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Bacteriocidal Activity of Some Plants Essential Oils Against *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia enterocolitica*

M. Bonyadian and H. Moshtaghi
Department of Food Quality Control, Faculty of Veterinary Medicine,
Shahrekord University, Iran

Abstract: This study was conducted to determine the effects of some plant essential oils on *B. cereus*, *S. typhimurium*, *L. monocytogenes* and *Y. enterocolitica*. In the first step Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of plant essential oils were determined by Tube Dilution Method in Luria-Bertani broth medium. In the second step the growth rate of each bacterium was assessed in presence of plant essential oils in concentration of less than MIC. The results showed that the essential oils of the plants used in this study have the acceptable antimicrobial activities against tested bacteria, the Caraway seed oils showed the most antimicrobial activity followed by Pennyroyal and Peppermint but Tarragon oils showed the least antimicrobial activity on tested bacteria. The results of the second step showed that, the plant essential oils were affected not only on lag phase but also on logarithmic phase of growth of bacteria.

Key words: Essential oils, antimicrobial activity, *B. cereus*, *S. typhimurium*, *L. monocytogenes*, *Y. enterocolitica*

INTRODUCTION

Foodborne illness are the main problem not only in developing countries but also in developed ones (Castro and Escartin, 2000; Hancock *et al*., 1997; Varnam, 1996) and have become a major topic of both public and scientific debate. Indeed the increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern (Meng *et al*., 1998; Miller *et al*., 2000; Stermitz *et al*., 2000). Therefore, there has been interest in some type of antimicrobial agents which, be effective and nontoxic.

Recently, plant essential oils have been considered as a natural preservative by most food safety researchers (Beuchat, 2000; Friedman, 1999; Hamilton-Miller, 1995; Kim *et al*., 1995; Mangena and Muyima, 1999).

Three main factors can influence the results of a test of antimicrobial activity of plant oil, the composition and solubility of the oil, the microorganism and the method of growing and surviving bacteria. For plant essential oils there are two or more active components that may interact additively or synergistically at low concentrations.

The general objective of this study was to determine the antimicrobial effects of essential oils of Tarragon (*Artemisia dracunculus*), Pennyroyal (*Mentha poulgum*), Peppermint (*Mentha pipperita*) and Caraway seed (*Carum carvi*) on some bacteria, which cause food infection (*Salmonella typhimurium*, *Yersinia enterocolitica*, *Listeria monocytogenes*) and food intoxication (*Bacillus cereus*). These plants are very common for preparing of many foods especially in Iran.

Corresponding Author: M. Bonyadian, Faculty of Veterinary Medicine, Shahrekord University, Saman Road, P.O. Box 115, Shahrekord, Iran Tel/Fax: +98 381 4424427

MATERIALS AND METHODS

This study was conducted from February to July 2006 in Food Hygiene Lab, Shahrekord University, Iran.

Plant Essential Oils

The following plants were obtained in spring and dried by putting in shadow on a flat surface: Tarragon, Pennyroyal, Peppermint and Caraway seed. The essential oils of the plants were extracted by Steam Distillation Method (Walton and Brown, 1999) in Research Institute of Drugs Plants (Tehran University). These oils were considered 100% pure.

Bacterial Strains

All of the strains used in this study were isolated from food by Department of Microbiology of Razi Institute of Iran.

Preparation of Plant Essential Oils for Bactericidal Assay

Most of the plant essential oils are immiscible in the aqueous media. A simplified shaking method was developed to prepare suspension of oil in Luria-Bertani broth; this method involved vigorous shaking of the sample followed by dilutions of each suspension in LB broth, with each dilution of sample being mixed carefully.

Determination of the MIC and MBC of Oils on Tested Bacteria

The assay to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was performed by Tube Dilution Method (Baron, 1990) in LB broth. One milliliter of a calibrated broth (10^6 cfu mL^{-1}) of strains was added to the each tube that contained oils. A dilution from 0.01 to 20% was prepared in LB broth. A tube without essential oils was chosen as control. Samples and control were kept at 37°C for 24 h, after that the tubes were checked for growth of bacteria and turbidity from control tube to higher concentrations. The first tube (concentration) without turbidity and lack of bacterial growth was chosen as MIC of the oils for tested bacterium.

To determine the MBC of oils, 1 mL of broth of MIC tube and higher concentrations were streaked on LB agar plates (Difco) and incubated at 37°C for 24 to 48 h. The plates were checked for growth of colonies, the first plate without growing of colonies was chosen as MBC of oils on tested bacterium.

Effect of Essential Oils Lower than MIC on Growth of Tested Bacteria

The assay to assess the growth of bacteria in presence of concentration lower than MIC of oils, were checked in LB broth. The concentration equal the MIC of each oils was prepared in 2 mL of LB broth and after shaking, 1 mL of aliquot was added to 1 mL of LB broth for a 1:2 dilution. A tube of 2 mL of LB broth without essential oils was considered as a control. 1 mL of a calibrated broth of strains (10^2 cfu mL^{-1}) was added to the each tube. The tubes were incubated at 37°C and the growth rate of bacteria were check at 0, 1, 2, 4, 8, 16 and 24 h, by preparing dilution and culture on LB agar plates and enumeration of colonies after incubation of plates at 37°C for 24 h.

RESULTS

MIC and MBC of Essential Oils on Tested Bacteria

Each of the strains tested was effectively sensitive in low concentration of essential oils. MIC and MBC of oils are different for each strain. For example, *S. typhimurium* showed more susceptibility to Pennyroyal and Peppermint oils than the others, but very resistant to Tarragon oil (MIC 15%, MBC 17%). The susceptibility of *L. monocytogenes* to Pennyroyal, Peppermint and Caraway seed was the same. *Y. enterocolitica* was shown the most susceptibility to all of the oils (Table 1).

As the results show the essential oils of Caraway seed showed the most and Tarragon oils showed the least antimicrobial activity on tested bacteria.

The Growth Rate of Tested Bacteria in the Presence of Oils Concentration Lower than MIC

The effects of plants essential oils were determined on growth rate of tested bacteria in concentration lower than (1:2) MIC of oils and compared with the normal growth rate. The results showed that the essential oils affected not only growth rate in lag phase but also in logarithmic phase of growth of bacteria (Fig. 1-4).

Table 1: MIC and MBC of plant essential oils on tested bacteria

	Oils (%)							
	Pennyroyal		Peppermint		Caraway seed		Tarragon	
Strains	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. typhimurium	0.20	0.3	0.2	0.3	0.40	0.5	15.00	17.0
L. monocytogenes	0.10	0.2	0.1	0.2	0.10	0.2	4.00	5.0
Y. enterocolitica	0.07	0.1	0.1	0.2	0.05	0.1	0.07	0.1
B. cereus	0.20	0.5	0.3	2.0	0.07	0.1	0.40	1.0

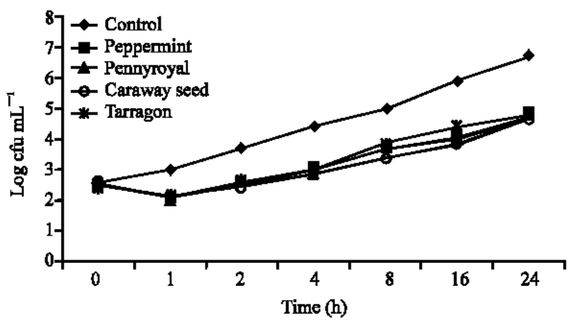

Fig. 1: Growth rate of *B. cereus* in presence of plants essential oils (50% MIC)

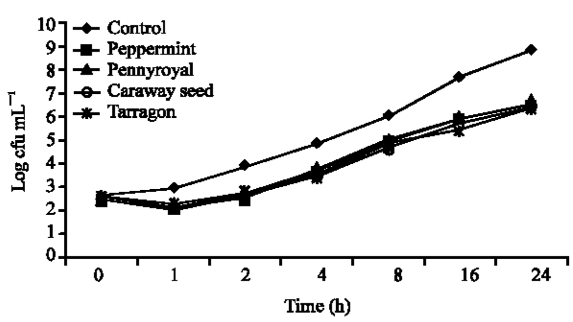

Fig. 2: Growth rate of *S. typhimurium* in presence of plants essential oils (50% MIC)

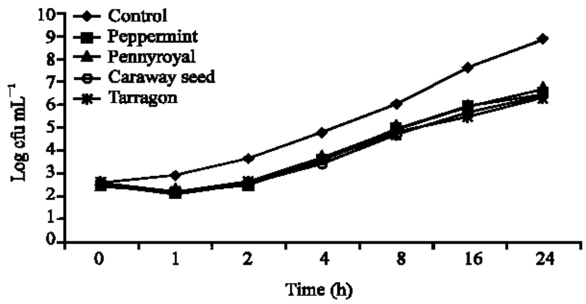

Fig. 3: Growth rate of *L. monocytogenes* in presence of plants essential oils (50% MIC)

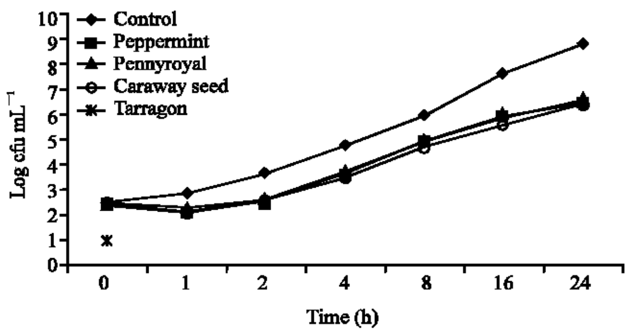

Fig. 4: Growth rate of *Y. enterocolitica* in presence of plants essential oils (50% MIC)

DISCUSSION

In present study, we have shown that some plant essential oils are bactericidal against multiple strains of *S. typhimurium*, *B. cereus*, *L. monocytogenes* and *Y. enterocolitica* that associated with foodborne outbreaks and human illness.

Many types of assays for measuring the antimicrobial activities of plant derivatives have been described and inconsistencies in results for different assay methods.

Types of assays described include the measurement of (i) the zone of inhibition of bacterial growth around paper disks containing a plant antimicrobial compound on tryptic soy agar (Kim *et al*., 1995), (ii) the minimum concentration necessary to inhibit the growth of bacteria (MIC) (Hammer *et al*., 1999) and (iii) the inhibition of bacterial growth on an agar medium with an antimicrobial compound diffused in the agar (Mangena and Muyima, 1999) and tube dilution method (Baron , 1990).

The method described and used in this study is tube dilution method in a suspension for antimicrobial activity against bacterial pathogen. The assay was easy to perform and the results were reproducible. The bacterial strains we tested had been isolated from different kinds of foods.

The nature of the plants from which some essential oils have been derived necessitates consideration of the sensory properties (e.g., flavor, aroma, color) of compounds to be added to food or water. The flavor of oils can vary widely; therefore, some oils would be more appropriate than other for foods such as fresh products, juices, dairy products, poultry and red meat. Some plants such as Peppermint, Tarragon, Caraway seed and Pennyroyal are very popular in Iran and add to many foods for induce flavor and taste.

The results of this study revealed that the essential oils of the tested plants can inhibit and kill the microorganisms in low concentration also the concentration lower than MIC affected on the growth rates of the tested microorganisms by 1.5 to 2 log reduction of microbial population after 20 h.

Several reports on antimicrobial effectiveness of essential oils in foods suggested that the use of these oils may improve food safety. For example, Koutsoumanis *et al*. (1999) inoculated a salad with several concentrations of oregano oil and with *Salmonella enteritidis* and then evaluated the influence of several environmental factors in microbial population. The death rate of the pathogen depended on the pH, the storage temperature and time and the essential oil concentration.

Lin *et al.* (2000) showed that vapors generated from plant-derived isothiocyanates resulted in an 8-log reduction in *E. coli* and *Salmonella montevideo* on iceberg lettuce and tomato skins inoculated with these pathogens.

It is unlikely that the observed antimicrobial activities of the oils are due to physical phenomenon such as aggregation or concentration of the bacteria. However, detailed examination of possible mechanisms involving inhibition of essential enzymes, chelating of essential trace elements such as iron, interference with cell membrane biosynthesis and disruption of cell membrane are described by Bank *et al*. (1986) and Friedman *et al*. (2002).

This study was conducted to determine the effects of some plants essential oils on *B. cereus*, *S. typhimurium*, *L. monocytogenes* and *Y. enterocolitica*. We found that these strains are susceptible in low concentrations of the tested oils, but the susceptibility of *Y. enterocolitica* was much greater than the others. In contrast, *S. typhimurium* showed the least susceptibility to tested oils.

The ultimate goal of this study is to develop safe, effective and inexpensive food formulations and processes to reduce pathogens in food.

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