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Biocontrol Efficiency of Medicinal Plants Against *Pectobacterium Carotovorum*, *Ralstonia Solanacearum* and *Escherichia Coli*

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Abstract: The spread of antibiotic resistant pathogens is one of the most serious menaces to successful treatment of microbial diseases. Medicinal and aromatic plants are widely used as traditional medicines and constitute a major source of natural organic compounds. In this research essential oils of *Coriandrum sativum*, *Thymus vulgaris, Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* were evaluated for their antibacterial activities, against *Pectobacterium carotovorum*, *Ralstonia solanacearum* and *Escherichia coli*. The essential oils were used at different concentrations 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 75 or 100 % (v/v). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by two-fold broth dilution method for the tested pathogens and the zone of inhibition was determined by agar disk diffusion method. Means were compared using Duncan's Multiple Range Test at the 1% level of significance by MSTATC software. Results showed that the most active essential oils against tested bacteria was thyme oil with the inhibition zone of 34.8 mm against *R. solanacearum* and the MIC of 1 µl/ml while this value was higher than Streptomycin and Erythromycin inhibition used as positive control. Essential oils of *Coriandrum sativum*, *Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* were in the next positions. The efficacy of essential oils from *E. globulus* was insignificant.

Keywords: Antibacterial activity, Essential oil, Pectobacterium carotovorum, Ralstonia solanacearum.

INTRODUCTION

Antimicrobial effects of essential oils (EOs) have gained research interests in the recent years. There are increases in the demand of safe foods and organic crops. Harmful effects of various chemical preservatives and antimicrobial agents are being recognized to cause some kind of allergic reactions, poisoning, cancer, and chronic diseases. EOs are biologically active compounds, which have shown antimicrobial activity against a broad range of microorganisms in research trials. For examples several studies have tested the antifungal and antibacterial effects of essential oils on Gram positive and negative bacteria [1, 2]. EOs are also used in cosmetics, sanitary, food, antiseptic compounds and insecticide. Traditionally these oils have been used to treat infections and diseases all over the world [30]. Phenols compounds, extracted from EOs, have demonstrated potential to be used as alternatives for food additives without risks of chemical compounds [2].

Recently, numerous studies on antimicrobial activities of EOs and plant extracts have been published including those related to the genus rosemary [3-7]; thyme [7-9]; cumin [3, 10]; eucalyptus [11-13] and coriander [15, 19]. Nychas [26] reported antimicrobial affects of thyme, rosemary and coriander on fungi and bacteria [15]. Many studies have been specified the bactericidal properties of thyme and demonstrated that it is effective against a wide range of bacteria [7, 14, 16]. Gachkar *et al.* [3] reported that EOs from hydrodistillation *Rosmarinus officinalis* and *Cuminum cyminum* have bactericidal effects on *E. coli*, *Staphylococcus aureus* and *Listeria. monocytogenes* [3].

It is also known that antimicrobial effects or biological activities of natural EOs may vary in the chemical composition depending on their origin, locality, environmental conditions, and the stage of development of the collected plant material [17].

Plant extracts can inhibit different human pathogenic bacteria and fungi, however, reports on phytopathogenic bacteria are fewer [16]. *R. solanacearum* and *P. carotovorum* infect tomatoes and potatoes and damage the crops. As healthy agricultural crops are very important to produce safe food, it seems necessary to study the prevention of potatoes and tomatoes infections from these diseases without using chemical agents. In this research the antibacterial activity of EOs extracted from *Coriandrum sativum*, *Thymus sativum, Cuminum cyminum* and *Rosmarinus officinalis* against *P. carotovorum, R. solanacearum* and *E. coli* was evaluated.

2. MATERIALS AND METHODS

2.1. Plant Materials

The aerial parts (leaf) of *Thymus vulgaris*, *Rosmarinus officinalis* and *Eucalytptus globulus* were collected from Agricultural Institute of Zabol University in October 2010. Seeds of *Cuminum cyminum* and *Coriandrum sativum* were

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collected from Khorasan Science and Technology Park, Mashad, Iran in January 2011. All plant materials/sections were dried under shade at room temperature.

2.2. Essential oil Extraction

The essential oil of all air-dried samples (100 g) was isolated by hydro-distillation for 3 h using a Clevenger -type apparatus according to the description of European Pharmacopoeia (European Pharmacopoeia, 2002). The distillated oils were dried over anhydrous sodium sulfate (Na_2SO_4) and stored in tightly closed dark vials at 4 ºC.

2.3. Bacterial Strains

The bacterial pathogens of *P. carotovorum* subsp. *carotovorum* (Pcc) and *R. solanacearum* (race 3, biovar 2) were provided by the Department of Plant Pathology, Isfahan University of Technology, Iran. *E. coli* (PTCC1330) was obtained from the Persian Type Culture Collection (PTCC). All the bacteria were maintained in Nutrient Broth (NB) mixed with 30% glycerol and stored at -70 ºC.

2.4. Antimicrobial Activity Assays (*In Vitro*)

2.4.1. Disc Diffusion Method

Standard agar disc diffusion method was used for antibacterial assays [18]. Sterile 9 cm petri plates were prepared by pouring 20 ml of medium and allowed to solidify. The plates were dried and 0.1 ml of standardized inoculums containing 10^8 CFU/ml of bacterial suspension was poured and uniformly spread, and the inoculums was allowed to dry for 5 min. A Sartorius No.388 sterile filter paper disc (6 mm diameter) was impregnated with 10 µl/disc of leaf essential oil which was allowed to dry in an open sterile petri dish in a biological safety cabinet with vertical laminar flow. Different concentrations, 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 75 and 100% solutions, of the essential oils in absolute ethanol were applied to the discs. Negative control was prepared using the same solvent (absolute ethanol). Standard reference antibiotics, streptomycin (10 µg/disc) and erythromycin (15 µg/disc) were used as positive controls for the tested bacteria. The plates were incubated at 28 ºC for 24 h. Antibacterial activities were evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Each assay in this experiment was performed in triplicates.

2.4.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were assessed according to the modified procedure of Kivanc and Akgul [19].

MIC was determined by a two-fold broth dilution method in test tubes as follows. Bacterial strains were cultured in NB overnight at 28 °C. A 5 ml volume of 10^8 CFU/ml microbial suspensions was incubated in a series of tubes containing 50 µl of decreasing concentrations of the oil (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 75 and 100%). The tubes were incubated at 28 ºC for 24 h. The highest dilution (the lowest concentration) with no visible growth was regarded as MIC. Cells from the tubes showing no growth were subcultured on agar plates to determine whether the inhibition was reversible or permanent. MBC was the highest dilution (the lowest concentration) at which no growth occurred on the plates after 24 h.

2.5. Statistical Analysis

Data was statistically analyzed using Analysis of Variance (ANOVA). Duncan's multiple range test (MSTATC software) was used to determine the differences among the means at $P<0.01$.

RESULTS

Antimicrobial Activity

Table **1** summarizes the results of antimicrobial activity assay by the disc diffusion method. All the EOs exhibited antibacterial characteristics; however, different kinds and concentrations showed variation in the extent of activity. Presence or absence of inhibition zone and their values are given in the Table **1**. Among the EOs tested, thyme showed the maximum inhibition with a zone of 34.8 mm against *R. solanacearum* and a zone of 30 mm against *E. coli*. The zone size was 16.5 mm against *P. carotovorum*. Antibacterial effect was enhanced by increasing the oil concentration. The highest antibacterial activity was observed when pure oils used i.e. 100% concentration. The concentrations of 0.01% and 0.05% had no effects on microbial growth. *R. solanacearum* was the most sensitive bacteria to EOs presence, followed by *E. coli* and *P. carotovorum*. Eucalyptus oil exhibited a weak antibacterial activity against all of the three bacteria and in this case the concentration of 75% or lower had no deterrent effect on bacterial viability. Control discs containing absolute ethanol (zero concentration, negative control) did not show any antibacterial activity. All three bacteria were sensitive to the antibiotic streptomycin (*R. solanacearum* 22 mm; *E. coli* 16 mm; and *P. carotovorum* 8.5 mm). *E. coli* and *P. carotovorum* showed resistance to erythromycin. Thyme essential oil was stronger than streptomycin in antimicrobial action.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Table **2** shows MIC and MBC values of the EOs. Based on the results given in Table **2**, the EOs displayed remarkable antibacterial activities against the tested strains with MIC and MBC values ranging from 1 µl/ml for T. vulgaris to 1000 µl/ml for E. globulus. Thyme had lowest MIC with 1, 5 and 5 µl/ml on *R. solanacearum*, *P. carotovorum* and *E. coli* respectively. However, E. globulus oil exhibited a lower antibacterial activity against P. carotovorum (750 µl/ml) and R. solanacearum (1000 µl/ml), and no effect on E. coli at any concentrations (Table **2**) when compared to other oils.

DISCUSSION

EOs are potential source of exquisite antimicrobial compounds, especially, those possess antimicrobial properties against bacterial pathogens [20]. Plant EOs and extracts are in use for thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is useful to investigate the characteristics of these plants and their EOs scientifically to improve the quality of healthcare [21]. Our study showed that the EOs

Table 1. Antimicrobial Activity of the Essential Oils Using Disc Diffusion Method

EOs	Zone of Inhibition (mm)		Concentration (%)	
Coriandrum Sativum	*R. Solanaceaum*	*P. Carotovorum*		*E. Coli*
Coriandrum sativum	- ^{e}	- ^{e}	0	- ^{e}
Coriandrum sativum	- ^{e}	- ^{e}	0.01	- ^{e}
Coriandrum sativum	- ^{e}	- ^{e}	0.05	- ^{e}
Coriandrum sativum	- ^{e}	- ^{e}	0.1	- ^{e}
Coriandrum sativum	- ^{e}	- ^{e}	0.5	- ^{e}
Coriandrum sativum	- ^{e}	- ^{e}	1	- ^{e}
Coriandrum sativum	- ^{e}	8 ^{XYZ}	5	- ^{e}
Coriandrum sativum	- ^{e}	9.1 ^{TU}	10	- ^{e}
Coriandrum sativum	6.8 ^{cd}	10 ^{QR}	25	- ^{e}
Coriandrum sativum	7.1 ^{bc}	10 ^{QR}	50	8 ^{XYZ}
Coriandrum sativum	7.8 ^{YZa}	10.6 ^{NO}	75	8.8 ^{UV}
Coriandrum sativum	9.1 ^{TU}	11.1 ^{M}	100	12 ^{KL}
Cuminum cyminum	- ^{e}	- ^{e}	0	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	0.01	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	0.05	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	0.1	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	0.5	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	1	- ^{e}
Cuminum cyminum	- ^{e}	- ^{e}	5	- ^{e}
Cuminum cyminum	6.5 ^{d}	6.6 ^{d}	10	- ^{e}
Cuminum cyminum	7.1 ^{bc}	7.1 ^{bc}	25	- ^{e}
Cuminum cyminum	8 ^{XYZ}	7.1 ^{bc}	50	9.1 ^{TU}
Cuminum cyminum	9 ^{U}	7.5 ^{ab}	75	10.1 ^{PQ}
Cuminum cyminum	9.6 ^{RS}	7.5 ^{ab}	100	11 ^{MN}
Thymus vulgaris	- ^{e}	- ^{e}	0	- ^{e}
Thymus vulgaris	- ^{e}	- ^{e}	0.01	- ^{e}
Thymus vulgaris	- ^{e}	- ^{e}	0.05	- ^{e}
Thymus vulgaris	6.5 ^{d}	- ^{e}	0.1	- ^{e}
Thymus vulgaris	7.8 ^{YZa}	7.1 ^{bc}	0.5	8.3 ^{WX}
Thymus vulgaris	8.5 ^{VW}	7.6 ^{Za}	1	9.6 ^{RS}
Thymus vulgaris	10.5 ^{OP}	9.5 ST	5	12.3 ^{K}
Thymus vulgaris	11.8 ^{L}	10.5 ^{OP}	10	13.1 ^{J}
Thymus vulgaris	13.1 ^{J}	14 ^{I}	25	23.6 ^{E}
Thymus vulgaris	22.8 ^{F}	15.6 ^{H}	50	25.3 ^{D}
Thymus vulgaris	29.6 ^{B}	16 ^{H}	75	27.8 ^{C}
Thymus vulgaris	34.8 ^{A}	16.5 ^{G}	100	30 ^{B}
Rosmarinus officinalis	- ^{e}	- ^{e}	0	- ^{e}
Rosmarinus officinalis	- ^{e}	- ^{e}	0.01	- ^{e}
Rosmarinus officinalis	- ^{e}	- ^{e}	0.05	- ^{e}
Rosmarinus officinalis	- ^{e}	- ^{e}	0.1	- ^{e}
Rosmarinus officinalis	- ^{e}	- ^{e}	0.5	- ^{e}

Table 1. contd…

EOs	Zone of Inhibition (mm)		Concentration (%)	
Coriandrum Sativum	***R. Solanaceaum***	***P. Carotovorum***		***E. Coli***
Rosmarinus officinalis	- [e]	- [e]	1	- [e]
Rosmarinus officinalis	- [e]	- [e]	5	- [e]
Rosmarinus officinalis	- [e]	- [e]	10	- [e]
Rosmarinus officinalis	6.5 [d]	6.5 [d]	25	- [e]
Rosmarinus officinalis	8 [XYZ]	7.1 [bc]	50	11.1 [M]
Rosmarinus officinalis	8.8 [UV]	7.1 [bc]	75	11 [MN]
Rosmarinus officinalis	11.8 [L]	8.1 [WXY]	100	11.8 [L]
Rosmarinus officinalis	- [e]	- [e]	0	- [e]
Eucalyptus globulus	- [e]	- [e]	0.01	- [e]
Eucalyptus globulus	- [e]	- [e]	0.05	- [e]
Eucalyptus globulus	- [e]	- [e]	0.1	- [e]
Eucalyptus globulus	- [e]	- [e]	0.5	- [e]
Eucalyptus globulus	- [e]	- [e]	1	- [e]
Eucalyptus globulus	- [e]	- [e]	5	- [e]
Eucalyptus globulus	- [e]	- [e]	10	- [e]
Eucalyptus globulus	- [e]	- [e]	25	- [e]
Eucalyptus globulus	- [e]	- [e]	50	- [e]
Eucalyptus globulus	- [e]	6.8 [cd]	75	- [e]
Eucalyptus globulus	6.5 [d]	7.6 [Za]	100	- [e]
SM[b]	22	8.5	10	16
EM[b]	14	-	15	-

Diameter of inhibition zones of essential oil including diameter of disc 6 mm; (–) no antimicrobial activity

a Concentration %0 is paper disc containing just ethanol (Negative control)

b Standard antibiotic (positive control): SM (Streptomycin, 10 µg/disc) and EM (Erithromicin, 15 µg/disc) values followed by the same letter indicates no significant difference according to Duncan's multiple range test at $P < 0.05$

Table 2. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of the Essential oils Against the Bacteria

EOs		MIC (µl/ml)			MBC (µl/ml)	
	P. Carotovorum	***R. Solanacearum***	***E. coli***	***P. Carotovorum***	***R. Solanacearum***	***E. Coli***
Coriandrum sativum	50	250	500	50	250	500
Cuminum cyminum	100	100	500	100	100	500
Thymus vulgaris	5	1	5	5	1	5
Rosmarinus officinalis	250	250	500	500	500	500
Eucalyptus globulus	750	1000	-	750	1000	-

(–) no antimicrobial activity

inhibited the growth of pathogen bacteria but variations were observed in the effectiveness depending on the type of oil, concentration and targeted organism. The antimicrobial activity of EOs has been reviewed previously [12, 14, 16, 22].

In the past the antimicrobial activity of thyme essential oil has been demonstrated [23], specific reports showed the antimicrobial effects of thyme oil against different bacteria, including *Xanthomonas axonopodis* pv. *vesicatori* [16], *X. citri* pv. *citri* [24] and *Clavibacter mishiganensis* subsp. *mishiganensis* [25, 26].

It has been found that phenolic compounds of vegetable oils exhibit the highest antibacterial properties. Bagamboula *et al*. [27] showed that phenolic antimicrobial substances such as thymol, carvacrol and p-cymene have a higher antimicrobial effect than other plant materials. Thyme oil consists of 43% thymol and 36% P-cymene from phenol group [28], therefore the strong antimicrobial effects of thyme seems a reasonable outcome.

After thyme, coriander showed a good antibacterial effect on all the tested bacteria. Coriander has no phenolic

compounds but 60-70% linalool [29]. Linalool is an alkyl compound so the antibacterial activity of coriander can be justified. Results reported by Wan *et al*. [29] showed that antibacterial effect of basil oil was mainly due to its linalool. Lixandru *et al*. [7] tested the antibacterial activity of some EOs and found thyme and coriander had the highest antibacterial effect on the tested bacteria.

Cumin essential oil consists of 25% cuminaldehyde and secondary compounds of α-Pinene and Sabinene are antibacterial [30]. Moderate activity of this EO in our study might be related these antimicrobial compounds.

Eucalyptus essential oil had no considerable antibacterial properties. Amount of phenolic compounds in this oil is not enough to expose antibacterial effect. One of the main compounds of this EO is eucalyptol which is not a bactericidal. Observing the inconsiderable amount of bactericidal effect might be due to borneol. Daferera, *et al*. [22] showed that rosemary oil compared to thyme oil had a very weak antibacterial effect against pathogens *Fusarium* sp., *Botrytis cinerea* and *Clavibacter mishiganensis* subsp. *Mishiganensis*. Prabuseenivasan *et al*. [31] observed the lowest inhibitory activity of eucalyptus on the bacteria used in the study. In the research of Bendaoud *et al*. [13] the MIC produced by eucalyptus EO against *Agrobacterium tumefaciens* was 750-1000 µl/ml, which is similar to our observations. High MIC in the both researches demonstrates the lower efficiency of this EO in prohibiting bacterial growth [13].

Many other studies have revealed that using whole EOs produced greater antibacterial activities than mixing major components [32, 33], which suggest that presence of minor components in EOs are also critical in presenting antimicrobial effects. There is a strong possibility that the major and the minor components in EOs have synergistic effects or potentiating influence. Activity of rosemary is caused by borneol and other phenolics in the terpene fraction. The volatile terpene, carvacrol and p-Cymene are reported to be probably responsible for the antimicrobial activity of some EOs. In rosemary a group of terpenes (borneol, camphore, 1,8 cineole, α-pinene, camphone, verbenonone and bornyl acetate) were responsible for antimicrobial properties [34].

An important characteristic of EOs and their components is the hydrophobicity, which enables them to partition into the lipid bilayer of the bacterial cell membranes and mitochondria, hence potentially disrupting cell structures and rendering them more permeable [35]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [35].

CONCLUSION

In this research, we found that some of the tested EOs including *Thymus vulgaris*, *Coriandrum sativum*, *Cuminum syminum* have the potential antibacterial properties against *Pectobacterium carotovorum* subsp. *carotovorum*, *Ralstonia solanacearum* (race 3, biovar 2) and *E. coli* (PTCC1330). We believe that the present investigation together with previous studies on this subject provide useful information on the antibacterial properties of different EOs, in particular thyme oil. Therefore, these EOs should be considered as potential alternatives to synthetic bactericides or as a lead compounds for new classes of natural bactericides.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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