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Antibacterial activity of essential oils from *Thymus vulgaris, Trachyspermum ammi* and *Mentha aquatica* against *Erwinia carotovora in vitro*

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ABSTRACT

Background & Aim: The aim of this study was to assess the bactericidal effect of three medicinal plant species, including *Thymus vulgaris*, *Trachyspermum ammi*, and *Mentha aquatica* on *Erwinia carotovora* growth.
Experimental: This research was done in a factorial to completely randomized design with three concentrations, and three replicates on nutrient agar culture medium.
Results &Discussion: Results of this study indicated that the essential oils from the studied plants were effective against *Erwinia carotovora*. At 300 ppm concentration, diameter of growth inhabitation was 43 mm. Results indicated the essential oil from *T. ammi*, especially at 300 and 400 ppm concentrations had the highest antibacterial activity against *Erwinia carotovora* with 43.00 and 46.67 mm diameter of growth inhabitation. In addition, there was no significant difference between 300 and 400 ppm concentrations of *M. aquatica* and at concentrations of 200, 300, and 400 ppm for *T. vulgaris*.
Recommended applications/industries: The overall results indicated the essential oils, especially *Trachyspermum ammi* have antibacterial activity against *Erwinia carotovora*; it seems that the essential oils can be employed in manufacturing desirable bactericidal agents.

1. Introduction

In recently years, due to increased interest of the public in consuming natural substances also prevalence of digestive and respiratory diseases and types of cancer, extensive investigations have been performed to use essential oils. Essential oils, also called ethereal or volatile oils, are in fact aromatic oily liquids obtained of plants. Phenolic compounds are mainly responsible for exhibiting bactericidal properties of essential oils. Essential oils, in addition to exhibiting bactericidal properties also show anti-parasite characteristics, a property related to the type of active ingredient of the essential oil (Burt, 2004).

Trachyspermum ammi is an herbaceous annual from the Apiaceae (Umbelliferae) family also known as *Carum copticum*. Medicinal part of this plant is the fruit that containing 2% essential oil. Its fruit essential oil called Ajowan is yellow-colored with a thymol scent (Amin, 1993). Analysis of the Persian anise

reveals such ingredients as thymol, *p*-cymene, β-pinene, γ-terpinene, and sabinene (Mirzavand, 1991).

Thyme is one of the oldest medicinal and condiment plants officially introduced from 16th century as a medicinal plant. This woody perennial belongs to the family Lamiaceae. The genus *Thymus* includes numerous species being very different from the viewpoint of their chemical type, quality and quantity of active substances. Thyme essential oil is a yellow liquid, lighter than water, aromatic, with a strong, acrid taste exhibiting bactericidal and fungicidal properties. Chemical analysis of essential oil from thyme reveals existence of various phenolic compounds, monoterpene hydrocarbons and alcohols however, its bactericidal properties is mostly attributed to thymol and carvacrol both being phenolic compounds with bactericidal properties which at the same time are irritant and caustic (Ghasemi Pirbalouti, 2009).

Mentha aquatica is a medicinal plant belonging to the family Lamiaceae. Plants of this family include about 160 genera and over 3000 species grown almost all over the world especially in the Mediterranean region. Menthol in essential oil has antibacterial activity (Omid-Beiguy, 2009).

Erwinia carotovora (Sym. *Pectobacterium carotovorum*) is a plant pathogen belonging to the Family Enterobacteriaceae. It is a Gram- negative, rod-shaped bacterium (Barras *et al*., 1994). The species is a plant pathogen with a diverse host range, including many agriculturally and scientifically important plant species. It produces pectolytic enzymes that hydrolyze pectin between individual plant cells. This bacterium is a ubiquitous plant pathogen with a wide host range (carrot, potato, tomato, leafy greens, squash and other cucurbits, onion, etc.), able to cause disease in almost any plant tissue it invades. It is a very economically important pathogen in terms of postharvest losses, and a common cause of decay in stored fruits and vegetables (Toth *et al*., 2003).

In Iran, in spite of frequency of natural resources, not much attention has been paid to medicinal plants. Therefore, paying scientific attention to this subject is crucial. In this study, antibacterial activity of the essential oils from *Trachyspermum ammi*, *Thymus vulgaris*, and *Mentha aquatica* against growth of the bacterium *Erwinia carotovora* was investigated.

2. Materials and Methods

2.1. Plant materials

In this study, essential oils of three medicinal plants, including *Trachyspermum ammi*, *Thymus vulgaris*, and *Mentha aquatica* (growing at Zagros mountains, Iran) prepared from Atran Daru Agro-Industry Co., Isfahan, Iran.

2.2. Antibacterial test

Gram-negative *Erwinia carotovora* bacterium (PTCC: 1675) was obtained from the Iranian organization of Scientific and Industrial Research, Biotechnology Research Institute. To study the antibacterial properties of essential oils, the pitted-plate method was used. To do so, the nutrient agar (NA) culture medium was prepared and via carbibauer procedure, the intended bacterium was cultured on it in solid form. Before culture, to assess the level of microorganisms via carbibauer procedure, standard microbial suspension with 0.5 Mc-Farland was prepared in which suspension there are about 1.5×10^8 CFU/ml bacteria. Then the above said suspension was diluted to reach the 1.5×10^6 CFU/ml density. Then 5 µl of the above said solution equal to 10^4 microorganisms was with-drown and transferred to the plates and employing sterile swab was solid or broom cultured. Then using the sterile Pasteur pipette bottom, pits were made in the culture medium and then the bottom of each pit was closed using a drop of molten NA culture medium. Then, 100, 200, 300, and 400 ppm of each essential oil were added to each of the pits. Plates were refrigerated for 1-2 h and then incubated for 16-20 h at 27 ºC. As a result around some of the pits no-growth halos were formed resulting from diffusion of essential oils from the pits to the culture medium preventing growth of bacteria sensitive to such substances. The diameter of growth inhabitation was measured employing calipers and the best concentration of each concentration was determined.

2.3. Statistical analyses

The data was statistically analyzed by SPSS (19.0) software, using a completely randomized design. Means of the traits were separated by Duncan's multiple range test at $p \leq 0.05$ level.

3. Results and discussion

Results indicated that species, concentrations, and interaction of essential oils × species were significant ($p \leq 0.05$) against *Erwinia carotovora* bacterium. The essential oil from the studied plants against *Erwinia* was effective at 300 and 400 ppm concentrations with diameter of growth inhibition 43.00 and 43.67 mm, respectively.

Results this study indicated the essential oil from *T. ammi*, especially at 300 and 400 ppm concentrations had the highest antibacterial activity against *Erwinia carotovora* with 43.00 and 46.67 mm diameter of growth inhabitation (Table 2). In addition, there was no significant difference was seen between the concentrations of the essential oil from *T. ammi* at 300 and 400 ppm concentrations. Compared with other plants at different concentrations and the controls however, there was a significant difference (Table 2). Iacobellis et al. (2005) reported the antibacterial activity of the essential oil from anise by agar diffusion method against different bacteria (*Rhodotorula*, *Erwinia*, *Xanthomonas*, and *Agrobacterium*).

Table 1. Variance analysis of essential oils at different concentrations against *E. carotovora*

Source variation	d.f	DI (mm)
Species	2	1457.22**
Concentration	4	1306.81**
Concentration × Species	8	143.02**
Error	30	5.28
CV	-	11.86

Table 2. Comparison means of antibacterial activity of essential oils (EOs) at various concentrations against *E. carotovora*

DI (mm)			Concentrations of EO (ppm)
T. vulgaris	*M. aquatica*	*T. ammi*	
0^{f}	0^{f}	0^{f}	0
22.67^{c}	6.33^{e}	27.33^{b}	100
13.33^{d}	13.67^{d}	31.00^{b}	200
26.33^{bc}	14.33^{d}	43.00^{a}	300
30.67^{b}	15.33^{d}	46.67^{a}	400

The essential oil from *M. aquatica* at 300 and 400 ppm concentrations had 14.33 and 15.33 mm diameters of growth inhabitation against *Erwinia carotovora*, respectively. No significant difference ($p \leq 0.05$) was seen between these concentrations, however, the best treatment was found at 300 ppm concentration (Table 2). Results a study by Preuss et al. (2005) indicated the inhibitory and lethal effect of mountain mint and some other essential oils against *Escherichia coli*, *Helicobacter pylori*, *Anthracis* and *Mycobacterium* of leek. The essential oil from mountain mint revealed, except on *Anthracis bacillus*, a lathed effect on other tested microorganisms.

The essential oil from *T. vulgaris* at 300 and 400 ppm concentrations had antibacterial activity with 26.33 and 30.67 mm diameter of growth inhabitation against studied bacteria, respectively. Results a study by Karami et al. (2010) indicted the essential oils from thyme and savory having carvacrol and thymol, as the main constituents, at 0.05 M concentration in the culture medium had 5.6 to 7 mm diameter of growth inhabitation against *Erwinia*.

Phenolic compounds present in essential oils exhibited the most bactericidal properties. These compounds both diffuse in the cell membrane and also can play a role in clotting of cellular contents (Dorman & Dianne, 2000). For instance, thymol and cravacrol as the main constituents in the essential oils from *Trachyspermum ammi* and *Thymus vulgaris* have antimicrobial activity (Nostro *et al*., 2000).

4. Conclusion

According to the findings of this study, the essential oil *T. ammi* and *T. vulgaris* had antibacterial activity against *Erwinia carotovora*. The present study suggests that the essential oil of *T. ammi* is a potential source of natural antibacterial agents. After this screening experiment, further work should be performed to describe the antibacterial activities in more detail as *in vivo*.

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