolic compounds which are widespread in some plants acting as antioxidants and free radical scavengers (20). In view of these, the present investigation was undertaken to screen for the efficacy of antifungal potency of certain plant extracts including; thyme *(Thymus vulgaris* L.), ajowan *(Trachyspermum ammi*) and fenugreek *(Trigonella foenum-graecum*) against one of the important pathogenic fungi in agriculture, medicine and veterinary science named

Fusarium solani. Ajowan is a highly valued medicinally important seed spice which contains compounds that reportedly exhibit insecticidal, antifungal, nematicidal and antibacterial activities from the aromatic and medicinal points of view (21–24). Thyme is one of the most widely known from the aromatic and medicinal points of view and has been described as having germicidal, antispasmodic, antifungal properties (25). Fenugreek also is well known for its multiple pharmacological properties such as antidiabetic, antioxidative, anti-inflammatory, antibacterial an antifungal (26). Therefore, the purpose of this work was to evaluate the antioxidant potential, total phenolic contents and antimicrobial properties of the reference plants against *F. solani*.

Materials and Methods

Preparation of extracts

Leaf samples of thyme and fenugreek were collected from greenhouses and natural sites located in Pakdasht in Tehran province, Iran, during April and June 2018. Ajowan seeds were procured from a local market located in Tehran. The samples were thoroughly washed in running water and dried away from direct sunlight under laboratory conditions. Subsequently, the dried plant materials were powdered using pistol and mortar (Table 1).

Aqueous extract

10 grams of grounded tissue was macerated with 100 ml of sterile distilled water and put in a warm bath for an hour and then 24 hours in laboratory condition. The extract obtained was filtered by Whatman filter paper (No: 1) and concentrated with a rotary evaporator at room temperature (27).

Hydro-ethanolic extract

Hydro-ethanolic extraction was prepared by mixing 10 grams of grounded tissue in 100 ml of ethanol: water (75:25, v/v) mixture and shaking for 24 hours at 20°C and at 250 rpm. Then extract was separated from plant debris by Whatman filter paper (No: 1) and concentrated with a rotary evaporator at room temperature (27).

Methanol extract

10 gram of dry powder materials of each plant were added to 100 ml of absolute methanol (≥99/5%) and homogenized for 1 h with the help of a magnet. After two days' mixtures were passed through Whatman filter paper (No: 1) and then shaken at 160 rpm at room temperature to obtain clear extracts. The methanol was completely removed from clear solutions using a rotary evaporator (28).

Two extract concentrations (1000 and 1500 ppm) were prepared in aqueous, hydro-ethanolic and methanolic solvents.

Isolation of the pathogen

F. solani (GenBank accession No. MF588957) as a fungal pathogen used in this study was obtained from mycology laboratory, University of Tehran, Iran. To make sure species identification, the purified isolates of *Fusarium* were identified according to their cultural and morphological characteristics as described by Leslie and Summerell (29). The isolates were grown on PDA medium to determine their growth rate and colony pigmentation and the cultures were incubated at 26ºC and 30ºC for 7–10 days in the dark condition. To investigate the presence and shape of the macroconidia, microconidia and chlamydospores, isolates were also placed on CLA and SNA medium and then incubated for 14 days under fluorescent and near-ultraviolet lights conditions.

Antifungal screening

Mycelial growth inhibition assay

In order to evaluate the effect of different extracts on mycelial growth of *F. solani*, poisoned food technique was used. PDA medium was autoclaved at 121°C for 20 minutes and kept under the sterilized hood to cool up to 40°C. The extracts were mixed with sterile molten PDA to obtain final concentrations of 1000 and 1500 ppm. 15-20 ml of each media was separately poured into petri dishes, allowed to cool and solidify. After complete solidification of the medium, 5mm disc of the seven-day-old culture of the *F. solani* was inoculated into petri dishes. The plates were incubated at 25± 1°C for seven days. The petri dishes containing media devoid of the extract but with the same amount of distilled water served as a negative control and carbendazim was used as the positive control. The measurements of the mycelial growth dynamic of the fungus were recorded on a daily basis, beginning with 24 hours after inoculation. Three replicates were used per treatment. The percent inhibition of fungal growth was estimated by using the following formula:

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

Where C; average diameter of the fungal colony of the control plate and T; average diameter of the fungal colony treated with the treatment plate.

Conidia germination assay

A broth dilution method was applied to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The minimum inhibitory concentration of different extracts was determined as recommended by Wiegand *et al*. (30). MIC method was performed in sterile, flat-bottomed 96-well microplates. All the extracts dissolved in PDB were first diluted to the highest concentration (30 mg/ml) to be tested and then two-fold serial dilution was made in the concentration range from 0.94 to 30 mg/

Table 1. Test plants used for antifungal activity assay.

Scientific name	Common name	Family	Plant part used
Thymus vulgaris L.	Thyme	Lamiaceae	Leaf
Trachyspermum ammi	Ajowan	Apiaceae	Seed
Trigonella foenum-graecum	Fenugreek	Fabaceae	Leaf

ml. In each well, 100 µl of each extract dilution was mixed with 100 µl of the PDB. The sample was subjected to a six-fold dilution series in order to give final amounts of the original suspension. Distilled water and carbendazim served as a negative and positive control, respectively. For broth dilution, 50 µl of 10^3 CFU/ml suspension of pathogen strain separately was added to each well containing various extracts at concentrations of 0 (control), 0.94, 1.88, 3.75, 7.5, 15 and 30 mg/ml in broth medium. The microplates were incubated at 25°C and observed for visible growth after 24 h. Also, 10 µl of each well was poured onto the slide and examined by microscope to the determination of germination or non-germination of spores. The lowest concentration of extracts in a microplate that failed to show any visible growth was considered as the MIC.

The minimum fungicidal concentration (MFC) was determined by sub-culturing the negative wells on potato dextrose agar (PDA) medium. For determined of MFC, 20 µl of contents of each well that showed complete inhibition (an optically clear well) was sub-cultured onto PDA plates. The plates were incubated at 25°C in the dark until growth was seen in the growth control subculture. The lowest concentration of extract with no visible growth after 48 h was defined as the MFC, indicating 99.9% killing of the original inoculum compared to Carbendazim which used as a positive control (31).

Determination of antioxidant activity

The antioxidant activity was measured in terms of radical scavenging ability using the stable radical DPPH (2,2-Diphenyl-1-picrylhydrazyl) according to the method of Blois (32). Briefly, a volume of 1 ml of the methanolic, hydro-ethanolic and aqueous stock solution of the extracts were put into test tubes and 1 ml of 1 mM DPPH solution was added. The tubes were covered with parafilm and kept again in the dark for 1 h. The absorbance of all samples was measured at 517 nm using a SPECTRAmax-PLUS384 UV–vis spectrophotometer and compared to an ascorbic acid calibration curve. The results were expressed as mg ascorbic acid/g dried sample. Each assay was performed in triplicate. The percentages of inhibition of the DPPH radical was calculated using the following formula:

$$I\% = \frac{A0-A}{A} \times 100$$

where I = DPPH inhibition (%), A0 = absorbance of control sample (t = 0 h) and A = absorbance of a tested sample at the end of the reaction (t = 1 h). The IC_{50} value was expressed as the concentration of sample that reduced the initial DPPH concentration by 50% (mg ascorbic acid/g dried sample).

Determination of total phenols

Total phenolic contents in the methanolic, hydro-ethanolic and aqueous extracts were measured by the Folin–Ciocalteu assay, with gallic acid as a standard (33). 0.2 ml of the extract solution (1 mg/ml) was introduced into the test tubes followed by 0.5 ml of Folin-Ciocalteu reagent (diluted 1/10). The solution was then kept at dark for 5 min and then 0.8 mL saturated sodium carbonate (7.5% w/v) was added. The tubes were kept at dark for 30 min for color development. Absorbance was measured using the same spectrophotometer described previously at 760 nm. A reagent blank was prepared using distilled deionized water. The results were expressed as mg gallic acid/g dried sample. All determinations were performed in triplicate.

High performance liquid chromatographic (HPLC) quantization of thymol

Sample preparation

Quantification of thymol content was performed in six different samples including a methanolic, hydro-ethanolic and aqueous extract of ajowan and thyme according to the modified method of aghamohammdi *et al.* (34). Briefly, 10 ml of each six samples was added to a volumetric flask and diluted to 100 ml (1:10) using methanol, hydro-ethanol and water as solvents (10^2 µg/ml) and injected three times to evaluate the quantification of thymol. Thymol standard materials (≥ 99.9% purity) were purchased from Merck (Germany).

Instrumentation and Analysis

HPLC analyses were performed with Waters® 2695 Separations Module consists of four solvent delivery system (25VE181ESJ Eurospher 100-5 C18) equipped with a photodiode array detector model Waters 996 (HPD011) set at 210 nm (Knauer Assoc., Germany). The analysis was done by using an ODS-C18 column (259 × 4.6 mm i.d., 5 µm particle size), and the corresponding precolumn. All solvents were filtered and degassed earlier entering the column. The mobile phase was selected an isocratic combination of acetonitrile: H2O (50:50 ratio V:V). Injection volume was 10 µl. The mobile phase flow rate was 1.0 ml/min, and all the measurements were done at 25˚C.

Statistical analysis

Statistical analysis of the data obtained in the present study was carried out in a completely randomized design layout using SPSS ver. 19 and grouping of the treatments was done by Tukey's range test ($p<0.05$) where the comparison of means of different treatment was performed using factorial design. The experimental results were expressed as mean values (n=3). The data were subjected to one-way analysis of variance (ANOVA).

Results

Mycelial growth inhibition

The percentage of growth inhibition has been summarized in table 2. The methanolic extract of *T. vulgaris* and *T. ammi* at 1500 ppm demonstrated the highest antifungal activity against mycelial growth of *F. solani* that recorded 90.33% and 85.73%, respectively. On the other hand, the aqueous extract at different concentrations had the lowest effect on the fungal growth, ranged between 13.9- 19.56% as shown in figure1. In other words, at 1000 ppm concentration, the inhibitory growth of 16.1, 15.63 and 13.9% were recorded for *T. ammi*, *T. vulgaris* and *T. foenum-graecum*, respectively. The hydro-ethanolic extracts of *T. vulgaris* showed inhibition of 42.4% and 39.2% at 1000 and 1500 ppm, respectively. In addition, the hydro-ethanolic extracts

Table 2. Mean comparison of growth inhibition of three plant extracts at two concentrations on PDA.

Treatments		Concentration (ppm)	Growth inhibition (%)		
			Aqueous extract	Hydro-ethanolic extract	Methanolic extract
T. vulgaris		1000	15.63$^{*\&ED}$	39.2$^{*\&ED}$	81.3 $^{*\&GF}$
		1500	18.83$^{*\&B}$	42.13$^{*\&ED}$	90.33^{*IH}
T. ammi		1000	16.1$^{*\&CB}$	36$^{*\&D}$	76.06$^{*\&F}$
		1500	19.56$^{*\&B}$	42.4$^{*\&E}$	85.73$^{*\&HG}$
T. foenum-graecum		1000	13.9$^{*\&B}$	16.3$^{*\&B}$	19.5$^{*\&CB}$
		1500	16$^{*\&B}$	19.66$^{*\&CB}$	24.16$^{*\&C}$
Control Group	Negative	1000	0 $^{\&A}$	0 $^{\&A}$	0 $^{\&A}$
		1500	0 $^{\&A}$	0 $^{\&A}$	0 $^{\&A}$
	Positive	1000	91.13^{*HI}	91.13^{*HI}	91.13^{*HI}
		1500	93^{*I}	93^{*I}	93^{*I}

Numbers within a column followed by the same letter are not significantly different at ($p< 0.05$). * Significantly different by negative control ($p< 0.05$). & Significantly different by positive control ($p< 0.05$). Values are means (n=3).

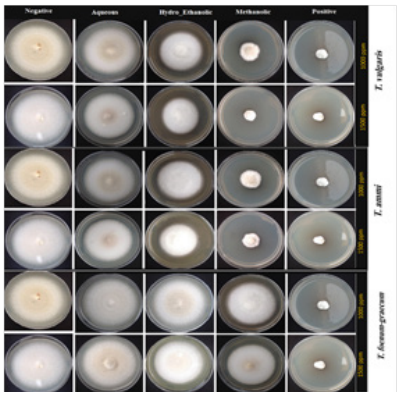

Figure 1. Effect of aqueous, hydro-ethanolic and methanolic extracts of three test plants (*T. vulgaris*, *T. ammi* and *T. foenum-graecum*) at two concentrations (1000 and 1500 ppm) on the growth of *F. solani* on PDA.

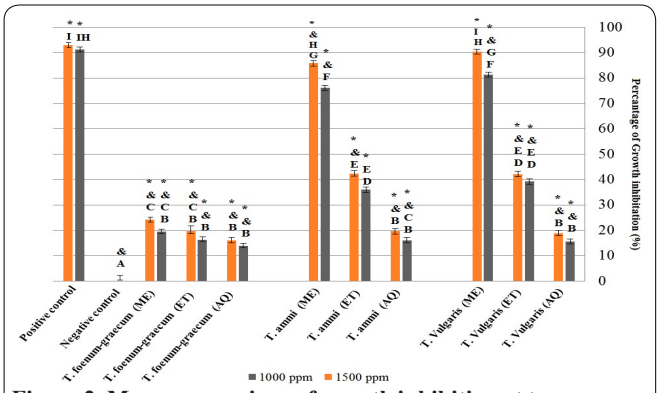

Figure 2. Mean comparison of growth inhibition at two concentrations of three plant extracts on growth of *F. solani* on PDA. ME: methanolic extract, ET: hydro-ethanolic extract, AQ: aqueous extract. Numbers within a column followed by the same letter are not significantly different at ($p< 0.05$). * Significantly different by negative control ($p< 0.05$). & Significantly different by positive control ($p< 0.05$). Error bars indicate SEM.

of *T. ammi* showed 42.4% and 36% at 1000 and 1500 ppm, respectively. (Table 2). Carbendazim 50 WP as a positive control, showed the highest growth inhibition at both concentration 1000 and 1500 ppm, 91.13 and 93%, respectively (Table and figure 2).

Conidia germination

MIC values of different extracts were determined using the broth dilution method. Extracts showing antifungal activity in poison food assay were further evaluated for MIC values. The MIC value for methanolic extract of *T. vulgaris* and *T. ammi* against *F. solani* were 3.75 mg/ml followed hydro-ethanolic extract having 7.5 mg/ml in reference plants. Aqueous extracts have MIC value of 15 mg/ml against *F. solani* in all used plants. The results of MIC values determination are shown in table 2.

The minimum fungicide concentration (MFC) of methanolic extract which caused total inhibition of *F. solani* was found to be 3.75 mg/ml in *T. vulgaris* and *T. ammi* and 15 mg/ml MFC for hydro-ethanolic and aqueous extracts in reference plants. While MFC value of different extracts of *T. foenum-graecum* was 30 mg/ml (Table 3).

DPPH radical scavenging activity

The DPPH inhibition of six different plant extracts is summarized as IC_{50} in Table 4. All methanolic extracts have the highest antioxidant activity ranging between 16.50 to 90.55 mg ascorbic acid/g. In contrast, the aqueous extracts had the lowest potential antioxidant activity ranging between 35.50 to 103.80 mg ascorbic acid/g. Significant differences ($P < 0.05$) in DPPH scavenging activity were found between all treatments except hydro-ethanolic and aqueous extracts of *T. foenum-graecum*.

Table 3. MIC and MFC of *T. vulgaris* and *T. ammi* and *T. foenum-graecum* extracts against *F. solani.*

	MIC in mg/ml			MFC in mg/ml		
	Methanolic	Hydro-ethanolic	Aqueous	Methanolic	Hydro-ethanolic	Aqueous
T. vulgaris	3.75	7.5	15	7.5	15	15
T. ammi	3.75	7.5	15	7.5	15	15
T. foenum-graecum	15	15	15	30	30	30

Table 4. Free radical scavenging activities of plant extracts expressed as IC_{50}.

	IC_{50} (mg ascorbic acid/g dried sample)		
	Methanolic	**Hydro-ethanolic**	**Aqueous**
T. vulgaris	16.50 [a]	27.68 [b]	35.50 [c]
T. ammi	33.60 [bc]	38.45 [cd]	53.70 [e]
T. foenum-graecum	90.55 [f]	101.60 [g]	103.80 [g]

Values are means of IC_{50} (n = 3). Numbers followed by the same letter are not significantly different at ($p< 0.05$).

Total 5. Phenolic contents (mg GAE/g DM) in different extracts of *T. vulgaris*, *T. ammi* and *T. foenum-graecum.*

	Total phenols (mg GAE/g DM) [a]		
	Methanolic	**Hydro-ethanolic**	**Aqueous**
T. vulgaris	70.55 [a]	68.34 [ab]	54.21 [c]
T. ammi	71.45 [a]	63.80 [b]	58.01 [bc]
T. foenum-graecum	46.70 [d]	43.90 [de]	37.60 [e]

[a] Total phenols are expressed as gallic acid equivalents; mgs of gallic acid per g of dried extract. Values are means (n = 3). Numbers within a column followed by the same letter are not significantly different at ($p< 0.05$).

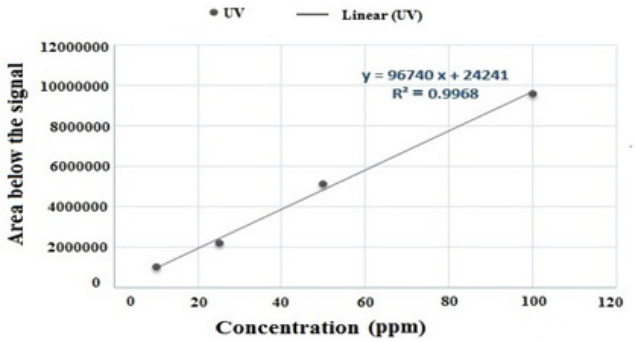

Figure 3. Thymol calibration curve.

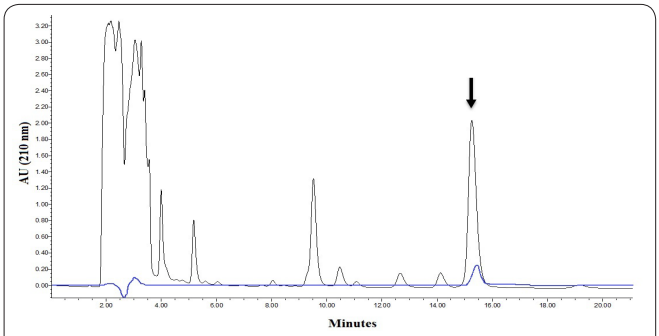

Figure 4. HPLC chromatogram of methanolic extract of *T.vulgaris* (black line) *vs.* standard thymol (blue line). Black arrow indicates peak of thymol.

Total phenolic content

The total phenolic contents of six plant extracts are summarized as in Table 5. The highest total phenolic content was determined in methanolic extract of *T. ammi*, however, the aqueous extract of *T. foenum-graecum* had the lowest amount of phenol (Table5).

Quantification of thymol

Calibration graph was performed using the external standard technique following linear regression analysis by plotting concentration against peak area. The linearity of the thymol method in a wide range of concentrations of 10-100 ppm was determined. The coefficient of correlation was calculated for the thymol $R^2 = 0.9981$, while the equation of direction was y= 96740x + 24241 (Figure 3). The highest and lowest amount of thymol was determined in the methanolic extract of thyme and aqueous extract of ajowan by 4.50 and 0.0021 mg/ml, respectively (Table 6 and Figure 4). The results indicated that there is a significant difference between methanolic extracts of thyme and ajowan. The chromatogram of standard thymol and the methanolic extract of thyme is shown in Figure 4, with associated retention times and area below the signal.

Table 6. Thymol content (mg/ml) in different extracts of *T. vulgaris* and *T. ammi.*

	Thymol in mg/ml		
	Methanolic	**Hydro-ethanolic**	**Aqueous**
T. vulgaris	4.50 [a]	0.84 [c]	0.0046 [d]
T. ammi	2.08 [b]	0.85 [c]	0.0021 [e]

Discussion

In this study, a total of nine extracts including methanolic, hydro-ethanolic and aqueous extracts of three medicinal plants including *T. vulgaris*, *T. ammi* and *T. foenum-graecum* were screened for their antifungal properties at two different concentrations (1000 and 1500 ppm). In general, growth inhibition percentage increase with increasing the concentration of extract. Among the treatments, fungi growth was not completely suppressed (100% inhibition) even by carbendazim as the positive control. Moreover, there is a significant difference between all treatments and negative control (without extract). The growth inhibition percentages of the methanolic extracts of all test plants showed highest inhibition efficiency ranging from 19.5 to 90.33% followed by hydro-ethanolic extracts. On the other hand, the lowest growth inhibition was recorded by aqueous extracts, ranged between 13.9-19.56%. The results indicated the significant potential of thyme. In other words, there was no significant difference between methanolic extract of thyme at 1500 ppm with positive control. Also, there is no significant difference between methanolic extract of thyme and ajowan at 1500 ppm. Methanolic extracts of *T. vulgaris* and *T. ammi* at 1000 ppm were found to be effective and suppressed the pathogen's mycelial growth with no statically significant difference, 81.33% and 76.06%, respectively. There were no statically differences between fenugreek extracts except for the methanolic extract at 1500 ppm.

Our results showed MIC values ranging from 3.75

to 15 mg/ml against pathogen isolate. MFC values were higher than MIC values, ranging from 7.5 to 30 mg/ml. According to the results, MIC and MFC values of *T. vulgaris* and *T. ammi* are identical ranged between 3.75 to 15 and 7.5 to 15 mg/ml against pathogen isolate, respectively. The present study revealed that *T. vulgaris* and *T. ammi* extracts have both fungistatic and fungicidal activities. Similar studies have been carried out on the antifungal activity of plant extracts on the mycelial growth and spore germination. Zabka *et al*. reported mycelial growth inhibition of *F. oxysporum* and *F. verticillioides* using *T. vulgaris* essential oils. In their study, essential oil of *T. vulgaris* showed a MIC of 1.2 µl/ml (*F. oxysporum*) and 1.8 µl/ml (*F. verticillioides*) (35). Previous studies have also indicated that thymol as a major component of other herbal plants including *Thymus vulgaris* was responsible for the strong antifungal activity against a variety of pathogenic yeasts and filamentous fungi (36,37).

Additionally, we determined the IC_{50} values of extracts which is the effective concentration of the sample at which DPPH radicals were reduced by %50 of extracts (Table 4). The lower the IC_{50} value, the higher the antioxidant activity. Significant differences ($P < 0.05$) in antioxidant activity were found between the nine extracts ranging from 16.50 to 103.80 mg ascorbic acid/g dried sample. The wide range of antioxidant capacity may be attributable to the different bioactive compounds, including phenolics, flavonols, etc. Methanolic extracts of thyme possessed the highest DPPH scavenging activity (16.50 mg ascorbic acid/g) followed by a hydro-ethanolic extract of thyme and methanolic extract of ajowan, 27.68 and 33.60 mg ascorbic acid/g, respectively. Fenugreek extracts showed the minimum percentage of antioxidant capacity ranged between 90.55 and 103.80, respectively. The significant antioxidant activity of methanolic and hydro-ethanolic extracts of thyme and ajowan could be due to the high amount of total phenolics and flavonoids in these species. Several research has shown that leaves of thyme and seeds of ajowan contain large amount of antioxidants among them phenolic acid and flavonoids are the most important compounds (38,39).

Table 5 shows a summary of the phenolics contents data. The results showed that methanolic and hydro-ethanolic extracts of thyme and methanolic extract of ajowan contained the highest amount of phenolics compounds. On the other hand, aqueous extract of fenugreek showed the lowest amount of phenolics contents. It could be noticed from the results that methanol and hydro-ethanol were better solvents in extracting phenolic compounds because of their polarity and good solubility for phenolic components (38). The lower polarity solvents such as water showed much lower ability in extracting the phenolic compounds in comparison with the polar solvents.

Moreover, the results of evaluation of thymol showed that methanol is the best solvent among the other solvents using in this study. The results demonstrated that methanolic extract of thyme and ajowan had the maximum amount of thymol which is in accordance with the other studies (40,41). It has been proved that thymol, a monoterpene phenol is the major phenolic compound present in some plants having antioxidant, anti-inflammatory, local anaesthetic, antinociceptive, antiseptic and antimicrobial activity (42,43). The genus *Thymus*, member of the Lamiaceae family, has numerous species and varieties. From *T. vulgaris*, some chemotypes have been reported; Geraniol, Linalool, γ(gamma)-terpineol, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol, p-cymene, 1,8-cineole (44). Apparently, due to various studies, the antimicrobial activity of the reference plant is related to the presence of phenolic compounds (thymol) which is the most dominant of all identified compounds of thyme.

The present study concludes that methanolic extracts of *T. ammi* exhibited remarkable antifungal activity against *F. solani*, while hydro-ethanolic and aqueous extracts had less antifungal activity, respectively. The studies on the ethanolic extracts of *T. ammi* have been reported to inhibit *Aspergillus* and *Candida* (22,45). Previous studies showed that *T. ammi* contained a mixture of components, major including; thymol (63.4%), p-cymene (19%) and g-terpinene (16.9%). The major components present in the extract are phenolic components which are considered to be mostly antifungal agents (46,47). In this study, methanolic extract of ajowan had a high amount of thymol (2.08 mg/ml), phenolic content (71.45 mg GAE/g DM) and antioxidant ability (33.60 mg ascorbic acid/g) compared to other treatments. Apparently, it is reasonable to speculate that the activity of *T. ammi* extract can be related to the presence of phenolic content such as thymol.

Although in previous studies of *T. foenum-graecum* showed considerable antibacterial and antifungal activities, the reference plant in the present study was not found to be effective and showed little ability to inhibit *F. solani strain*. Thomas *et al*. reported aqueous and methanolic extract of leaves and seed of 75 different *Trigonella* accessions was not effective against a range of enteric bacteria and yeast (48). On the other hand, Nahar *et al.* revealed that *T. foenum-graecum* displayed antibacterial activity against *Bacillus subtilis, Sarcina lutea, Xanthomonas campestris, Proteus vulgaris and Pseudomonas denitrificans* (49). According to our results, fenugreek extracts showed the lowest antioxidant activity. In addition, we proved that fenugreek extracts had a low amount of phenolic contents such as thymol. It can be concluded that low antifungal activity can be associated with low phenolic content in fenugreek extracts. It should be noted that the methanolic extract of fenugreek showed higher antifungal activity compared to the aqueous extract. According to this result, it can be suggested an insight into the dependence of antimicrobial activity on the solvent system being chosen for the extract preparation and type of microorganisms (50). It has been suggested that sensitivity of the extracts towards microorganism is dependent not only on the solvent system but also on the type of microorganisms (51). In addition, several research showed that the biosynthesis of both primary and secondary metabolites in medicinal and aromatic plants are strongly influenced by environmental factors which in turn can lead to change in chemical composition of essential oils or extracts of plants (52,53). In conclusion, further experiments are suggested to investigate the *in vivo* effects of these extracts as compared to some commercial chemical fungicides for the management of *F. solani.* The

findings of the present study should stimulate studies on toxicity, improved formulations and the determination of optimal concentrations for management applications.

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Conflicts of Interest
The authors declare no conflict of interest.

References

1. Saremi H, Okhovvat SM. Major Fusarium diseases on crops and their control management with soil solarization in northwest Iran. Commun Agric Appl Biol Sci 2008; 73:189-99.
2. Georgiadou SP, Velegraki A, Arabatzis M, Neonakis I, Chatzipanagiotou S, Dalekos GN *et al.* Cluster of *Fusarium verticillioides* bloodstream infections among immunocompetent patients in an internal medicine department after reconstruction works in Larissa, Central Greece. J Hosp Infect 2014; 86: 267-71.
3. Knutsen HK, Alexander J, Barregard L, Bignami M, Brüschweiler B, Ceccatelli S *et al.* Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA J 2017; 15:e04655.
4. Desjardins AE. Fusarium mycotoxins: chemistry, genetics, and biology, APS Press, USA, St Paul, 2006, pp. 260.
5. Covarelli L, Stifano S, Beccari G, Raggi L, Lattanzio VMT, Albertini E. Characterization of *Fusarium verticillioides* strains isolated from maize in Italy: Fumonisin production, pathogenicity and genetic variability. Food Microbiol 2012; 31:17-24.
6. Nalim FA, Samuels GJ, Wijesundera RL, Geiser DM. New species from the *Fusarium solani* species complex derived from perithecia and soil in the Old World tropics. Mycologia 2011; 103:1302-30.
7. O'Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME *et al.* Molecular phylogenetic diversity, multilocus haplotype nomenclature, and In Vitro antifungal resistance within the *Fusarium solani* species complex. J Clin Microbiol 2008; 46:2477-90.
8. Pandey A, Juwantha R, Chandra S, Kumar A, Kannojia P, Khanna D *et al*. First report of *Fusarium solani* causing wilt of *Melia dubia*. Forest Pathol 2018; 48:e12398.
9. Yan K, Han G, Ren C, Zhao S, Wu X, Bian T. *Fusarium solani* infection depressed photosystem performance by inducing foliage wilting in apple seedlings. Front Plant Sci 2018; 9: 479.
10. Babic MN, Zalar P, Ženko B, Schroers H-J, Džeroski S, Gunde-Cimerman N. *Candida* and *Fusarium* species known as opportunistic human pathogens from customer-accessible parts of residential washing machines. Fungal Biol 2015; 119: 95-113.
11. Krcmery V, Jesenska Z, Spanik S, Gyarfas J, Nogova J, Botek R *et al.* Fungaemia due to *Fusarium* spp. in cancer patients. J Hosp Infect 1997; 36:223-8.
12. Nucci M, Anaissie E. Fusarium Infections in Immunocompromised Patients. Clin Microbiol Rev 2007; 20:695-704.
13. Edris AE, Farrag ES. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Nahrung-Food 2003; 47:117-21.
14. Lopez-Reyes JG, Spadaro D, Prelle A, Garibaldi A, Gullino ML. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits In Vivo. J Food Protect 2013; 76:631-9.
15. Mirghazanfari SM, Hosseinzadeh L, Shokoohinia Y, Aslany M, Kamali-Nejad M. Acute and subchronic toxicological evaluation of *Echinophora platyloba* DC (Apiaceae) total extract in wistar rats. Clinics 2012; 67:497-502.
16. Zarei B, Kahrizi D, Sayfi T, Movahedi R, Ghaheri M, Kazemi E. The antibacterial effect of alcoholic extraction of *Satureja bachtiarica* on four bacterial human pathogens. Agric Biotech 2017; 8:81–6.
17. Mirghazanfari SM, Abbassian A, Kamalinejad M, Karimian SM, Massoud A. The effect of methanolic and ethanolic extracts of *Ruta graveolens* L. leaves on formalin-Induced inflammation in Rats. Trad Integr Med 2016; 1:136-41.
18. Larijani B, Esfahani MM, Moghimi M, Shams Ardakani MR, Keshavarz M, Kordafshari G *et al.* Prevention and treatment of flatulence from a Traditional Persian Medicine perspective. Iran Red Crescent Med J 2016; 18: e23664.
19. Kalhor R. Contribution of Persian physicians to the development of Islamic medicine. Med Arh 1997; 51:9-12.
20. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci 2015; 9:449–54.
21. Seo SM, Park HM, Park IK. Larvicidal activity of Ajowan (*Trachyspermum ammi*) and Peru Balsam (*Myroxylon pereira*) oils and blends of their constituents against mosquito, *Aedes aegypti*, acute toxicity on water flea, *Daphnia magna*, and aqueous residue. J Agric Food Chem 2012; 60:5909-14.
22. Kim E, Oh CS, Koh SH, Kim HS, Kang KS, Park PS *et al.* Antifungal activities after vaporization of Ajowan (*Trachyspermum ammi*) and allspice (*Pimenta dioica*) essential oils and blends of their constituents against three *Aspergillus* species. J Essent Oil Res 2016; 28:252-9.
23. Park IK, Kim J, Lee SG, Shin SC. Nematicidal activity of plant essential oils and components from Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*) and Litsea (*Litsea cubeba*) essential oils against pine wood nematode (*Bursaphelenchus Xylophilus*). J Nematol 2007; 39:275-9.
24. Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. Iran J Microbiol 2011; 3:194-200.
25. Debonne E, Van Bockstaele F, De Leyn I, Devlieghere F, Eeckhout M. Validation of in-vitro antifungal activity of thyme essential oil on *Aspergillus niger* and *Penicillium paneum* through application in par-baked wheat and sourdough bread. LWT - Food Scie Technol 2018; 87:368-78.
26. Wani SA, Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci 2018; 17:97-106.
27. Bahraminejad S. In vitro antifungal activity of 63 Iranian plant species against three different plant pathogenic fungi. Afri J Biotechnol 2011; 10:16193-201
28. Zaker M, Mosallanej H. Antifungal activity of some plant extracts on *Alternaria alternata*, the causal agent of Alternaria leaf spot of potato. Pak J Biol Sci 2010; 13:1023-9.
29. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. John Wiley and Sons, USA, Ames, Iowa, Blackwell Publishing, 2006, pp. 370.
30. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008; 3:163-75.
31. Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Dalla

Rizza M. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chil J Agr Res 2011; 71:231-9.
32. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181:1199–200.
33. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J-P, Pihlaja K, Kujala TS *et al.* Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999; 47:3954–62.
34. Aghamohammadi A, Azadbakht M, Hosseinimehr SJ. Quantification of thymol content in different extracts of *Zataria multiflora* by HPLC method. Pharm Biomed Res 2016; 2:8–13.
35. Zabka M, Pavela R, Slezakova L. Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. Ind Crop Prod 2009; 30:250-3.
36. Soković M, Vukojević J, Marin P, Brkić D, Vajs V, Van Griensven L. Chemical composition of essential oils of *Thymus* and *Mentha* Species and their antifungal activities. Molecules 2009; 14:238-49.
37. Shokri H, Sharifzadeh A, Ashrafi Tamai I. Anti- Candida zeylanoides activity of some Iranian plants used in traditional medicine. J Mycol Med 2012; 22:211-6.
38. Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. Ind Crop Prod 2013; 43:827–31.
39. Amamra S, Elena Cartea M, Elkheir Belhaddad O, Soengas P, Abderrahmane B, Kaabi I *et al*. Determination of total phenolics contents, antioxidant capacity of *Thymus vulgaris* extracts using electrochemical and spectrophotometric methods. Int J Electrochem Sci 2018; 3:7882–93.
40. Fachini-Queiroz FC, Kummer R, Estevão-Silva CF, Carvalho MD de B, Cunha JM, Grespan R *et al*. Effects of thymol and carvacrol, constituents of *Thymus vulgaris* L. essential oil, on the inflammatory response. Evid Based Complement Alternat Med 2012; 1–10.
41. Dubey S, Kashyap P. *Trachyspermum ammi*: A review on its multidimensional uses in Indian folklore medicines. Res J Med Plant 2015; 9:368–74.
42. Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF *et al*. Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chem 2016; 210:402–14.
43. Meeran MFN, Javed H, Al Taee H, Sheikh A, Ojha SK. Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development. Front Pharmacol 2017; 8:380.
44. Boskovic M, Zdravkovic N, Ivanovic J, Janjic J, Djordjevic J, Starcevic M *et al*. Antimicrobial activity of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against some foodborne microorganisms. Procedia Food Sci 2015; 5:18-21.
45. Sharifzadeh A, Khosravi AR, Shokri H, Sharafi G. Antifungal effect of *Trachyspermum ammi* against susceptible and fluconazole-resistant strains of *Candida albicans*. J Mycol Med 2015; 25:143-50.
46. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 2001; 91:453-62.
47. Singh G, Marimuthu P, Heluani CS de, Catalan C. Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. (Aril Part). J Food Sci 2005; 70:141-8.
48. Thomas JE, Basu SK, Acharya SN. Identification of *Trigonella* accessions which lack antimicrobial activity and are suitable for forage development. Can J Plant Sci 2006; 86:727-32.
49. Nahar N, Rahman MS, Mizanur Rahman S, Moniruzzaman M. GC-MS analysis and antibacterial activity of *Trigonella foenumgraecum* against bacterial pathogens. Free Rad Antioxidants 2016; 6:109-14.
50. Sheikhlar A, Alimon AR, Daud HM, Saad CR, Shanagi H. Screening of *Morus alba*, *Citrus limon* and *Trigonella foenum-graecum* extracts for antimicrobial properties and phytochemical compounds. J Biol Sci 2013; 13:386-92.
51. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S *et al.* Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014; 22:296-302.
52. Mostafaie A, Kahrizi D, Mohammadi M, Yari K, Rostami H, Yaghotipoor A *et al*. Effect of planting times and different levels of application of vermicompost on the proteomic pattern of fenugreek (*Trigonella foenum-graecum*). Cell Mol Biol 2018; 64:46-51.
53. Onofrei V, Benchennouf A, Jancheva M, Loupassaki S, Ouaret W, Burducea M *et al*. Ecological foliar fertilization effects on essential oil composition of sweet basil (*Ocimum basilicum* L.) cultivated in a field system. Sci Hortic 2018; 239:104–13.