



Investigating the Antifungal Properties of *Trachyspermum Ammi* Essential oil with the Approach of Application in Food Warehouses and Landscape

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ABSTRACT

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Solar The purpose of this study was to assess the antifungal activity in vitro of Ajwain essential oil through addition to the fungal growth medium of three pathogens (*Botrytis cinerea*, *Aspergillus niger* and *Penicillium digitatum*) isolated from grapes, tomato, and orange. In addition, the study aimed to determine its antifungal properties in vivo as a potential food preservative. Essential oil of *Trachyspermum ammi* was extracted using a Clevenger-type apparatus and quantified and identified by gas chromatography-mass spectroscopy and gas chromatography. Analysis of the total essential oil showed that thymol (60.50%), terpinene (15.65%) and p-cymene (12.51%) were the major constituents of this oil. The oil completely inhibited the growth of *A. niger*, *B. cinerea* and *P. digitatum* at 400–600 µl/l on the PDA medium. Exposure to Ajwain oil at 600 µl/l reduced fruit mold incidences by more than 90%. In conclusion, this study demonstrated that Ajwain oil was highly effective against post-harvest diseases, and supports its application in phytopathology for its antiseptic properties.



Introduction

Globally, fungi are the dominant contaminants of spices, but such microbial populations may be considered more commensal inhabitants on the plant during storage and drying [1; 2]. Postharvest diseases of plants are one of the greatest causes of the postharvest loss of horticultural fresh produce [3]. Regarding the fact that fungi are omnipresent plant pathogens, they are important corruption and contamination agents of the feedstuffs and foods. The spoilage of plants by various species of fungi leads to a diminution in product quantity and quality with significant economic damages [4].

Planting medicinal plants in green spaces and agricultural lands creates very beneficial effects on the surrounding environment, industry and economy of a society. In other words, the use of these plants in the green space has an important role in supplying wood, providing food, controlling air pollution, medicinal compounds, and bioherbicides, and offering aesthetic value. Medicinal plants used in green open spaces could serve the surrounding population by providing environmental services, one of which is the provision of herbal medicines through the vegetation they possess [5].

Essential oil from plants is one of the very useful groups of natural products for the development and production of a wide range of cheaper and safer antifungal agents [6; 7]. However the use of synthetic fungicides in plant pathology has made an effective contribution to protecting plants, and many are not usually used due to the economic, health or environmental concerns or the development of resistant varieties. Fungicides initially used for controlling postharvest diseases are newly considered to pose a potential cancerous risk [8]. Therefore, scientific communities worldwide are looking for safe alternative products from plants for effective management of diseases during storage. Usually occurring, biological compounds from plants are generally considered to be more acceptable and less dangerous than synthetic compounds and demonstrate a wealthy source of potential disease-control agents in the plant [9]. *Trachyspermum ammi* L. is a native of Egypt and is cultivated in Iraq, Afghanistan, Iran, India and Pakistan. This plant is a highly valued medicinally important seed spice, belonging to the family Apiaceae [10; 11]. Ajwain, with its fragrant smell and spicy taste, is extensively used as a condiment in curries. Its seeds are used for the production of essential oil in perfumery, medicine, and in small quantities for flavouring foods [12]. *T. ammi* has been demonstrated to possess nematicidal antimicrobial properties as well as the detoxification of aflatoxins [10; 13]. Thymol—the main phenolic compound present in *T. ammi* is an antispasmodic, antifungal and germicide agent [14; 15].

The main compounds present in the Ajwain (i.e. Cymene, thymol, terpinene, ocimene and sabinene) have been reported by several researchers [13; 16].

Antifungal and antibacterial activities of this plant against *Aspergillus flavus*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Curvularia lunata*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Candida albicans* are reported by other researchers [17-19]. Furthermore, the possibility of application of essential oils as botanical fungicides for the control of certain fruit postharvest spoilage fungi has been studied by several researchers [20-22].

This study is an attempt first, to find the chemical composition of *Trachyspermum ammi* as a medicinal plant in the surrounding environment and green space, and secondly, to find the antifungal effects of its essential oil against *Aspergillus niger*, *Botrytis cinerea* and *Pinicillium digitatum* as some important post-harvest fungal disease agents.

Material and method

Fungi

Aspergillus niger, *Botrytis cinerea* and *Pinicillium digitatum* were isolated from tomato, grape and orange, respectively. Isolates of fungi were detected according to descriptions by valid fungal identification keys and results [23-25]. For the preservation of fungi, the methodologies suggested by the Commonwealth Mycological Institute were used [26]. Code numbers A941, B941 and P942 represent *A. niger*, *B. cinerea* and *P. digitatum*, respectively.

Plant material

Ajwain Seeds (*T. ammi*) were collected from Lorestan, Iran in January 2017. The seeds were thoroughly washed and soon after dried at room temperature for 24 hours. Dried *T. ammi* seeds were ground and powdered with the help of a pounder and pestle. These seeds were used for extracting the essential oil.

Essential oil

Dried *T. ammi* seeds were subjected to hydrodistillation in a conventional glass Clevenger-type apparatus at 80°C for 4 h according to the European Pharmacopoeia [27]. One glass bulb had one capacity. Ajwain essential oil was dried over anhydrous sodium sulphate and was stored in a dark glass bottle at 4°C until experimental use.

Analysis conditions

A gas chromatography-mass spectrometer (GC/MS-QP5050) was used to detect *T. ammi* oil for the crude extraction of methanol and hexane. Before analysis, the extracts were filtered using a PTFE filter membrane. The crude extract was analyzed by Shimuzu gas chromatography (GC-17A) system

equipped with Flame Ionization Detection (FID). BP-X5 capillary column 5% phenyl-95% methylpolysiloxan (30 m × 0.25 mm id), 0.25 µm film thickness was used. Helium gas was used as a carrier with a flow rate of 81.6 mL min⁻¹ at 33 kPa. The injector and detector temperatures were set and maintained at 280°C and 260 °C, respectively. Injection was performed in splitless mode with a volume of 1 µL. The oven temperature of GC was programmed with a run time of 51.67 min. The temperature of the oven of GC/MS was programmed as follows:

- 40°C (1 min hold) to 100°C at the rate 20°C min⁻¹.
- Increased to 220°C at the rate 3°C min⁻¹.
- Increased to 280°C at the rate 30°C min⁻¹.
- It was kept for another 5 min at 280°C

Injection temperature was maintained at 280°C, interface temperature at 260°C, quadruple temperature at 100°C and ion source temperature at 200°C. Injection was performed in splitless mode and the volume used was 1.00 µL. The mass spectra of the compound sample were acquired by Electron Ionization (EI) at 69 eV, and the detector operated in scan mode from 35 to 400 amu. Compounds were recognized by direct comparison of their chromatogram retention time with those of standard compounds. In addition, the identification of *T. ammi* oil was performed by the NIST software library.

Antifungal effects of essential oil on mycelial growth

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Antifungal effect of essential oil on mycelial growth

Potato-Dextrose-Agar (PDA) medium was used to investigate the antifungal properties of the Ajwan essential oil against *P. digitatum*, *B. cinerea* and *A. niger*.

The antifungal activity of Ajwain essential oil against these fungi was undertaken using the inverted petri plates technique [28]. The petri plates cultivated with respective test fungi were inverted upside down and a pre-sterilized filter paper disc, Whatman no.1, 10 mm, d, was placed on the center of its inverted lid. The required doses of the undiluted sample (Ajwain oil) were soaked on paper discs. In the control treatment, the essential Ajwain oil was superseded with an equal amount of distilled water. Radial growths of fungi in the period of average diameter (mm) were recorded. During the entire experiment, complete sterile conditions were conserved. Petri plates were then incubated at 27 °C. This experiment was conducted in a completely randomized design (CRD) with four replications and eight treatments including 0, 50, 100, 200, 400, 600, 800, and 1000 µl/l of oil for each fungus. The mycelial growth

inhibition percentage was calculated according to the following Deans and Svoboda formula [29]:

$$I_r = \frac{T_c - T_t}{T_c} \times 100 \quad (1)$$

Where I_r =percentage mycelial inhibition, T_c =average colony diameter of control sets, and T_t =average colony diameter of treatment sets.

Antifungal effect of essential oil on healthy fruits

Tomato, strawberry, and orange fruits were obtained from the mercantile market. The fruits were selected in a way that was free of any mechanical damage, stain, decay, and fungal infection and were the same size. The fruit surfaces were sterilized with 2% sodium hypochlorite for 3 minutes and followed by washing with distilled water. Conidia of *A. niger*, *B. cinerea* and *P. digitatum* were recovered from 4, 5, and 7-day-old cultures respectively by adding 10 ml of sterile distilled water to each petri plate. The suspension was filtered through four layers of sterile cheesecloth. The concentration of each suspension was adjusted to 2×10^6 conidia per ml, and a drop of Tween 20 was added to the suspension. The concentration of the Ajwain oils, including 200, 400, and, 600 $\mu\text{l/l}$ was provided by dissolving the requisite amounts in 25 ml of 0.05% Tween 20 and then mixing with 475 ml of sterile distilled water. The control sets were prepared similarly using equal amounts of sterilized distilled water in place of the Ajwain oil. On each fruit tissue, 3-4 scratches were made to a depth of 0.5 mm and a length of 2 cm. The wounded fruits were immersed into the oil solution in various concentrations for 2 min at room temperature and air dried. The control set fruits were dipped into sterile distilled water. Both essential oil-treated and untreated sets were subjected to conidial suspension. The uninfected control set was excepted from adding any conidial suspension and stored in closed sterile containers at room temperature for 15 days [28]. This portion of the experiment was conducted in a completely randomized design (CRD) with four replications and three treatments including 200, 400, and 600 $\mu\text{l/l}$ of oil for each fruit and also infected and uninfected control sets. The entire experiment was repeated twice. The percentage of infected fruits was then recorded.

Result and discussion

The results of the Gas chromatography/mass analysis of Ajwain oil are presented in Figure 1 and Table 1. The oil was revealed to contain an admixture of components, mainly thymol and several other components in minor percentages. Five components such as thymol, terpinene, p-cymene, carene and

terpinyl acetate were recognized, which represented 98.79% of the total essential oils. As indicated in Table 1, thymol (60.5%), terpinene (15.65%), p-cymene (12.51%), careen (1.65%) and terpinyl acetate (0.93%) were recognized as the main components of the Ajwain essential oil (Table and Figure 1). Similarly, a previous study demonstrated that thymol and terpinene are the major components of *T. ammi* essential oil [13; 14; 30; 31]. Other studies have reported that thymol is a major component of this oil [31-33]. In the Mahboubi and Kazempour [17] and Khajeh *et al.* [27] studies, γ -terpinene was the second most abundant component of Ajwain oil. Terpinene was identified as the second most common compound in Ajwain oil in the present study.

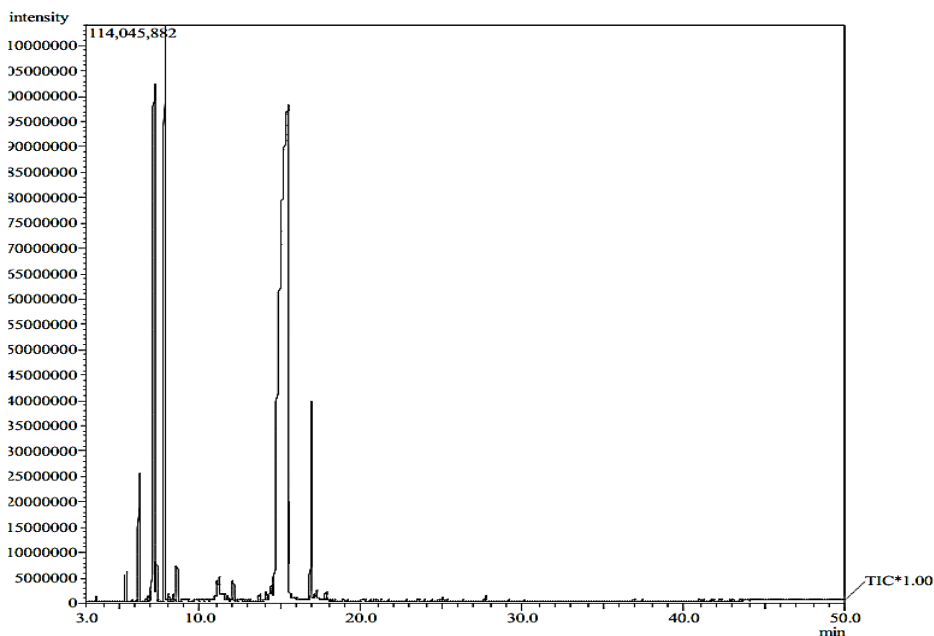


Figure 1. GS/MS analyses of *T.ammi* essential oil.

Table 1. Chemical compositions of *Trachyspermum ammi* essential oil.

Compound name	Similarity	RI(STD)	RI(Calc)	t'R(A)	Area%
Hexanal	95%	800	808.13	3.82	0.01
Thujene (alpha)	96%	931	932.00	5.28	0.21
Camphene	94%	953	963.33	5.75	0.24
Sabinene	90%	976	984.00	6.05	0.01
Carene	95%	1011	1020.05	6.72	1.65
Terpinene (alpha)	95%	1018	1027.40	6.87	0.07
Gamma terpinene	86%	1046	1046.00	7.27	15.65
P-cymene	84%	1095	1085.00	8.08	12.51
Terpinolene	93%	1088	1099.76	8.38	0.08

Compound name	Similarity	RI(STD)	RI(Calc)	t'R(A)	Area%
Linalool acetate	93%	1275	1273.64	13.68	0.03
Thymol	93%	1309	1324.00	15.50	60.50
Terpinyl acetate (alpha)	95%	1350	1362.01	16.92	0.93
Eugenol	93%	1365	1370.45	17.24	0.08
Geranyl acetate	93%	1383	1384.96	17.79	0.24
Eugenyl acetate	88%	1524	1529.01	23.44	0.11
Nerolidol	93%	1564	1568.96	25.01	0.04

Essential oil of *T. ammi* was tested against three important food spoilage fungi including *A. niger*, *B. cinerea* and *P. digitatum* by measuring the diameter of the fungi colony in a PDA medium. The final results of the antifungal activity of essential oil on the growth of fungi species are presented in Table 2 and Figure 2. As can be observed, the essential oil in this plant showed inhibitory effects on the growth of fungi at all tested concentrations compared with the controls ($P < 0.01$). The inhibition of growth was found to be 29.06, 66.88, 92.19, 99.06 and 100% for *A. niger*, 29.06, 67.50, 88.13, 99.69 and 100% for *P. digitatum* and 20.94, 59.38, 82.81, 98.13 and 100% for *B. cinerea* at the essential oil concentrations of 50, 100, 200, 400 and 600 $\mu\text{l/l}$, respectively.

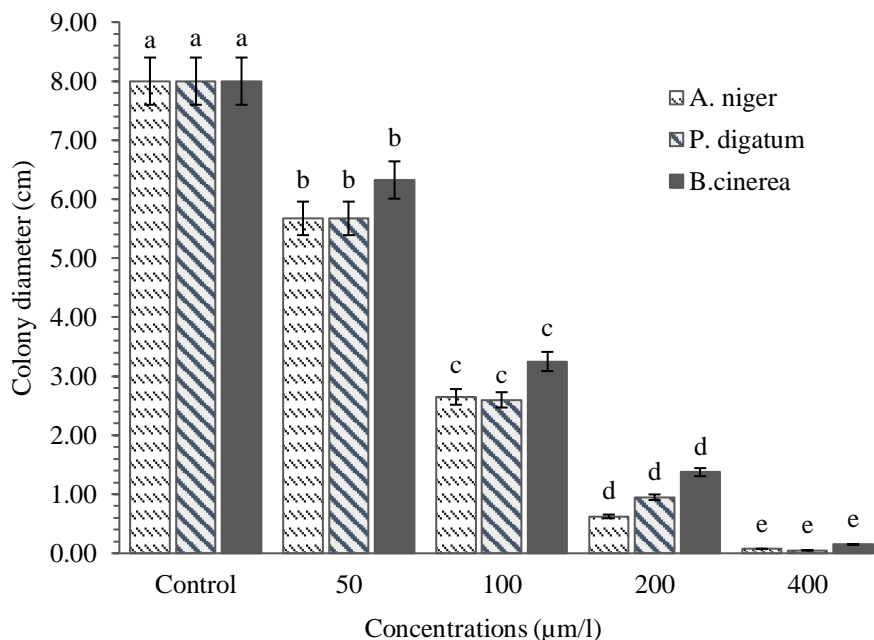


Figure 2. The effect of Ajwain oil in inhibition of growth fungi on the PDA medium.

The effect of *T. ammi* oil on the control of *A. niger*, *B. cinerea* and *P. digitatum* growth in inoculated tomatoes, strawberries and oranges were evaluated,

respectively. Ajwain oil volatiles was highly effective in reducing mold incidences in fruits caused by *A. niger*, *B. cinerea* and *P. digitatum* (Figure 3).



Figure 3. The effect of *T. ammi* oil on reducing fruit molds.

The results shown in Figure 4 demonstrate that when unwounded fruits were treated with Ajwain oil, all concentrations significantly inhibited *A. niger*, *B. cinerea* and *P. digitatum* on tomatoes, strawberries and oranges ($p < 0.01$). Inhibition of mold growth in fruits subjected to 200, 400 and 600 $\mu\text{l/l}$ of Ajwain oil was significantly higher ($P < 0.01$) compared to the infected controls. Exposure to the essential oil of Ajwain at 600 $\mu\text{l/l}$ reduced mold incidences by more than 90% after 2 weeks of storage. The percentages of decay in fruits compared to their control, infected fruits without essential oil, were found to be 41.67, 75 and 91.67% for *B. cinerea*, 53.33, 81.25 and 95.83% for *A. niger* and 50, 72.92 and 93.75% for *P. digitatum* at the essential oil concentration levels of 200, 400 and 600 $\mu\text{l/l}$, respectively (Figure 4).

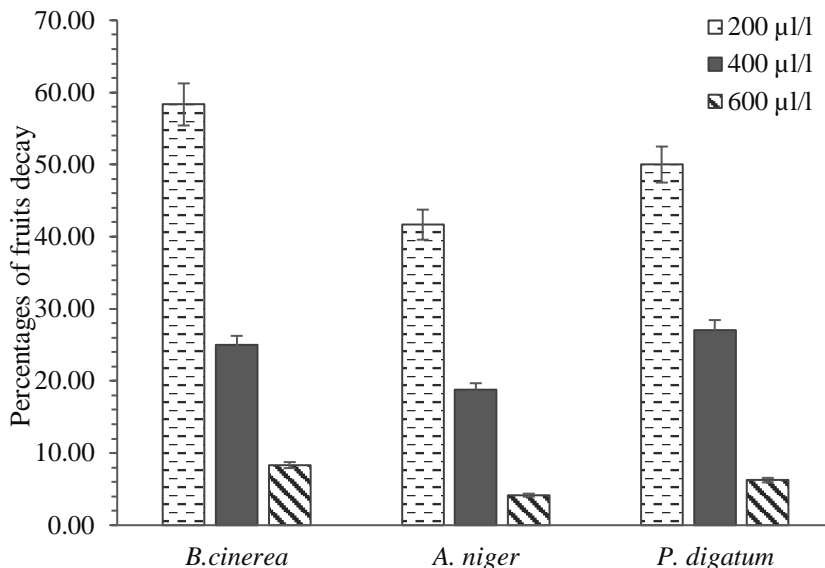


Figure 4. The effect of Ajwain oil on reducing fruit decay.

In the present study, a low concentration range of *T. ammi* essential oil—100 to 400 µl/l—showed fungistatic activities against *A. niger*, *B. cinerea* and *P. digitatum*. This is in agreement with other studies which reported this essential oil has anti-fungal activities against *A. parasiticus*, *A. niger* and *A. fumigatus* [17; 34], and *Penicillium citrinum* and *Penicillium chrysogenum* [14; 35]. Some studies have reported that Ajwain is among the most potent medicinal plants due to its antimicrobial properties against fungi and bacteria [36; 37]. It has previously been reported that the essential oil of *T. ammi* displays antifungal effects as well as antibacterial activity [33; 38]. In addition, other research has stated the inhibitory effects of *T. ammi* extract on anti-toxigenic, spore germination and mycelial growth of fungi *Aspergillus ochraceus* [39]. It is believed that the inhibitory effect of Ajwain oil on fungi is the outcome of their indirect effects on mycelium fungi [14].

Analysis of the total essential oil of *T. ammi* in the study showed that thymol (60.50%), terpinene (15.65%) and p-cymene (12.51%) were the major constituents of this oil. The essential oil of *T. ammi* exhibited antifungal activities which might be attributed to the presence of thymol, γ -terpinene and p-cymene. In several previous studies, these compounds have been demonstrated to possess potent antifungal effects [34; 40-42]. Other research has reported that γ -terpinene and p-cymene do not possess any antimicrobial activity [43-45]. The p-cymene does not have antifungal activity but it increases the antifungal activity of thymol in *T. ammi* oil [17; 46].

Thymol in Ajwain oil is a phenolic compound that plays an important role in antimicrobial activities [17]. The antimicrobial effect of thymol is due to damage in membrane entirety cells with change in pH and equilibrium of inorganic ions [46; 47]. Similarly, a previous study has demonstrated that thymol and Trpinene are the major components of essential oils of *T. ammi* [13; 48]. Other studies have reported that thymol is a major component of this oil [19; 32]. Furthermore, it has been demonstrated that thymol has a remarkably antifungal potential against *Aspergillus niger*, *Alternaria alternate*, *Botrytis cinerea*, *Penicillium chrysogenum*, *Fusarium oxysporum* and *Rhizopus oryzae* [49-51].

Conclusion

In conclusion, the present research has demonstrated that Ajwain oil is highly effective against post-harvest diseases which supports its use in phytopathology for its antiseptic properties. The outcomes of this study showed the use of the essential oil of *T. ammi* as a significant potential antifungal during the transport or storage period against *A. niger*, *B. cinerea* and *P. digitatum*. However, the use of essential oils can not only ameliorate food safety by its antimicrobial activity against post-harvest fungi. Some supplementary experiments are required for organoleptic characteristics such as fruit colour, firmness, aroma, and smell and to standardize the safety limit of the *T. ammi* essential oil (Figure 5).

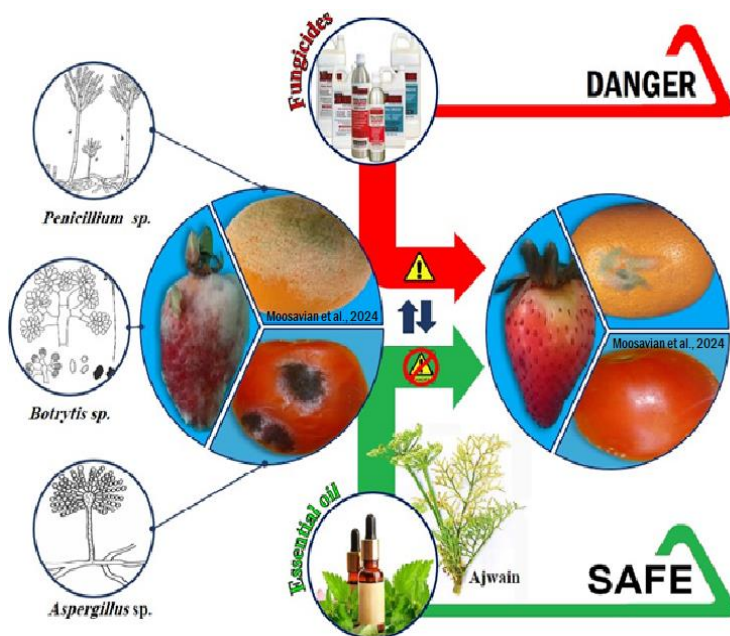


Figure 5. The result of using *T. ammi* essential oil in controlling pathogenic fungi compared to the use of fungicides.

Disclosure statement and funding

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