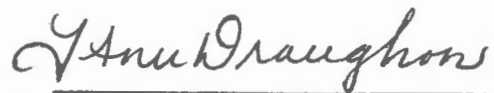


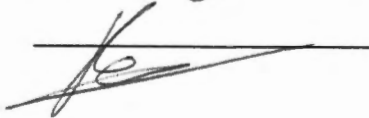
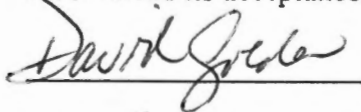
To the Graduate Council:

I am submitting herewith a thesis written by Mona Ahmed Elgayyar entitled "Antimicrobial Activity of Essential Oils from Herbs and Spices and Application of Black Cumin to Rainbow Trout Fillets." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.



F. Ann Draughon, Major Professor

We have read this thesis and  
recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor and  
Dean of The Graduate school

**ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM HERBS AND  
SPICES AND APPLICATION OF BLACK CUMIN TO RAINBOW  
TROUT FILLETS**

A Thesis  
Presented for the  
Master of Science  
Degree  
The University of Tennessee, Knoxville

Mona Ahmed Elgayyar  
May 1999

AG-VET-MED.

Thesis  
99  
.E43

## **DEDICATION**

I dedicate This work to the spirit of my father (Ahmed Elgayyar, 1935-1984) and to my mother who have given me invaluable educational opportunities and to my husband and my children.

## ACKNOWLEDGMENTS

First of all I want to thank almighty God, the most passionate, the most merciful for all his blessings. I would like to thank my major professor Dr. Ann Draughon for her guidance, assistance and patience. I would also like to thank the other committee members, Dr. David Golden and Dr. Riette Van Laack, for their advise, constructive comments, and assistance in the completion of this work.

I thank the publisher, Hesham and Mohamed Hafez, who sponsored me financially to complete this study in the United States.

There are a number of other people whose assistant should be recognized. I want to thank Aminta Martinez, Robert Williams, Yusuf Yalmaz, Sima Kasraie, Mondonna Cate, Paul Angelino, C.Tony Shao, Philipus Pangloli, for their friendship and help. I also wish to thank Willie Taylor for his assistant in the laboratory. I also would like to thank Mary Zaher for her help and patience with me.

I thank Dr. John Mount for his continuous encouragement and help. I also want to thank Dr. Arnold Saxton for assistance with statistical analysis.

Lastly, I wish to express my thanks to my husband, Ahmed, for his help and patience. I would like to thank my children, Zeinab, Ahmed, and Mohamed.

## ABSTRACT

This research was conducted for two purposes. In the first study, The antimicrobial activity of selected herb and spice essential oils for control of growth and survival of microorganisms was examined. Inhibition of growth was determined by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum lethal concentration (MLC) was determined by the tube dilution method. Essential oils from anise, angelica, basil, carrot, celery, cardamom, coriander, dill weed, fennel, oregano, parsley, and rosemary were evaluated. Inhibition ranged from complete with oregano to no inhibition with carrot oil for each of the test strains which included: *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O:157:H7, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Aspergillus niger*, *Geotrichum*, and *Rhodotorula*. Oregano essential oil showed the greatest inhibition (zone 370-80 mm) (MLC ~ 8 ppm). Coriander and basil were also highly inhibitory (MLC = 25 - 50 ppm) to *E. coli* O: 157:H7 and other bacteria tested. *Pseudomonas* was the most resistant microorganism to the spice oils but still had a MLC in the 100 to 200 ppm range using oregano, basil and coriander oils. Anise oil was not particularly inhibitory to bacteria (inhibition zone ~ 25 mm), however anise oil was highly inhibitory to molds. Basil, coriander, and oregano were also highly inhibitory to the fungi assayed.

In the second study, the effectiveness of aqueous, ethanolic and petroleum ether extracts of black cumin (*Nigella sativa*) for control of growth and survival of common spoilage and pathogenic microorganisms was investigated. More over, the applications of

black cumin oil on fresh Rainbow trout fillet were tested. In vitro, the ethanolic extract of black cumin seed was found to be highly inhibitory (zone = 35-55 mm) against *Listeria monocytogenes*, *Staphylococcus aureus*, , *Yersinia enterocolitica*, and *Rhodotorula*, moderately inhibitory (zone =16-28mm) against *Escherichia coli* O:157:H7, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, and *Geotricum*, and only weakly or not inhibitory (zone<12 mm) against *Aspergillus niger* by the paper disc agar diffusion method. Freshly harvested rainbow trout fillets were stored at 0°C (on ice) and at 4°C using five marinate treatments. Treatments evaluated were a plain teriyaki marinate (control), and four marinates containing black cumin and/or dimethyl dicarbonate (DMDC). Trout treated with marinate using black cumin oil had significantly lower aerobic plate counts (< 3 log), coliform counts, *Listeria* and yeasts and mold counts ( $p < 0.05$ ). DMDC marinated trout was not significantly differ than control marinate for all counts ( $p < 0.05$ ). Fresh trout treated with either ground seeds or the combination of black cumin oil and DMDC marinate exhibited moderate reduction (>1 log) in all counts ( $p < 0.05$ ). Since a marinate containing black cumin essential oil was highly inhibitory to both spoilage and pathogenic microorganisms and also significantly ( $p<0.0001$ ) increased shelf-life by almost two weeks, it may be useful for extending shelf-life of fresh refrigerated fish.

## TABLE OF CONTENTS

| PART                                                                                                                         | PAGE |
|------------------------------------------------------------------------------------------------------------------------------|------|
| I. Literature Review .....                                                                                                   | 1    |
| Introduction .....                                                                                                           | 2    |
| Essential oils .....                                                                                                         | 4    |
| Physical and Chemical Characteristics .....                                                                                  | 5    |
| Antimicrobial Activity .....                                                                                                 | 11   |
| Regulation of Herbs, Spices and Essential oils .....                                                                         | 13   |
| General Characteristics - Microorganisms .....                                                                               | 14   |
| Antimicrobials in Foods .....                                                                                                | 18   |
| Dimethyl Dicarbonate .....                                                                                                   | 19   |
| Antibiotic Sensitivity .....                                                                                                 | 20   |
| Fish as food .....                                                                                                           | 22   |
| Purpose of Current Study .....                                                                                               | 25   |
| References .....                                                                                                             | 25   |
| II. Antimicrobial Activity of Essential Oils from Plants against Selected Pathogenic<br>and Saprophytic Microorganisms ..... | 31   |
| Abstract .....                                                                                                               | 32   |
| Introduction .....                                                                                                           | 33   |
| Materials and Methods .....                                                                                                  | 35   |
| Results and Discussion .....                                                                                                 | 38   |
| References .....                                                                                                             | 49   |
| III. Use of Black Cumin Essential Oil to Inhibit Spoilage and Pathogenic<br>Microorganisms in Rainbow Trout .....            | 52   |
| Abstract .....                                                                                                               | 53   |
| Introduction .....                                                                                                           | 54   |
| Materials and Methods .....                                                                                                  | 55   |
| Results and Discussion .....                                                                                                 | 61   |
| References .....                                                                                                             | 77   |
| Appendix .....                                                                                                               | 80   |
| Vita .....                                                                                                                   | 85   |

## LIST OF TABLES

| FIGURE                                                                                                                               | PAGE |
|--------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>Part I: Literature Review</b>                                                                                                     |      |
| 1. Breakdown of major chemical components by herb and plant family . . . . .                                                         | 6    |
| <b>Part II: Antimicrobial Activity of Essential Oils from Herbs and Spices to Selected Pathogenic and Saprophytic Microorganisms</b> |      |
| 1. Inhibition zones (mm) of growth of tested microorganisms by essential oils . . . . .                                              | 39   |
| 2. Inhibition zones (mm) of growth of tested microorganisms by antibiotics . . . . .                                                 | 41   |
| 3. Minimum lethal concentration (MLC) of essential oils for tested bacteria . . . . .                                                | 47   |
| <b>Part III: Use of Black Cumin Essential Oil to Inhibit Spoilage and Pathogenic Microorganisms in Rainbow Trout.</b>                |      |
| 1. Inhibition zones (mm) of growth of tested microorganisms by black cumin essential oils . . . . .                                  | 62   |
| 2. Minimum lethal concentration (MLC) of black cumin essential oils for tested bacteria . . . . .                                    | 65   |

## LIST OF FIGURES

| FIGURE                                                                                                                 |                                                                                                                    | PAGE |
|------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|------|
| <b>Part III : Use of Black Cumin Essential Oil to Inhibit Spoilage and Pathogenic Microorganisms in Rainbow Trout.</b> |                                                                                                                    |      |
| 1.                                                                                                                     | Change in aerobic plate counts (APC) of five treated trout fillet during storage period (60 days) at 4°C .....     | 67   |
| 2.                                                                                                                     | Change in aerobic plate counts (APC) of five treated trout fillet during storage period (60 days) at 0°C .....     | 68   |
| 3.                                                                                                                     | Change in coliform counts of five treated trout fillet during storage period (60 days) at 4°C .....                | 69   |
| 4.                                                                                                                     | Change in coliform counts of five treated trout fillet during storage period (60 days) at 0°C .....                | 70   |
| 5.                                                                                                                     | Change in <i>L. monocytogenes</i> counts of five treated trout fillet during storage period (60 days) at 4°C ..... | 72   |
| 6.                                                                                                                     | Change in <i>L. monocytogenes</i> counts of five treated trout fillet during storage period (60 days) at 0°C ..... | 73   |
| 7.                                                                                                                     | Change in yeast and mold counts of five treated trout fillet during storage period (60 days) at 4°C .....          | 75   |
| 8.                                                                                                                     | Change in yeast and mold counts of five treated trout fillet during storage period (60 days) at 0°C .....          | 76   |

## **Part I**

### **Literature Review**

## INTRODUCTION

Beneficial activity of extracts from many types of plants, used as flavoring and seasoning agents in foods and beverages in reducing infection, has been claimed for centuries (Ayres et al., 1980; Charalambous, 1994). Some of these essential oils have antimicrobial properties (Davidson and Branen, 1993). Spices and herbs have played an important role in human life, not only for flavoring foods, but also for preventing food deterioration and diseases (Arora et al., 1991; Charalambous, 1994). The antimicrobial activities of mustard, cinnamon and cloves have been tested at the end of last century and their oils were thought to hamper microbial spoilage in foods (Uda et al., 1993; Saxena and Vyas, 1986). Recently, the use of natural preservatives in food system has increased. Garlic, onion, nutmeg, clove, pepper, paprika, anise, dill, rosemary, cloves, cumin and oregano, have been investigated as possible antifungals (Conner and Beuchat; 1984, Paster et al., 1995)

Fish is the most abundant protein resource in the U.S. The increase in fish consumption is due to the fact that fish meat is considered healthy since it is lower in fat than other meats (Ayres, 1980). Fish also has many biotechnological applications, which include production of enzymes, gelatin, and pharmaceutical protein with antimicrobial and antitumor activity (Benjakul et al, 1996). Aquaculture is a rapidly growing industry in the U.S., but shelf-life problems interfere with marketing and distribution, thus, hampering profits (Draughon et al., 1998).

Many researchers have evaluated the application of partial freezing, addition of chemicals; mixture of crushed ice and NaCl, and modified atmosphere storage to fish and

shellfish to extend the storage life. However, little is known about the mechanism by which these methods influence quality during storage (Jiang et al, 1990). The most popular method of fish storage is crushed ice, which reduces fish quality by producing a soft and smelly product. New methods need to be established to improve fish quality and inhibit microbial growth (Ayres, 1980).

Fish is a highly perishable food, even under refrigerated storage or on ice.

Nutrients in fish promote the growth of spoilage and pathogenic microorganisms, such as: *Listeria*, *Salmonella*, *Escherichia coli*, *Staphylococcus*, *Pseudomonas*, *Clostridium perfringens*, *C. botulinum* Type E, *Vibrio parahaemolyticus*, yeasts and molds (Hobbs, 1987; Gram and Huss, 1996). Some pathogens are of special concern in fishery products, because of their association with foodborne diseases. Many foodborne diseases associated with fish and fish products have been reported (Huss, 1992; Jay, 1996).

Recently, with new HACCP regulations, the need for control of pathogenic and spoilage bacteria in both fresh and processed fish has increased. Any chemicals, which interfere with the metabolism and the availability of nutrient, will inhibit or prevent growth of microorganisms (Maragal et al., 1988). Most of the antimicrobial agents are not very useful in neutral pH foods such as fish since many are weak organic or inorganic acids (Davidson and Branen, 1993). Since most antimicrobial products now available are not very effective at neutral pH, antimicrobial products that can be used effectively on neutral pH foods are needed.

The purpose of this thesis was to examine the effectiveness of selected herb and spice essential oils on survival and growth of key foodborne pathogenic and spoilage

microorganisms, and to develop a method of storing fresh trout using an essential oil which might eliminate foodborne pathogens and/or spoilage microorganisms, and extend fish shelf-life.

## **ESSENTIAL OILS**

Herbs and spices are widely used to contribute flavor to foods (Davidson and Branen, 1993). Spices are defined as the roots, bark, buds, seeds or fruits of aromatic plants which commonly grow in tropical countries (Gould, 1995). This is a somewhat narrow definition since herbs grow throughout the world. The oil extraction from several types of plants either by steam distillation or volatile organic solvents have been recognized for years (Gould, 1995; Conner and Beuchat, 1984). Essential oils are defined, as being a group of odorous compounds, soluble in alcohol and somewhat soluble in water (Davidson and Branen, 1993). They comprise a mixture of esters, aldehydes, ketones and terpenes (Gould, 1995). It is generally thought that certain herbs and spices have antimicrobial activity and may influence the quality and safety of foods to which they have been added (Arora et al., 1991). The antimicrobial compounds in spice and herb plant material are commonly in the essential oil fraction (Gould, 1996).

The earliest recorded use of herbs and spice extracts as preservatives was by the ancient Egyptians, who used these materials for mummification (Davidson and Branen, 1993). It was not until the eighteenth century that the first scientific information on the preservative effects of spice extract were recorded. However, this was before people truly

understood what caused food spoilage and infections. Recently, Wilkins and Board (1989) wrote that more than 1,340 plants might be plausible sources of antimicrobial compounds.

## PHYSICAL AND CHEMICAL CHARACTERISTICS

Physical and chemical characteristics of spices and herbal oils used in our study can be found in several reference texts (Furia and Bellanca, 1975; Charalambous, 1994). Key characteristics and family of each herb are summarized in Table 1. Anise seed (*Pimpinella anisum* L.) is an herbal seed that is cultivated in the eastern Mediterranean region, southern Europe, Middle East, India, former USSR, Mexico and western Asia (Furia and Bellanca, 1975; Phillipson, 1994). Anise seed essential oil (*Oleum anisi*) is derived in approximately 3% yields by steam distillation of dried and minutely crushed fruits and can also be obtained by solvent extraction (Furia and Bellanca, 1975). The oil is a colorless clear liquid with a strong, characteristic sweet odor and flavor reminiscent of anethole. The oil consists mainly of trans-anethole (80-95%) and other components (Table 1). Angelica root (*Angelica archangelica* L.), is a herbaceous plant which is cultivated in Europe and Asia (Phillipson, 1994). The essential oil yields approximately 0.35-1.9% and is derived from roots (Furia and Bellanca, 1975). The oil is pale-yellow to deep-amber exhibiting a warm, pungent odor with a bitter sweet flavor (Furia and Bellanca, 1975). The essential oil is comprised of 80-90% monoterpene hydrocarbons and other components (Table 1) (Furia and Bellanca, 1975; Ochocka and Lamparczyk, 1993;

Table 1. Breakdown of major chemical components by herb and plant family

| Chemicals     | Umbelliferae     |          |        |        |           |      |        | Labiatae |       |         | Zingiberaceae | Ranunculaceae |             |
|---------------|------------------|----------|--------|--------|-----------|------|--------|----------|-------|---------|---------------|---------------|-------------|
|               | Anise            | Angelica | Carrot | Celery | Coriander | Dill | Fennel | Parsley  | Basil | Oregano | Rosemary      | Cardamom      | Black cumin |
| Anethol       | 90% <sup>1</sup> |          |        |        |           |      | 70%    |          |       |         |               |               |             |
| P-anol        | +                |          |        |        |           |      |        |          |       |         |               |               |             |
| Chavicol      | +                |          |        |        |           |      |        |          | +     |         |               |               |             |
| Estragole     | +                |          |        |        |           |      |        |          |       |         |               |               |             |
| Anisaldehyde  | +                |          |        |        |           |      |        |          |       |         |               |               |             |
| Anise alcohol | +                |          |        |        |           |      |        |          |       |         |               |               |             |
| Carvone       | +                |          |        |        |           | +    |        |          |       |         |               |               |             |
| Linalool      | +                |          |        |        | 70%       |      |        |          | 75%   |         |               | +             |             |
| Limonene      | +                |          |        | 60%    | +         | +    | +      |          |       |         |               | 85%           |             |
| Monoterpene   |                  | ~80%     |        |        |           |      |        |          |       |         | +             |               |             |
| Phellanderene |                  | ~13%     |        |        |           | +    | +      |          |       |         |               |               |             |
| α-pinene      |                  | ~10%     |        |        | +         | +    |        |          |       |         | 25%           |               |             |
| Eugenol       |                  |          |        |        |           |      |        |          | 20%   |         |               |               |             |
| Cineol        |                  |          |        |        |           |      |        |          |       |         | 30%           | +             |             |

1. Numbers indicate percentage of chemicals in essential oils

| Chemicals        | Umbelliferae |          |        |        |           | Labiatae |        |         | Zingiberaceae | Ranunculaceae |          |          |             |
|------------------|--------------|----------|--------|--------|-----------|----------|--------|---------|---------------|---------------|----------|----------|-------------|
|                  | Anise        | Angelica | Carrot | Celery | Coriander | Dill     | Fennel | Parsley | Basil         | Oregano       | Rosemary | Cardamom | Black cumin |
| d-terpineol      |              |          |        |        |           |          |        |         |               |               |          | +        |             |
| Terpinyl acetate |              |          |        |        |           |          |        |         |               |               |          | +        |             |
| Carotenol        |              |          | 18%    |        |           |          |        |         |               |               |          | +        |             |
| Terpenes         |              |          | +      |        | 20%       |          |        |         |               |               |          |          |             |
| Terpen alcohol   |              |          | +      |        |           |          |        |         |               |               |          |          |             |
| Sesquiterpenes   |              |          | +      | +      |           |          |        |         |               |               |          |          |             |
| Selinene         |              |          |        | 10%    |           |          |        |         |               |               |          |          |             |
| Palmitic acid    |              |          |        | +      |           |          |        |         |               |               |          |          |             |
| Transephenol     |              |          |        | +      |           |          |        |         |               |               |          |          |             |
| Dipentene        |              |          |        |        |           | +        |        |         |               |               |          |          |             |
| Camphor          |              |          |        |        |           |          | +      |         |               |               | 25%      |          |             |
| Melanthine       |              |          |        |        |           |          |        |         |               |               |          |          | +           |
| Tannin           |              |          |        |        |           |          |        |         |               |               |          |          | +           |
| Nigellidine      |              |          |        |        |           |          |        |         |               |               |          |          | +           |
| Thymol           |              |          |        |        |           |          |        |         |               | +             |          |          |             |
| Carvacrol        |              |          |        |        |           |          |        |         |               | +             |          |          |             |
| Apiol            |              |          |        |        |           |          |        | +       |               |               |          |          |             |
| Myristicin       |              |          |        |        |           |          |        | +       |               |               |          |          |             |
| Coumarins        |              |          |        |        |           |          |        | +       |               |               |          |          |             |

1. Numbers indicate percentage of chemicals in essential oils

Phillipson, 1994).

Carrot seed (*Daucus carota* L.) is a herbaceous plant very common in Europe and many other countries. The essential oil is obtained from the seeds. It is an amber liquid with a characteristic warm, spicy sweet fatty odor and piquant flavor (Furia and Bellanca, 1975). The main constituent identified in carrot seed oil is carotenol (14-18%) (Furia and Bellanca, 1975; Ochocka and Lamparczyk, 1993). Celery seed (*Apium graveolens* L.) is a herbaceous plant, which is native through Europe, Western Asia to India, North, and South Africa (Charalambous, 1994; Phillipson, 1994). The yield of the essential oil from seeds is about 2-3%. The oil is pale-yellow to yellow-brownish, exhibiting a characteristic spicy, aromatic odor and a warm, burning taste (Furia and Bellanca, 1975; Charalambous, 1994). The main component of celery seed oil is *d*-limonene (60%) (Table 1) (Charalambous, 1994; Furia and Bellanca, 1975; Phillipson, 1994).

Coriander (*Coriandrum sativum* L.) is a herbaceous plant originally from the Middle East (Furia and Bellanca, 1975). The essential oil is obtained from the fruits (seeds); yields range between 0.3-1.1%. The essential seed oil is a clear, colorless to light-yellow liquid with a characteristic odor of linalool. The oil contains 60-70% D-linalool (Table 1) (Furia and Bellanca, 1975; Ochocka and Lamparczyk, 1993; Phillipson, 1994). Dill (*Anethum graveolens* L.) is a herbaceous plant native to the Orient, which grows wild almost everywhere in Southern Europe, Asia, and Africa (Furia and Bellanca, 1975). Dill seed oil is slightly yellow with a strong, fresh, spicy, aromatic odor and a warm, somewhat burning taste (Furia and Bellanca, 1975). The main component of dill essential oil is carvone (Table 1) (Furia and Bellanca, 1975; Vera and Ming, 1998).

Fennel (*Foeniculum vulgare* Mill) is a herbaceous plant native to the Mediterranean region (Phillipson, 1994). The essential oil is obtained from seeds and yields 2-6%. The oil is yellowish-green with a characteristic anise odor (Phillipson, 1994). The major constituent of the oil is sweetish anethole (50-70%) (Table 1) (Furia and Bellanca, 1975; Ochocka and Lamparczyk, 1993).

Parsley (*Petroselinum crispum*) is cultivated throughout the Mediterranean region, Europe, and U.S. (Phillipson, 1994; Furia and Bellanca, 1975). Parsley essential oil is obtained from seeds with a yield of 1.5-6%. It is yellowish to yellow-amber with a warm spicy bitter and aromatic taste (Furia and Bellanca, 1975). The main component of the oil is apiol (Table 1) (Charalambous, 1994; Phillipson, 1994; Manderfeld et al., 1997).

Basil (*Occimum basilicum* L.) is a herbaceous plant native to Asia and the Mediterranean region (Phillipson, 1994; Charalambous, 1994). The essential oil components vary according to variety and chemotype. The oil yields from 1.5-3% and is derived from the leaves (Furia and Bellanca, 1975). It is a pale-yellow to amber liquid with spicy, herbaceous, slightly earthy odor. The main component of the oil is linalool (up to 75%) and other components (Table 1) (Furia and Bellanca, 1975; Charalambous, 1994; Phillipson, 1994). Oregano (*Origanum vulgare* L.) is a herbaceous plant that grows widely in the Mediterranean and Latin America (Charalambous, 1994). The essential oil is obtained by steam distillation of the plant's flowering tops with yields of less than 1%. It is a yellowish-green clear liquid with fresh, herbaceous odor and warm burning flavor (Charalambous, 1994; Furia and Bellanca, 1975). The major components

of oregano oil are thymol and carvacrol (Table 1) (Salmeron et al., 1990; Furia and Bellanca, 1975).

Rosemary (*Rosmarinus officinalis* L.) is cultivated in the Mediterranean region and Europe (Phillipson, 1994; Furia and Bellanca, 1975). The essential oil is obtained from the leaves with yields of approximately 1.0-2.5% of pale yellow liquid with a characteristic camphoraceous odor and strong, fresh, woody, herbaceous spicy flavor (Furia and Bellanca, 1975). The major constituent of the oil is cineole (15-30%) (Phillipson, 1994).

Cardamom (*Elettaria cardamomum* M) is cultivated in India and Guatemala. The essential oil obtained from the seeds with an average yield of 4-6% (Furia and Bellanca, 1975; Charalambous, 1994). It is a greenish-yellow liquid with a warm, spicy odor. The main constituent of the oil is limonene (Table 1) (Furia and Bellanca, 1975; Charalambous, 1994).

Black cumin (*Nigella sativa* L.) is cultivated in the Eastern Mediterranean region, Central Europe, and Western Asia (Nergiz and Otles, 1993; Charalambous, 1994). The essential oil is obtained by either steam distillation or organic solvent (petroleum ether, chloroform, or ethanol) extraction which yields 0.5-1.5% of yellowish-brown liquid that tends to darken on aging (Charalambous, 1994). The major constituent of the oil saponin glucoside (melanthine) (Table 1) (Charalambous, 1994; Rahman et al., 1995; Nergiz and Otles, 1993).

## ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

Many spices, herbs, and their extracts have antimicrobial activity, almost consistently due to the essential oil fraction (Gould, 1995). Wilkins and Board (1989) reported that more than 1,340 plants may be potential sources of antimicrobial compounds.

Ela and his co-workers (1996) screened sixteen essential oils for their antimicrobial activity by using the agar diffusion technique and measuring their inhibition zones. They classified the tested oils according to their activity, black cumin was categorized as strongly active (inhibition zone >8mm) against *Staphylococcus aureus*, *Candida albicans* and *C. olivacum*, moderately active (inhibition zone >6-<8mm) against *Asperigillus niger*, and inactive (no inhibition) against *Escherichia coli*. Basil and parsley showed potent activity against *Staphylococcus aureus* and *Candida* spp. Black cumin essential oil has been tested by several authors; Rathee et al. (1981) recorded that the essential oil of black cumin possesses very strong antimicrobial (inhibition zone >20 mm) properties against *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Shigella shigae*. Other findings showed that, black cumin essential oil had potent inhibitory activity against bacteria (*Bacillus cereus*, *Vibro cholerae*, *S. aureus*, *E. cloi*, *S. typhimurium*, *P. aeruginosa*, *Corynebacterium pyogens*, *Streptococcus pyogenes*) and fungi (*A. niger*, *Candida albicans*, *A. falvours*, *Penicillium* spp.) (Agarwal et al., 1979). This agreed with the work done by Toppozada and his co-workers (1964), and Kandil and his co-workers (1994). Some ampicillin, co-trimoxazole and tetracycline resistant *E. coli*

showed sensitivity to nigella essential oil and the MIC of the volatile oil for the one tested strain was between 50-400µg / ml (Ferdous et al., 1992)

Conner and Beuchat (1984) investigated the antimicrobial effect of thirty-two essential oils on thirteen food-spoilage and industrial yeasts. Oregano was the most effective. Anise, basil, cardamom, celery, dill, fennel, parsley, and rosemary were less effective. Previous research showed that basil and anise essential oils are potent antimicrobial and antifungal against wide range of foodborne Gram-positive, Gram - negative and acid-tolerant organisms (*Lactobacillus* and *Saccharomyces* spp.) (Lachowicz et al., 1998). Essential oils from coriander and basil showed less activity against *A. niger*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae* and no effect on *Bacillus cereus* (Meena and Sethi, 1994). Oil of fennel, anise, or basil in combination with either benzoic acid or methyl-paraban possess a synergistic inhibitory effect against *Listeria monocytogenes* and *S. enteritidis* (Fyfe et al., 1998). Earlier studies demonstrated that oregano and rosemary progressively delayed growth and acid production of lactic acid bacteria (Zaika et al., 1983). Parsley also showed inhibitory effect against *E. coli*, *L.monocytogenes*, *P. fragi* and other spoilage microorganisms (Manderfeld et al., 1997). Babic et al. (1994) reported that purified ethanolic extracts of peeled and shredded carrot have an antimicrobial effect against a range of foodborne microorganisms. In general, Gram-positive bacteria are more sensitive to essential oils than Gram-negative, with the lactic acid bacteria being the more resistant among gram-positive bacteria (Jay, 1996).

One of the negative aspects of spices and herbs is that, many spices and herbs

were found to be heavily contaminated by mold flora. This mold flora were dominated by an *Aspergillus* spp. which may produce aflatoxin (Garrido et al., 1992).

## **REGULATION OF HERBS, SPICES AND ESSENTIAL OILS**

The spices, herbs and their essential oils have been regulated by the Food and Drug Administration (FDA) Title 21, Volume 3, Parts 170 to 199 which revised as of April 1, 1998 (CFR, 1998). Section 21CFR182 states that spices, other natural seasonings and flavorings, essential oils oleoresins, and natural extractives (including distillates) are Generally Recognized As Safe. Also, FDA passed another regulation for labeling of the spices and essential oils as used in food. This act is found in Title 21, Volume 2, Parts 100 to 169 which was also revised as of April 1, 1998. Section 21CFR101.22 defines spice and essential oils as: "The term spice means any aromatic vegetable substance in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed. Spices include the spices listed in Sec. 182.10 and part 184 of this chapter, such as the following: Allspice, Anise, Basil, Bay leaves, Caraway seed, Cardamom, Celery seed, Chervil, Cinnamon, Cloves, Coriander, Cumin seed, Dill seed, Fennel seed, Fenugreek, Ginger, Horseradish, Mace, Marjoram, Mustard flour, Nutmeg, Oregano, Paprika, Parsley, black Pepper, white Pepper, red Pepper, Rosemary, Saffron, Sage, Savory, Star

aniseed, Tarragon, Thyme, Turmeric, Paprika and saffron or other spices which are also colors, shall be declared as “spice and coloring” unless declared by their common or usual name.

The term “natural flavor” or natural flavoring means the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis (CFR, 1998), which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in Section 182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter, and the substances listed in Sec. 172.510 of the CFR”. By these definitions, spices and their essential oils are GRAS and must be labeled as spice, coloring or by their names.

## **GENERAL CHARACTERISTICS - MICROORGANISMS**

Despite progress improving the quality and safety of foods, zero risk of microbiological hazards is not possible and no one method will eliminate all pathogens or toxins from the food chain. Foodborne microorganisms get into the foods during preparation, cooking, serving and storage (Jay, 1996). According to the best available estimates by public health and food safety experts, millions of illnesses and thousands of deaths each year in this country can be traced to contaminated food. The General

Accounting Office, in a May 1996 report, stated that there are between 6.5 million and 81 million cases of foodborne illness a year. The National Center for Health Statistics estimates the number of deaths per year from foodborne illness to be 9,100. Food and Drug Administration (FDA) estimates that about 2 to 3 percent of all foodborne illness cases lead to secondary long-term illnesses as in case of *E. coli* O157:h7, *Salmonella*, *Listeria*, *Yersinia enterocolitica*, and *Staphylococcus aureus*. Foodborne illness costs the U.S. economy billions of dollars each year in lost productivity, recalled products, hospitalization, legal liability, long-term disability and even death as these statistics reveal (Anonymous, 1999). The Gram positive pathogenic bacteria selected for this study included both infectious bacteria such as *Listeria monocytogenes* and toxigenic bacteria such as *Staphylococcus aureus*. Both *L. monocytogenes* and *S. aureus* are found in cooked ready-to-eat foods, and better methods are needed to control their growth and survival. The Gram negative bacterial pathogens selected for our study included all of the major agents causing bacterial foodborne infections in the United States except for *Campylobacter jejuni*. *C. jejuni* was not included in this study since it does not grow under refrigeration or at room temperature.

Many microorganisms have been involved in food spoilage, thus economic loss. Representative spoilage organisms were selected for this study based upon the frequency of their occurrence in foods of various types. The most common gram negative spoilage bacterium generally found in refrigerated foods is *Pseudomonas spp.* (Jay, 1996). *Lactobacillus plantarum* and other lactobacilli are frequently the cause of spoilage of vacuum packaged food and dairy products. *Aspergillus spp.* are found worldwide and are

the most commonly isolated mold along with *Penicillium* spp. *Geotrichum candidum*, is commonly found in food processing plants and has been given the name “machinery mold” (Jay, 1996). *Rhodotorula* are incriminated in spoilage of many refrigerated foods, especially in highly perishable refrigerated meat, dairy, vegetable and fruit products (Jay, 1996).

*Listeria monocytogenes* is a small Gram-positive, non-sporeforming, catalase positive, and nonacid-fast rod (Jay, 1996). *L. monocytogenes* is a psychrotrophic microorganisms and can grow at temperatures as low as 0.5°C with a maximum growth temperature of 45°C (Ryser and Marth, 1991; Jay, 1996) It can grow in the presence of 10-40% bile, 10% NaCl, 0.04% potassium tellurite, and 0.025% thallous acetate (Jay, 1996). *Listeria* grow at a pH range of 4.1-9.6 and a minimum  $a_w$  of 0.92 (Jay, 1996). *Listeria* is widely distributed in nature and can be found in soil, sewage, animal feces, and water (Ryser and Marth, 1991). Since *L. monocytogenes* has the potential to grow in a low pH product such as a fish marinade at low temperatures and has been isolated from fish (Draughon et al., 1999) from retail stores, it was included in this study.

*Staphylococcus aureus* is a small spherical, gram-positive, catalase-positive, coagulase-positive bacterium which has a diameter ranging from 0.5-1.5:μm (Doyle et al., 1997). It is a mesophile but can grow over a range of 7-47.8°C, and enterotoxins are produced between 10-46°C. *S. aureus* grows well at 7-10% NaCl concentration and can grow at 20% within a pH range of 4.0-9.83. *Lactobacillus plantarum* is a Gram-positive, catalase negative rod that is often found in long chains. It is typically microaerophilic but some anaerobic strains may exist (Jay, 1996). It is a mesophilic microorganism but can

grow below 5°C and at 45°C and in a pH range of 3.2-9.6.

*E. coli* O:157:H7, *S. typhimurium* and *Y. enterocolitica* are small, Gram-negative nonsporing, facultatively anaerobic rod. All are members of the large bacterial family, *Enterobacteriaceae*. They are widely distributed in nature, and are found in the intestinal microflora of human and warm-blooded animals (Ayres, 1980; Doyle et al., 1997). *E. coli* O:157:H7 has an optimum temperature for growth and toxin production at 37°C (12-42°C). This particular strain (O:157:H7) does not grow at 44.5°C, but can tolerate low pH (as low as 3.65) and high NaCl content ( $\geq 8.5\%$ ) (Jay, 1996). *E. coli* O:157:H7 causes hemolytic uremic syndrome (HUS) and hemorrhagic colitis (Doyle et al., 1997). *S. typhimurium* is also a mesophilic microorganism with an optimum temperature for growth of 37°C and a range of 6.2-45°C. The optimum pH is 7.0 with a range of 5.5-9. It is sensitive to high salt content and nitrite (Doyle et al., 1997).

*Y. enterocolitica* is a psychrotrophic organisms which grows at a temperature range of -2 to 45°C with the optimum of 22-29°C and a wide pH range of 4.6 to 9.6 (Doyle et al., 1997). *Pseudomonas aeruginosa* is a Gram-negative, non-fastidious, psychrotrophic, aerobic, fluorescent, long rod (Jay, 1996). It is widely distributed in nature (Jay, 1996). *Pseudomonas* spp. are incriminated in spoilage of refrigerated foods especially highly perishable refrigerated meat products. *Pseudomonas* spp. are not considered to be pathogenic and are primarily a spoilage problem (Anonymous, 1998).

*Aspergillus niger* and *Geotrichum candidum* are conidial fungi (Fungi imperfecti). Aspergilli appear yellow to green to black on foods. *Aspergillus* spp. are found in various habitats and under many different environmental conditions. Aspergelli are xerotolerant

and can withstand conditions of low moisture and may grow under refrigeration ( $a_w > 0.85$ ) (Jay, 1996). *G. candidum* is referred to as a “machinery mold” since it builds up on food contact equipment in food processing plants (Arora, 1991, Jay, 1996). It is widespread and has been incriminated in spoilage of fruits, vegetables, meat, and dairy products (Jay, 1996). *Rhodotorula* are non-fermenting yeasts (Jay, 1996). They have a mucous, pasty, dry or wrinkled appearance, and produce pink to red pigments (Ayres, 1980). They are sometimes called the “dairy yeast” because they cause spoilage of fermented dairy products such as sour cream. *Rhodotorula* spp. are psychrotrophic organisms and are a major spoilage yeast found on fish, shrimp, poultry, beef, and dairy products (Arora, 1991; Jay, 1996).

## ANTIMICROBIALS IN FOODS

The use of conventional antimicrobials in foods is to preserve the food, increase shelf-life, add flavor, and improve the texture and nutritional value (Davidson and Branen, 1993). FDA has defined chemical preservatives as “any chemicals that when added to foods tend to prevent or retard deterioration, but does not include common salt, sugar, vinegar, spices, and spice essential oils”. The antimicrobials that are used in food products must fulfill the legal requirement which are must be non-toxic, be metabolized and excreted by the body, no residues build-up, and overall safety (Davidson and Branen, 1993). The disadvantages of using chemicals preservatives in foods, are off-color, off-flavor, texture problems, side effects when used in a high dose (allergy problem and

toxicity for human and experimental animals) and decreased nutritional value, which explains why these chemicals are used in very low concentration (Davidson and Branen, 1993; Jay, 1996; Doyle et al., 1997).

Sodium benzoate and benzoic acid were the first chemical preservatives approved by FDA to be used in food (Jay, 1996). Benzoic acid has a low cost, is easy to conglomerate into food products, lacks color, and is almost non-toxic which made it widely used in foods as an antifungal agents (Doyle et al. 1997). It is Generally Recognized As Safe (GRAS) with a maximum use in foods of 0.1% (Jay, 1996). Sorbic acid is naturally occurring in berries and it also considered a GRAS food additive, and its permissible limit to use in food is 0.2% (Davidson and Branen, 1993). Other organic acids are permitted by FDA to be used in foods but in low concentration such as: acetic acid, citric acid, fumeric acid, lactic acid, malic acid, propionic acid, tartaric acid, also sulfite, and nitrite (Jay, 1996).

### **DIMETHYL DICARBONATE**

DMDC is a colorless, fruity smelling liquid, slightly soluble in water with a melting point of 15.2°C and a boiling point of 123-149°C (Davidson and Branen, 1993). DMDC activity is affected by the variables of pH, temperature, and the amount of other reactive substances during food processing (Jay, 1996). Terrell and his co-workers (1993) reported that low pH, high alcoholic content and high temperature (40°C) increase the microbial destruction activity of DMDC. Other workers showed that high

temperature (30°C) and high alcohol level were synergistic and made DMDC more effective as an antifungal agent (Porter and Ough, 1982). It is a very reactive compound, which will break down autocatalytically in the presence of any proton donor and is rapidly hydrolyzed into carbon dioxide and methanol (Jay, 1996).

Yeast is the primary target microorganism for DMDC, it also affects some bacterial species such as *Acetobacter pasteurianus*, *E.coli*, *P.aeruginosa*, *S. aureus*, *Lactobacillus*, and *Pediococcus cerevisiae*. Molds are more resistant to DMDC (Doyle et al., 1997). FDA has stated the legal limit of DMDC in wine at bottling to be 200mg/liter to inhibit fermentative yeasts.

## ANTIBIOTIC SENSITIVITY

Antibiotics may not be used as food additives in U.S. (Jay, 1996). Antibiotic resistance of foodborne pathogens is an increasing concern to public health officials (Doyle et al., 1997). Antibiotics are classified based on their mechanism of action, as follows: agents that inhibit synthesis of bacterial cell wall and often cell lysis, agents that act directly on the cell membrane of the microorganisms, agents that affect the function of the bacterial ribosomes, agents that alter protein synthesis in the microorganisms, and agents that affect nucleic acid metabolism (Gilman et al., 1990). Tetracyclin is a broad-spectrum antibiotic which enter microorganism by passive diffusion and affects the function of bacterial ribosomes to cause reversible inhibition of protein synthesis (Katzung, 1992). Resistant bacteria may lack passive permeability to tetracyclin (Gilman

et al., 1990). Chloramphenicol inhibits protein synthesis in bacteria. The drug is readily penetrates into bacterial cells by a process of facilitated diffusion which make this antibiotic possesses a fairly wide spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria (O'Grady et al., 1997; Gilman et al, 1990).

Penicillin and Ampicillin are  $\beta$ -lactam antibiotics and they are bactericidal for both Gram-positive and Gram-negative bacteria. They inhibit synthesis of or activate enzymes that disrupt bacterial cell wall to cause loss of viability and often cell lysis (Gilman et al., 1990; O'Grady et al., 1997). Some bacteria are resistant due to the production of  $\beta$ -lactamase enzyme (Katzung, 1992).

Neomycin is one of the aminoglycosides, which affect the function of bacterial ribosomes to cause a reversible inhibition of protein synthesis (Gilman et al., 1990). *E. coli* is highly sensitive to neomycin and most of the Gram-negative bacteria are highly resistant to it (Gilman et al., 1990). Nalidixic acid is one of the quinolones which inhibits the production of DNA gyrase and prevent DNA replication (Katzung, 1992). It is bactericidal to most of the common Gram-negative bacteria with the *Pseudomonas aeruginosa* is the most resistant, and it is not effective against Gram-positive bacteria due to the presence of the protective peptidoglycan coat which prevent passive entrance of nalidixic acid (Gilman et al., 1990; O'Grady et al., 1997).

Cephalothin is  $\beta$ -lactam antibiotic, it inhibits bacterial cell wall synthesis and activates autolytic enzymes in the cell wall which result in lesions that cause bacterial death (O'Grady et al., 1997). It is very active against Gram-positive cocci and *E. coli*, *Pseudomonas* spp. are resistant (Gilman et al., 1990). Bacitracin is an antibiotic that

formed from polypeptide mixture. It is highly active against many Gram-positive bacteria (including  $\beta$ -lactamase producing one) by inhibiting cell wall formation (Gilman et al., 1990).

## **FISH AS FOOD**

Fish is the most abundant protein resource in the U.S and constitutes a wide range of human foods (Hobbs, 1987). Increasing fish consumption is related to the fact that fish meat is considered healthy since it is low in fat (Ayres, et al., 1980). Fish also has many biotechnological applications, which include production of enzymes, gelatin, and pharmaceutical protein with antimicrobial and antitumor activity (Benjakul et al, 1996).

Since fish spoilage is a huge economical problem world wide, many methods of preservation have been postulated. These methods include chilling, freezing, mixtures, of crushed ice and NaCl, salt curing and fermentation, smoke curing, heat processing, irradiation, chemical preservation, and modified atmosphere packaging (Hobbs, 1987; Ayres et al., 1980). However, little is known about the mechanism by which these methods influence quality during storage. The most popular method is to use crushed ice for storage, which reduces fish quality by producing soft and smelly product. New methods are needed to improve fish quality and inhibit microbial growth.

Fish is a highly perishable food even under refrigerated storage or on ice (Jay, 1996). It is one of the products that has a limited shelf-life because of production, distribution and low temperature storage conditions which are critical for fish quality and

shelf-life (Stenstrom et al., 1990). Nutrients in fish promote the growth of spoilage and pathogenic microorganisms, these microorganisms are influenced by post-mortem spoilage processes. Common microflora of fish include: *Listeria* spp., *Salmonella* spp., *E.coli*, *Staphylococcus* spp., *Pseudomonas* spp., *Clostridium perfringens*, *C. botulinum* Type E, *Vibrio parahaemolyticus*, enteroviruses, yeasts and molds (Hobbs, 1987; Gram and Huss, 1996). The bacteria are very active metabolically, and produce proteases, lipases, and saccharolytic enzymes which result in fishy or rotten smells (Ayres et al., 1980; Garcia-Carreno et al., 1995; Jay, 1996).

The chemical composition of fish varies widely but in general water content of approximately 80%, protein content of 18%, carbohydrate content of 1%, a lipid content of 1-25 % (variation associated with the fish species and seasonal effect), and minor compounds such as minerals, vitamins, and free amino acids and nitrogenous compounds which contribute to the characteristic odor and flavor of fish species (Hobbs, 1987).

Quality loss can be a consequence of chemical, physical, microbial, or enzymatic reactions (Zwietering et al., 1992; Doyle et al., 1997). Immediately after fish is caught, cellular autolytic enzyme reactions start which eventually lead to spoilage and microbial invasion (Ayres, 1980). The microbial flora of the fresh water fish varies with the season, geographical area, and fish species (Hobbs, 1987). Gram-negative psychrotrophs are the predominant bacterial spoilage microflora in fresh fish and shellfish. The most frequent groups of bacteria found are *Pseudomonas*, *Alteromonas*, *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Vibrio*, *Aeromonas*, and *Cytophaga* spp (Windsor and Thma, 1974; Jay, 1996; Doyle et al., 1997). These spoilage bacteria, especially *Pseudomonas* and

*Moraxella* spp., produce proteases, which give fruity flavor, trimethylamine, hydrogen sulfide, dimethyl sulfide, and methyl mercaptan as end products. These end products cause off-flavor, off-color, and sensory changes which affect the shelf-life and consumer perception of such products (Hobbs, 1987).

Huss (1995) and Gram and Huss (1996) reported that during fish spoilage, the initial microbial contamination and growth is by aerobes which act on carbohydrate and protein, which cause the surface to become covered with slime. The condition becomes more favorable for anaerobes. The anaerobic and facultative microorganisms are incriminated in off-odor and off flavor due to the reduction of trimethylamine oxide into trimethylamine and break down of the sulfur containing amino acids.

Some studies have reported that fresh fish is generally pathogen free, only *C. botulinum* and *V. parahaemolyticus* were found (Hobbs, 1987). Other research has indicated the presence of pathogenic bacteria in fresh-shelled fish. Gobat and Jemmi, (1993) reported that approximately 10.5-14.3% of the tested raw and ready to eat fish are frequently contaminated with *Aeromonas* species. Hanninen and his co-workers (1997) identified *Aeromonas* species in 93% of the tested fish. Another investigation by Ayulo et al. (1994) *E. coli* in 10% of tested fish and *S. aureus* in 20% of tested samples.

Many chemical preservatives have been tested in fish and fish products to increase shelf-life, quality, and safety (Jorgensen et al., 1988). Organic acids (lactic, acetic, propionic, sorbic, cabrylic, formic) have also been used to extend shelf-life but not for microbial inhibition (Hobbs, 1987, Huss, 1992).

## PURPOSE OF CURRENT STUDY

Several study have investigated the antimicrobial activity of some spice or herb essential oils using a few selected microorganisms (Conner and Beuchat, 1984; Ela et al., 1996). However, comprehensive information on the effect of a wide-range of oils on key foodborne pathogenic and spoilage bacteria, yeasts and molds is needed. The antimicrobial activity of these oils in food systems has not been evaluated. Therefore, the objectives of this research are: (1) to produce an essential oil, alcohol or water extract of black cumin (*Nigella sativa*) and evaluate its antimicrobial activity in vitro and in fish and (2) to examine the effectiveness of some herbal and spices essential oils on selected pathogenic and spoilage microorganisms.

## REFERENCES

- Anonymous, 1999. Foodborne illness. available at:[http://dir.yahoo.com/health/Diseases\\_and\\_Conditions/Foodborne\\_illness/](http://dir.yahoo.com/health/Diseases_and_Conditions/Foodborne_illness/)
- Agarwal-R; Kharya-MD; Shrivastava-R. 1979. Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. . Ind. J. Exper. Biol. 17: (11), 1264-1265.
- Arora, D.K., Mukerji, K.G.,Marth, E.H. 1991. Handbook of Applied Mycology. Ch. 16. Liewen, M.B. (Ed.) Antifungal food additives. Marcel Dekker, Inc. N.Y.
- Ayres, J.C., Mundt, J.O., and Sandine, W.E. 1980. Microbiology of foods, Ch. 11, 18, Spices and Condiments, Fish and Shellfish. W.H. Freeman and company.
- Ayulo, A.M.R., Machado, R.A., and Scusse, V.M.. 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. Int. J. Food Microbiol. 24: 171-178.

- Babic, I., Nguyen-the, C., Amiot, M.J., and Aubert, S. 1994. Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeasts. *J Appl. Bacteriol.* 76:135-141.
- Benjakul, S., Seymour, T. A., Morrissey, M. T., and An, H. 1996. Proteinase in pacific whiting surimi wash water: Identification and characterization *J. Food Sci.* 61:1165-1170.
- Charalambous, G. 1994. Spices, herbs and edible fungi, Ch 3, 12, 19, 20. Elsevier Science. B.V. Amsterdam.
- Conner, D.E. and Beuchat, L.R. 1984. Effect of essential oils from plants on growth of spoilage yeasts. *J. Food Sci.* 49: 429-434.
- Davidson, P.M. and Branen, A.L. 1993. Antimicrobials in foods, Ch. 13 in D.E.Conner (ed.) Naturally occurring compounds. Marcel Dekker, Inc, New York.
- Doyle, M.P., Beuchat, L.R., and Montville, T.J. 1997. Food Microbiology Fundamentals and Frontiers. 1<sup>st</sup> ed. ASM Press Washington D.C..
- Draghon, F.A., Anthony, B., Denton, M.E. 1999. *Listeria* species in fresh Rainbow trout purchased from retail markets. *Dairy Food Env. San.*19: (2) 90-94.
- El-Alfy, T.S., El-Fataty, H.M., and Toama, M.A. 1975. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. *Phamazie* 30: (2) 109-111.
- Ela, M.A., El-Shaer, N.S., and Ghanem, N.B. 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. *Pharmazie* 51:(12) 993-995.
- FDA, 1999. Code of Federal Regulations. 21. available at:<http://frwebgate.access.gpo.gov/cgi-bin/multidb.cgi>
- Ferdous, A.J., Islam, S.N., Ahsan, M., and Hasan, C.M. 1992. In vitro antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug-resistant isolates of *Shigella* spp. and isolates of *Vibrio cholera* and *Escherichia coli*. *Phytotherapy research* 6: 137-140.
- Furia, T.E. and Bellanca, N. 1975. Fenarolis Handbook of Flavor Ingredients. 2<sup>nd</sup> ed. CRC Press Ohio.
- Fyfe, L., Armstrong, F., and Stewart, J. 1998. Inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic

acid : the development of synergistic antimicrobial combinations. Int. J. Antimicrobial Agents 9: 195-199.

Garcia-Carreno, F. L., Navarrete Deletro, M. A., Diaz-Lopez, M., Hernandez-cortes, M. P., and Ezquerro, J. M. 1995. Proteinase inhibition of fish muscle enzymes using legume seed extracts. J. Food Prot. 59:312-318.

Garrido, D., Jodral, M., and Pozo, R. 1992. Mold flora and aflatoxin-produced strain of *Aspergillus flavus* in spices and herbs. J. Food Prot. 55: (6) 451-452

Gilman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. Eighth edition. The pharmacological basis of therapeutics. Ch 11<sup>th</sup> chemotherapy of microbial diseases. Pergamon press, NY.

Gobat, P.F. and Jemmi, T. 1993. Distribution of mesophilic *Aeromonas* species in raw and ready to eat fish and meat products in Switzerland. Int. J. Food Microbiol. 20 : 117-120.

Gould, G.W. 1995. New Method Of Food Preservation, 1<sup>st</sup> ed. Blackie Academic and Professional, London.

Gould, G.W. 1996. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Prot. Supplement 82-86.

Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33: 121-137.

Hanninen, M.L., Ovianen, P., and Koski, V.H. 1997. *Aeromonas* species in fish, fish-egg, shrimp and fresh water. Int. J. Food Microbiol. 34: 17-26.

Hobbs, G. 1987. Microbiology of fish. Essays in Agricultural and Food Microbiology. John Wiley and Sons Ltd.

Huss, H.H. 1995. Development and use of HACCP cocept in fish processing. Int. J. Food Microbiol. 15 : 33-44.

Jay, J.M. 1996. Modern Food Microbiology, 5<sup>th</sup> ed. Van Nostrand Reinhold, New York.

Jiang, S. T., Tsao, Wang, Y. T., and Chen, C. S. 1990. Purification and characterization of proteases from milk fish muscle (*Chanos chanos*). J. Agric. Food Chem. 38:1458-1463.

Jorgensen, B.R., Gibson, D.M., and Huss, H.H. 1988. Microbiological quality and shelf life prediction of chilled fish. Int. J. Food Microbiol. 6: 295-307.

- Kandil, O., Radwan, N.M., Hassan, A.B., Amer, A.M., El-Benna, H.A., and Amer, M.M. 1994. Antimicrobial activity of *Nigella sativa* and *Zingiber officinale*. Vet. Med. J. Giza. 42: (1) 159-168.
- Katzung, B.G. 1992. Fifth edition. Basic and clinical pharmacology. Ch 8<sup>th</sup> in Jawetz, E. Chemotherapeutic drugs. Appleton and Lange Norwalk, Connecticut.
- Lachowicz, K.J., Jones, G.P., Briggs, D.R., Bienvenu, F.E., Wan, J., Wilcock, A., and Coventry. 1998. The synergistic preservative effects of essential oil of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. Lett.App. Microbiol. 26: 209-214.
- Mahmoud, H.M.A. 1993. Inhibitory action of black cumin (*Nigella Sativa*) against *Listeria monocytogenes*. Alex. J. Agric. Res. 38 (1):123-134.
- Maragal, M.M., Chandrasekhar, T.C., and Saralaya, K.V. 1988. Studies on the preservation of fish sausage in natural casing with chemical preservatives and smoking. J. Agric. Sci. 22: 530-537.
- Manderfeld, M.M., Schafer, H.W., Davidson, P.M., and Zottola, E.A. 1997. Isolation and identification of antimicrobial furocoumarins from parsley. J. Food. Prot. 60: (1) 72-77.
- Meena, M.R. and Sethi, V. 1994. Antimicrobial activity of essential oils from spices. J Food Sci.Technol. 31 (1): 68-70.
- Nergiz, C. and Otles,S. 1993. Chemical composition of *Nigella sativa* L. seeds. J. Food Chem. 48:259-297.
- Ochocka, R.J. and Lamparczyk, H. 1993. Evaluation of essential oils from the family umbelliferae using principle component analysis. Pharmazie 48:229-231.
- O'Grady, F., Lambert, H.P., Finch, R.G., and Greenwood, D. 1997. Antibiotic and chemotherapy.seventh edition. Churchill Livingstone, N.Y.
- Paster, N., Menasherov, M., Ravid, U. and Juven, B. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot. 58 (1):81-85.
- Phillipson, J.D. 1994. Herbal Drugs and Phytopharmaceuticals. 2<sup>nd</sup> ed. Med Pharm Scientific Publishers, Stuttgart.
- Poter, L.J. ang Ough, C.S.. 1982. The effect of ethanol, temperature, and dimethyl

- dicarbonate on viability of *Saccharomyces servevisae*. Montrachet No.522 in wine. Am. J. Enol. Vitic. 33: 222-225.
- Rahman, A.U., Malik, S., Hasan, S.S., Choudhary, M.I., Zhou, C., and Clardy, J. 1995. Nigellidine a new indazole alkaloid from the seeds of *Nigella sativa*. Tetrahedron Letters 36 (12) 1993-1996.
- Rathee, P.S., Mishra, S.H., and Kaushal, R. 1982. Antimicrobial activity of essential oil, fixed oil and unsaponifiable matter of *Nigella sativa* Linn. Ind. J Pharm. Sci. 44: (1) 8-10.
- Ryser, E.T. and Marth, E.H. 1991. *Listeria*, Listeriosis, and Food Safety. Marcel Dekker, Inc., New York.
- Salmeron, J., Jordano, R. and Pozo, R. 1990. Antimycotic and antiaflatoxinogenic activity of oregano *Origanum vulgare* L. and thyme (*Thymus vulgaris*, L.). J. Food Prot. 53: (8) 697-700.
- Saxena, A.P. and Vyas, K.M. 1986. Antimicrobial activity of seeds of ethnomedicinal plants. J. Econ Tax. Bot. 8 (2): 291-299.
- Sayed, M.D. 1980. Traditional medicine in health care. J. Ethnopharma 2: 19-22.
- Stenstrom, I.M. and Molin, G. 1990. Classification of the spoilage flora of fish, with special reference to *Shewanella putrefaciens*. J. Appl. Bacteriol. 68: 601-618.
- Terrell, F.R., Morris, J.R., Johnson, M.G., Gbur, E.E., and Makus, D.J.. 1993. Yeast inhibition in grape juice containing sulfur dioxide, sorbic acid, and dimethyldicarbonate. J. Food Sci. 58: (5) 1132-1134
- Toppozada, H.H., Mazloun, H.A., and El-Dakhakhny. 1964. The antibacterial properties of the *Nigella sativa* L. seeds. Active principle with some clinical applications. J. Egypt. Med. Assoc. 48: 178-202.
- Vera, R.R. and Ming, J.C. 1998. Chemical composition of essential oil of dill (*Anethum Graveolens* L.) growing in reunion island. J. Essent. Oil Res. 10: 539-542.
- Wilkins, K.M. and Board, R.G. 1989. Natural antimicrobial systems ch 11. In Gould G.W. Mechanisms of action of food preservation procedures. Elsevier Applied Science, London.
- Zaika, L.L., Kissinger, J.C., and Wasserman, A.E. 1983. Inhibition of lactic acid bacteria by herbs. J. Food Sci. 48: 1455-1459.

Zwietering, M.H., Wiltzes, T., Wit, J.C., and Riet, K.V. 1992. A decision support system for prediction of microbial spoilage in foods. *J. Food Prot.* 55: (12) 973-979.

**Part II**  
**Antimicrobial Activity of Essential Oils from Plants Against Selected Pathogenic  
and Saprophytic Microorganisms**

## ABSTRACT

The antimicrobial activity of extracts from many types of plants, which are used as seasoning agents in foods and beverages, has been claimed for centuries. The purpose of this study was to examine the effectiveness of selected herb and spice essential oils for control of growth and survival of microorganisms. Inhibition of growth was tested by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum lethal concentration (MLC) was determined by the tube dilution method. Essential oils from anise, angelica, basil, carrot, celery, cardamom, coriander, dill weed, fennel, oregano, parsley, and rosemary were evaluated. Inhibition ranged from complete with oregano, to no inhibition with carrot oil for each of the test strains which included: *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O:157:H7, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Aspergillus niger*, *Geotrichum*, and *Rhodotorula*. Oregano essential oil showed the greatest inhibition (zone  $\geq 70$ -80 mm) (MLC  $\sim 8$  ppm). Coriander and basil were also highly inhibitory (MLC  $\sim 25$  - 50 ppm) to *E. coli* O:157:H7 and other bacteria tested. *Pseudomonas* was the most resistant microorganism to the spice oils but still had a MLC in the 100 to 200 ppm range using oregano, basil and coriander oils. Anise oil was not particularly inhibitory to bacteria (inhibition zone  $\sim 25$  mm), however, anise oil was highly inhibitory to molds. Basil, coriander, and oregano were also highly inhibitory to the fungi assayed. Since some of the herbal and spice essential oils are highly inhibitory to selected pathogenic and spoilage microorganisms, they may provide alternatives and supplements to conventional

antimicrobial additives in foods.

## INTRODUCTION

Beneficial activity of extracts from many types of plants used as flavoring and seasoning agents in foods and beverages in reducing infection, has been claimed for centuries (Ayres et al., 1980; Charalambous, 1994). Some of these essential oils have antimicrobial properties (Davidson and Branen, 1993). The antimicrobial activities of mustard, cinnamon and cloves have been known since the end of last century, and their oils were thought to hamper microbial spoilage in foods (Saxena and Vyas, 1986). The antimicrobial activities of garlic, onion, mustard, cinnamon and cloves have been heavily studied at the end of last century and their oils were known to hamper microbial spoilage in foods (Saxena and Vyas, 1986; Uda et al., 1993).

The oil extraction from several types of plants either by steam distillation or volatile organic solvent, has been used for years (Conner and Beuchat, 1984; Gould, 1995). It is generally thought that certain herbs and spices have antimicrobial activity and may influence the quality and safety of foods to which they have been added (Arora et al., 1991). Many spices, herbs, and their extracts have antimicrobial activity, almost consistently due to the essential oil fraction (Gould, 1995). These compounds are responsible for the characteristic aroma and flavor of the spices (Gould, 1996). Wilkins and Board (1989) reported that more than 1,340 plants are known to be potential sources of antimicrobial compounds. However oil extracts from only few of these plants

have been scientifically studied. Ela and his co-workers (1996), screened sixteen essential oils for their antimicrobial activity against *S. aureus*, *E. coli*, and the fungi *A. niger* and *Candida albicans*, by using the agar diffusion technique and measuring their inhibition zones. They classified the tested oils according to their activity, strongly active (inhibition zone >8mm), moderately active (inhibition zone >6-<8mm), and inactive (no inhibition, zone < 6 mm). Basil and parsley showed potent activity against *S. aureus*, *E. coli*, *A. niger* and *Candida albicans*. Conner and Beuchat (1984) investigated the antimicrobial effect of thirty-two essential oils on thirteen food-spoilage and industrial yeasts. They categorized the essential oils as strongly active (inhibition zone >11mm), moderately active (inhibition zone >6-<11mm) or inactive (inhibition zone <6mm). They found that oregano was the most effective inhibitor against yeasts, anise, basil, cardamom, celery, dill, fennel, parsley and rosemary were less effective against yeasts.

Previous research has shown that, essential oils from anise, basil, fennel, coriander basil, parsley, carrot oregano, and rosemary have a variable antimicrobial and antifungal against wide range of foodborne organisms (Zaika et al., 1983; Babic et al., 1994; Meena and Sethi, 1994; Manderfeld et al., 1997; Fyfe et al., 1998; Lachowicz et al., 1998). The antimicrobial activity of the common spice essential oils which have not been thoroughly studied or which have been only briefly investigated is needed, especially in relation to foodborne pathogens.

This study was undertaken to investigate the effectiveness of selected herbal and spice essential oils on survival and growth of selected pathogenic and spoilage microorganisms.

## MATERIALS AND METHODS

### Essential Oils

The steam distilled essential oils of anise, angelica, basil, cardamom, carrot, celery, coriander, dill, fennel, parsley, oregano, and rosemary were purchased from the Essential Oil Company which supplies foodgrade oils. The oils were stored in dark bottles and kept refrigerated per manufacturer's recommendation until evaluation.

### Culture Maintenance and Inoculum Preparation

*Escherichia coli* O157:H7 (ATCC 35150, apple cider), *Lactobacillus plantarum* (ATCC 14917), *Listeria monocytogenes* Scott A (meat isolate), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 13563), *Yersinia enterocolitica* (ATCC 228), *Aspergillus niger* (UT stock culture), *Geotrichum candidum* (ATCC 34614), and *Rhodotorula* (ATCC 32765) were used. Test strains of bacteria were inoculated into Brucella broth (Difco; Detroit, MI) and incubated at 35°C for 24h. Cultures were subjected to three successive 24-h transfers before use. All cultures were kept refrigerated on tryptose soy agar slants during the experiment (up to two weeks) and were tested biochemically for viability and purity before each use.

*Aspergillus niger* and *Geotrichum candidum* cultures were maintained on potato dextrose agar (PDA) at 4 °C and revived and cultivated on PDA at 25 °C. *Rhodotorula* cultures were maintained on yeast and mold agar (YMA) and revived on YM broth at 25°C. Yeast and mold cultures were subjected to two successive transfers in YM broth

and tested for purity and viability using biochemical and morphological characteristic, before each use.

### **Assay for Antimicrobial Activity**

Antimicrobial activity was tested by the filter paper disc diffusion method as described by Meena and Sethi (1994). Standard method agar (SMA) (Difco, Detroit, MI) with 100 ppm of 2,3,5-triphenyl tetrazolium chloride (TTC) (Fisher, Fairlawn, NJ) to distinguish bacterial colonies and to clarify the zone of inhibition, was used for antibacterial assay. After sterilization and cool down, TTC was added and plates were poured and allowed to set at room temperature for 24h to enable the agar to solidify and dry slightly. SMA without TTC added was used for antifungal assay. Each plate was inoculated with bacterial and/or fungal culture directly from the broth (no more 48 hs old) and spread uniformly using a sterile loop to completely cover the agar surface. The plates were allowed to set undisturbed for 30 minutes to permit culture to be absorbed by the media for 30 minutes. One drop of each essential oil (average weight = 24 µg) was placed on each sterile 6-mm diameter absorbent blank filter paper discs (Difco, Detroit, MI) which was placed in the center of the inoculated plates. The plates were incubated at 32°C for 2-4 days, after which the inhibition zones were measured and recorded in millimeters (mm). The scale of measurement was as the following:  $\geq 28$  mm zone of inhibition is strongly inhibitory;  $< 28 - 16$  mm zone of inhibition is moderately inhibitory;  $< 16-10$  mm zone of inhibition is mildly inhibitory and  $< 12$  mm is non inhibitory inhibitoryControl plates were prepared by placing sterile water on disks for

negative controls, and antibiotic disks were used for comparison. The antibiotics used were cephalothin 30 µg, amoxicillin 20 µg and clavulanic acid 10µg, nalidixic acid 30µg, tetracycline 30 µg, and neomycin 5 µg from Difco (Detroit, MI). From BBL (Cockeysville, MD), penicillin 10 IU, tetracycline 5 µg, bacitracin 30µg, and chloramphenicol 30 µg. All tests were conducted in triplicates (3 samples / rep) with a minimum of three replications for each antibiotic.

### **MLC Setup**

The minimum lethal concentration was measured by using 2-fold serial broth dilution (with 2 ml oil initially added to 20 ml Brucella broth (Difco, Detroit, MI) and 2 ml Tween 80 emulsifier), for all oils that showed inhibitory activity to tested bacteria. All tubes were incubated at 32°C for 72 hs. All the tests were conducted in triplicates. Controls consisted of tween 80 emulsifier with addition of cultures to broth. The results were measured visually and by plating on SMA plates (incubated for 24-48 hs at 32°C) and recorded as (+) for growth or (-) for no growth. MIC was not determined due to the optical density variations and differences in cultures using different oils.

### **Microbial Analysis of Essential Oils**

All tested oils were subjected to microbial analysis for quality control. Each oil (0.1 ml) was plated onto the surface of SMA and Rose Bengal (RBA-C) (Difco Detroit MI) agar by using a sterile bent glass rod. SMA plates were incubated at 32°C for 48 hs and RB plates at room temperature ( $\cong$ 25°C for up to one week). All tests were conducted

with three replications.

### **Experimental Design and Statistical Analysis**

This experiment was performed as a completely randomized block by replications (3). Statistical analysis was conducted using microcomputer SAS version 6.1 (SAS institute; Cary, NC) to determine differences in antimicrobial activity of tested spice and herb essential oils in comparison to controls. Data were fit to a completely randomized design, with factorial treatment arrangement, and analyzed using PROC MIXED.

## **RESULTS AND DISCUSSION**

Antimicrobial inhibition zones resulting from paper disc assay for essential oils against foodborne pathogens, spoilage bacteria, yeasts and molds are shown in Table 1. Antimicrobial activity of herb and spice essential oils ranged from no inhibition to complete inhibition against the test microorganisms.

Oil of oregano completely inhibited growth of the Gram-negative pathogens tested (*S. typhimurium*, *Y. enterocolitica* and *E. coli*), *S. aureus* and fungi imperfecti (*A. niger*, *Geotrichum* and *Rhodotorula*). Oregano was significantly less effective in inhibiting *P. aeruginosa* ( $P < 0.001$ ), although still strongly inhibitory (zone = 45.7 mm). *L. monocytogenes* was also strongly inhibited (zone = 52.7 mm) but oregano was significantly less effective against *L. monocytogenes* compared to other microorganisms. These data confirm the findings that oregano inhibits *Aspergillus* spp. (Salmeron et al., 1990; Paster et al., 1995). Biondi et al. (1993) also reported that oregano exhibited

Table 1. Inhibition zones (mm<sup>1</sup>) of growth of tested microorganisms by essential oils

|           | <i>L.monocytogenes</i> | <i>L.plantarum</i> | <i>S.aureu</i>    | <i>E.coli</i>    | <i>S.typhimurium</i> | <i>Y.enterocolitica</i> | <i>P.aeruginosa</i> | <i>A.niger</i>   | <i>G.candidum</i> | <i>Rhodotorula</i> |
|-----------|------------------------|--------------------|-------------------|------------------|----------------------|-------------------------|---------------------|------------------|-------------------|--------------------|
| Anise     | 0 <sup>dw</sup>        | 25 <sup>eu</sup>   | 26 <sup>fu</sup>  | 19 <sup>ev</sup> | 22 <sup>cu</sup>     | 34 <sup>el</sup>        | 0 <sup>ew</sup>     | 56 <sup>el</sup> | 42 <sup>es</sup>  | 0 <sup>dw</sup>    |
| Angelica  | 24 <sup>br</sup>       | 0 <sup>n</sup>     | 21 <sup>br</sup>  | 11 <sup>ss</sup> | 12 <sup>es</sup>     | 23 <sup>br</sup>        | 12 <sup>es</sup>    | 0 <sup>n</sup>   | 12 <sup>hs</sup>  | 22 <sup>cr</sup>   |
| Basil     | 15 <sup>cu</sup>       | 0 <sup>fv</sup>    | 87 <sup>sar</sup> | 52 <sup>bl</sup> | 79 <sup>bs</sup>     | 87 <sup>ar</sup>        | 15 <sup>cu</sup>    | 87 <sup>ar</sup> | 77 <sup>bs</sup>  | 87 <sup>ar</sup>   |
| Cardamom  | 12 <sup>cv</sup>       | 43 <sup>bs</sup>   | 39 <sup>dl</sup>  | 12 <sup>ev</sup> | 18 <sup>du</sup>     | 57 <sup>cr</sup>        | 19 <sup>bu</sup>    | 18 <sup>du</sup> | 20 <sup>eu</sup>  | 0 <sup>dw</sup>    |
| Carrot    | 0 <sup>dr</sup>        | 0 <sup>fr</sup>    | 0 <sup>hr</sup>   | 0 <sup>hr</sup>  | 0 <sup>fr</sup>      | 0 <sup>hr</sup>         | 0 <sup>er</sup>     | 0 <sup>fr</sup>  | 0 <sup>ir</sup>   | 0 <sup>dr</sup>    |
| Celery    | 22 <sup>bu</sup>       | 0 <sup>fw</sup>    | 24 <sup>fu</sup>  | 13 <sup>fv</sup> | 13 <sup>fv</sup>     | 38 <sup>el</sup>        | 13 <sup>cv</sup>    | 16 <sup>dv</sup> | 41 <sup>cs</sup>  | 45 <sup>br</sup>   |
| Coriander | 21 <sup>bu</sup>       | 12 <sup>ew</sup>   | 87 <sup>ar</sup>  | 37 <sup>cl</sup> | 22 <sup>cu</sup>     | 67 <sup>bs</sup>        | 19 <sup>bv</sup>    | 87 <sup>ar</sup> | 87 <sup>ar</sup>  | 87 <sup>ar</sup>   |
| Dill      | 0 <sup>dv</sup>        | 0 <sup>fv</sup>    | 70 <sup>br</sup>  | 36 <sup>cr</sup> | 26 <sup>cl</sup>     | 47 <sup>ds</sup>        | 0 <sup>ev</sup>     | 12 <sup>eu</sup> | 38 <sup>dr</sup>  | 44 <sup>bs</sup>   |
| Fennel    | 0 <sup>dw</sup>        | 16 <sup>du</sup>   | 39 <sup>dl</sup>  | 14 <sup>fu</sup> | 18 <sup>du</sup>     | 57 <sup>cr</sup>        | 11 <sup>dv</sup>    | 60 <sup>br</sup> | 43 <sup>cs</sup>  | 0 <sup>dw</sup>    |
| Parsley   | 21 <sup>bu</sup>       | 0 <sup>fx</sup>    | 34 <sup>es</sup>  | 13 <sup>fw</sup> | 16 <sup>dv</sup>     | 30 <sup>n</sup>         | 16 <sup>cv</sup>    | 17 <sup>dv</sup> | 29 <sup>cl</sup>  | 44 <sup>br</sup>   |
| Oregano   | 53 <sup>ss</sup>       | 87 <sup>ar</sup>   | 87 <sup>ar</sup>  | 87 <sup>ar</sup> | 87 <sup>ar</sup>     | 87 <sup>ar</sup>        | 46 <sup>el</sup>    | 87 <sup>ar</sup> | 87 <sup>ar</sup>  | 87 <sup>ar</sup>   |
| Rosemary  | 0 <sup>dv</sup>        | 23 <sup>cl</sup>   | 45 <sup>cr</sup>  | 23 <sup>dl</sup> | 23 <sup>cl</sup>     | 35 <sup>es</sup>        | 13 <sup>cu</sup>    | 0 <sup>fv</sup>  | 24 <sup>n</sup>   | 0 <sup>dv</sup>    |

1. The diameter of the filter paper discs (6mm) is included.

2. 0 means no inhibition (<6 mm diameter).

3. Least square means in columns followed by different letters (a-i), are significantly different ( $p < 0.001$ ).

4. Least square means in rows followed by different letters (r-z), are significantly different ( $p < 0.001$ ).

5. Complete inhibition.

complete inhibition activity against *S. aureus* or *E. coli* and antifungal activity against *A. niger*. Conner and Beuchat (1984) reported that oregano essential oil was strongly inhibitory for thirteen yeasts at the 10% level. Our data showed that *L. plantarum* was completely inhibited by essential oil of oregano. Earlier studies have demonstrated that oregano progressively delayed growth and acid production of lactic acid bacteria (Zaika et al., 1983; Kivanc et al. 1991). Oil of oregano exhibited the highest antimicrobial activity of any essential oil in our study against all test microorganisms (inhibition zone = 45-87 mm).

Inhibition zones of oil of oregano and other essential oils, which were inhibitory, were significantly greater than all antibiotic positive controls ( $p < 0.001$ ) (Table 2). The powerful antimicrobial activity of oregano against Gram negative pathogens, *S. aureus* and to a lesser extent against *L. monocytogenes* suggests that oregano oil may be useful in some food formulations as an antimicrobial. *Salmonella* and *Yersinia* were resistant to neomycin, ampicillin, bacitracin, and penicillin as were most of the other gram-negative bacteria. As might be expected, the fungi and *Lactobacillus* were not inhibited by any of the antibiotic disks (negative controls).

Oil of coriander completely inhibited growth of the *S. aureus* and fungi imperfecti (*A. niger*, *Geotrichum* and *Rhodotorula*) (Table 1). Coriander was less effective in inhibiting *Y. enterocolitica* and *E. coli* ( $P < 0.001$ ), although still strongly inhibitory (zone = 66.7 and 36.7 mm respectively). Coriander was moderately inhibitory to *L. monocytogenes*, *S. typhimurium*, *P. aeruginosa* (zone = 20.7, 21.3, and 18.3 mm

**Table 2: Inhibition zones (mm<sup>1</sup>) of growth of tested microorganisms by antibiotics.**

|                | <i>L.monocytogenes</i> | <i>L.planarum</i> | <i>S.aureus</i> | <i>E.coli</i> | <i>S.typhimurium</i> | <i>Y.enterocolitica</i> | <i>P.aeruginosa</i> | <i>A.niger</i> | <i>G.candidum</i> | <i>Rhodotorula</i> |
|----------------|------------------------|-------------------|-----------------|---------------|----------------------|-------------------------|---------------------|----------------|-------------------|--------------------|
| Tetracycline   | 12                     | 0 <sup>2</sup>    | 28              | 19            | 22                   | 23                      | 11                  | 0              | 0                 | 0                  |
| Neomycin       | 0                      | 0                 | 0               | 0             | 0                    | 0                       | 0                   | 0              | 0                 | 0                  |
| Chloamphenicol | 10                     | 0                 | 30              | 26            | 26                   | 30                      | 20                  | 0              | 0                 | 0                  |
| Cephalothin    | 0                      | 0                 | 25              | 17            | 24                   | 14                      | 12                  | 0              | 0                 | 0                  |
| Ampicillin     | 0                      | 0                 | 0               | 0             | 0                    | 0                       | 0                   | 0              | 0                 | 0                  |
| Bacitracin     | 0                      | 0                 | 11              | 0             | 0                    | 0                       | 0                   | 0              | 0                 | 0                  |
| Naladixic acid | 10                     | 0                 | 0               | 30            | 29                   | 34                      | 18                  | 0              | 0                 | 0                  |
| Penicillin     | 0                      | 0                 | 0               | 0             | 0                    | 0                       | 0                   | 0              | 0                 | 0                  |

1. The diameter of the antibiotic discs (6mm) is included.

2. 0 means no inhibition (<6 mm diameter).

respectively). *L. plantarum* was highly resistant to the antimicrobial activity of coriander (zone = 11.3). These data confirm the reports that seeds and essential oil of coriander may have both antibacterial (*E. coli*, *L. plantarum*, *S. aureus*, *Y. enterocolitica*) and antifungal effect (Charalambous, 1994).

Conner and Beuchat (1984) stated that oil of coriander was only slightly inhibitory toward different genera of yeasts selected for evaluation. Our results showed that coriander completely inhibited *Rhodotorula* (zone > 87 mm). In addition, Meena and Sethi (1994) found that coriander has no inhibitory effect on *A. niger* and a mild inhibitory effect on *L. acidophilus* at both room temperature and at 37°C. Our results showed that coriander completely inhibited *A. niger* (zone > 87 mm), and that it had weak or no effect on *L. plantarum*. Inhibition zones of coriander were significantly greater than all tested antibiotics except against *S. typhimurium* and *P. aeruginosa*. Gould (1996) suggested that coriander contains high levels of antimicrobials with potential food use. However, the effects of coriander appear to be highly variable depending on cultural conditions and species. This variation may limit commercial applications.

Oil of anise (Table 1) was inhibitory for all organisms in our study except for *L. monocytogenes*, *Pseudomonas*, and *Rhodotorula*. Greatest inhibition of growth by anise was found against *Yersinia enterocolitica*, *A. niger*, and, *G. candidum* (zone =34, 56 and 41.3 mm respectively). Anise was also moderately inhibitory against *L. plantarum*, *S. aureus*, *E. coli* and *S. typhimurium* (zone =24.7, 25.7 and 22 mm respectively). Conner and Beuchat (1984) investigated the antimicrobial effect of essential oils of anise, and stated that these oils had only mild effects on all tested yeasts which is consistent with our

findings. Other research has shown that anise was strongly antimicrobial and antifungal against *L. plantarum*, *L. monocytogenes*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, *Y. enterocolitica*, *Rhodotorula*, *Saccharomyces* spp. and several molds (Lachowicz et al., 1998). Differences reported between the Lachowicz et al. (1998) study and ours may be due to a difference in concentration of anise essential oil or cultural characteristics of the organisms used.

Oil of angelica did not completely inhibit any of the bacteria used in our study and was only moderately inhibitory (Table 1) against *L. monocytogenes*, *S. aureus*, *Y. enterocolitica*, and *Rhodotorula* (zone =23.3, 20.3, 23 and 22 mm respectively). Oil of angelica was weakly inhibitory against *E. coli*, *S. typhimurium*, *P. aeruginosa*, *G. candidum* (zone =10.3, 11.7, 12 and 12 mm respectively), and had no inhibitory effect against *L. plantarum* and *A. niger*. This is the first scientific study to report antimicrobial activity of angelica. This was also one of the few oils which had antimicrobial activity against *L. monocytogenes* in our study.

Oil of basil (Table 1) completely inhibited growth (zone = 87 mm) of *S. aureus*, *Y. enterocolitica* and the fungi imperfecti (*A. niger* and *Rhodotorula*). Basil was also strongly inhibitory to *E. coli*, *S. typhimurium* and *G. candidum* ( $P<0.001$ )(zone = 51.3, 78.8 and 76.3 mm respectively). Basil was only mildly inhibitory against on *L. monocytogenes* and *P. aeruginosa* (zone = 14.7 and 14.7 mm respectively). No inhibitory effect was shown against *L. plantarum* which was highly resistant to the antimicrobial activity of basil. Therefore, the inhibitory effect of basil was highly dependent on the organisms evaluated. With the exception of *S. aureus*, the Gram-positive organisms were

inhibited less by basil than the gram-negative organisms.

Several investigators have studied antimicrobial effectiveness of basil with varying results. Lachowicz et al. (1998) found that essential oils of five different varieties of basil possessed a mild antimicrobial activity against three gram-positive bacteria (*L. plantarum*, *L. monocytogenes*, *S. aureus*) and several gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. typhimurium*, *Y. enterocolitica*), yeasts (*Rhodotorula*) and molds. Fyfe et al. (1998) reported that oil of basil at 0.2% was a potent inhibitor of *L. monocytogenes* and *S. enteritidis*. Meena and Sethi (1994) also found that basil essential oil has a moderate antimicrobial effect on *A. niger* and *L. acidophilus*. Our data expands and support the findings of Ela et al. (1996), who showed that basil essential oil had antibacterial and antifungal activity against *S. aureus*, *E. coli* and *A. niger*.

Our data showed that carrot essential oil (Table 1) was not inhibitory to any of the tested microorganisms. Babic et al. (1994) reported that purified ethanolic extracts of peeled and shredded carrot have an antimicrobial effect against a range of foodborne microorganisms such as *L. monocytogenes*, *S. aureus* and *E. coli*. It should be noted that the ethanol or water extract of carrot gives highly polar compounds whereas, the essential oil extract gives both non-polar and polar compounds. Steam distillation is used to prepare essential oils and gives more volatile compounds which are often associated with flavor or odor of herbs.

Oil of celery was strongly inhibitory against *Y. enterocolitica*, *G. candidum* and *Rhodotorula* (zone = 37.7, 40.3 and 44.3 mm respectively), moderately inhibitory against *L. monocytogenes* and *S. aureus* (zone = 22 and 24 mm respectively), had a weak

inhibitory effect on *E. coli*, *S. typhimurium*, *P. aeruginosa* and *A. niger* (zone = 13, 12.7, 13 and 15.3 mm respectively), and did not inhibit against *L. plantarum*. Conner and Beuchat (1984) reported weak inhibitory activity of essential oil of celery on yeasts.

Oil of dill had a strong antimicrobial effect against *S. aureus*, *E. coli*, *Y. enterocolitica*, *G. candidum* and *Rhodotorula* (zone = 69.3, 35.3, 46.3, 37.3 and 43.7 mm respectively), moderate inhibition against *S. typhimurium* (zone = 25.7 mm), weak inhibition against *A. niger* (zone = 11.7 mm), and no effect toward *L. monocytogenes*, *L. plantarum* or *P. aeruginosa*. Other studies have reported the mild antimicrobial activity of oil of dill against yeasts (Conner and Beuchat, 1984).

Both oil of fennel and oil of parsley were strongly inhibitory against *S. aureus*, *Y. enterocolitica*, and fungi (zone = 38.3, 57, and 42.7 mm respectively), moderately inhibitory against *S. typhimurium* (zone = 18 mm), and only weakly or not inhibitory to *L. plantarum*, *E. coli*, *P. aeruginosa*, *L. monocytogenes* and *Rhodotorula*. Oil of fennel was reported by Conner and Beuchat (1984) to be mildly effective against selected yeasts. Others have reported that fennel may be inhibitory in combination with known antimicrobials (Fyfe et al., 1998; Hodgson, 1998).

Oil of parsley had a potent antimicrobial effect against *S. aureus*, *Y. enterocolitica*, *G. candidum* and *Rhodotorula* (zone = 33.3, 29.3, 29 and 43.3mm respectively), moderate effect against *L. monocytogenes*, *S. typhimurium*, *P. aeruginosa* and *A. niger* (zone = 21, 16, 16 and 17 mm respectively), weak effect on *E. coli* (zone = 12.7 mm) and no effect against *L. plantarum*. Conner and Beuchat (1984) also reported mild antimicrobial activity of parsley against selected yeasts.

Oil of rosemary was not inhibitory to *L. monocytogenes*, *A. niger*, or *Rhodotorula* was only weakly inhibitory to *Pseudomonas*. Zones of inhibition for the other spoilage and pathogenic organisms exposed to oil of rosemary ranged from 22.3 to 44.7 mm which made rosemary moderately to strongly inhibitory to a wide range of organisms. This was the only oil, which was moderately inhibitory to one of the fungi imperfecti but not inhibitory to the other. Conner and Beuchat (1984) reported that oil of rosemary was mildly inhibitory to some of the yeasts, which they tested. Recent studies by another research group has shown that parsley showed inhibitory effect against *E. coli*, *L. monocytogenes*, *P. fragi* and other spoilage microorganisms (Manderfeld et al., 1997). Therefore, in general, oil of rosemary was only weakly or not inhibitory to fungi imperfecti or yeasts, but showed a fairly broad range of activity against gram positive and gram-negative bacteria.

The MLC values for the individual oils are shown in Table 3. Among the Gram-positive bacteria tested, *S. aureus* was the most sensitive bacterium. The MLC for *S. aureus* ranges from as low as 400 ppm for both oregano and coriander to 100,000 ppm with angelica essential oil. *L. monocytogenes* was, in general, the most resistant organism to the various essential oils with MLC ranging from 6250 ppm for oregano to greater than 100,000 ppm for several of the essential oils. The gram-negative pathogenic and spoilage bacteria had MLC's ranging from 400 ppm to >100,000 ppm depending on the essential oil. Essential oils of angelica, carrot, celery and parsley were not inhibitory to most gram-negative bacteria tested in our study. Jay, (1996) stated that, generally gram-positive bacteria are more sensitive than gram-negative to the spice essential oils,

**Table 3: Minimum Lethal Concentration (MLC) of essential oils for tested bacteria**

|           | <i>L.monocytogenes</i> | <i>L.plantarum</i> | <i>S.aureus</i> | <i>E.coli</i> | <i>S.lyphumum</i> | <i>Y.enterocolitica</i> | <i>P.aeruginosa</i> |
|-----------|------------------------|--------------------|-----------------|---------------|-------------------|-------------------------|---------------------|
|           | MLC (ppm)              |                    |                 |               |                   |                         |                     |
| Anise     | >100000                | 50000              | 50000           | 50000         | 25000             | 50000                   | >100000             |
| Angelica  | 50000                  | >100000            | >100000         | >100000       | >100000           | >100000                 | >100000             |
| Basil     | >100000                | >100000            | 1500            | 6000          | 6000              | < 800                   | 50000               |
| Cardamom  | >100000                | 25000              | 12500           | 50000         | 50000             | 3000                    | 12500               |
| Celery    | 50000                  | >100000            | 25000           | >100000       | >100000           | 50000                   | >100000             |
| Coriander | 50000                  | >100000            | 400             | 50000         | 50000             | 3000                    | 12500               |
| Dill      | >100000                | >100000            | 3000            | 12500         | 50000             | 12500                   | >100000             |
| Fennel    | >100000                | >100000            | 25000           | 50000         | 50000             | 12500                   | 25000               |
| Parsley   | 50000                  | >100000            | 50000           | >100000       | >100000           | 50000                   | >100000             |
| Oregano   | 6250                   | < 800              | < 400           | < 800         | < 800             | < 400                   | < 800               |
| Rosemary  | >100000                | 50000              | 25000           | 50000         | 25000             | 50000                   | 50000               |

MIC could not be determined because visual and instrumental detection of growth was impaired due to broth colloidal suspension and turbidity.

with the lactic acid bacteria being the more resistant among gram-positive bacteria. We would disagree with this statement based on our results since *S. aureus* was very sensitive to essential oils but *L. monocytogenes* was highly resistant. It is difficult to make such generalizations since each oil (or plant family) is unique and gram positive bacteria vary widely in structure and functionality.

Essential oil of oregano was the only oil in this study containing primarily thymol and carvacrol. Another interesting finding was that the only two oils containing linalool (coriander and basil) were both highly inhibitory to fungi, but not necessarily bacteria. Studies are needed to identify the active antimicrobial components in each oil.

The essential oil tested in our study had < 100 CFU/g of aerobic plate count and yeast and mold count since no growth was recorded from any plate. One of the negative aspects of using spices and herbs is that some types have been found to be heavily contaminated by mold flora. This mold flora were reported to be dominated by *Aspergillus* spp. which may produce aflatoxin (Garrido et al., 1992). However, our data shows that the essential oil, which is derived by extraction or steam distillation, is relatively free of microorganisms probably due to the harsh environmental conditions during preparation of the oil and to the antimicrobial activity of the pure oil after preparation.

Since many of the essential oils have very potent antimicrobial effects against some of the foodborne pathogens, a variety of studies should incorporate these oils into appropriate food formulations, especially associated with meat products. Since essential oils are primarily oil soluble not water-soluble, they will probably work best with

refrigerated foods and food that are naturally high in fat. It has to be emphasized that these oils will probably not work in foods that will receive heat treatment unit operations since it is very likely that the antimicrobial compounds would be evaporated.

## REFERENCES

- Anonymous, 1999. Foodborne illness. available at: [http://dir.yahoo.com/health/Diseases\\_and\\_Conditions/Foodborne\\_illness/](http://dir.yahoo.com/health/Diseases_and_Conditions/Foodborne_illness/)
- Arora, D.K., Mukerji, K.G., Marth, E.H. 1991. Handbook of Applied Mycology. Ch. 16. Liewen, M.B. (Ed.) Antifungal food additives. Marcel Dekker, Inc. N.Y.
- Ayres, J.C., Mundt, J.O., and Sandine, W.E. 1980. Microbiology of foods, Ch. 11, 18, Spices and Condiments, Fish and Shellfish. W.H. Freeman and company.
- Babic, I., Nguyen-the, C., Amiot, M.J., and Aubert, S. 1994. Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeasts. J Appl. Bacteriol. 76: 135-141.
- Biondi, D., Cianci, P., Geraci, C. and Ruberto, G. 1993. Antimicrobial activity and chemical composition of essential oils from sicilian aromatic plants. Flavor and Fragrance Journal 8: 331-337.
- Charalambous, G. 1994. Spices, herbs and edible fungi, Ch 3, 12, 19, 20. Elsevier Science B.V. Amsterdam.
- Conner, D.E. and Beuchat, L.R. 1984. Effect of essential oils from plants on growth of spoilage yeasts. J. Food Sci. 49: 429-434.
- Davidson, P.M. and Branen, A.L. 1993. Antimicrobials in foods, Ch. 13 in D.E. Conner (ed.) Naturally occurring compounds. Marcel Dekker, Inc, New York.
- Ela, M.A., El-Shaer, N.S., and Ghanem, N.B. 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. Pharmazie 51:(12) 993-995.
- FDA, 1999. Code of Federal Regulations. 21. available at: <http://frwebgate.access.gpo.gov/cgi-bin/multidb.cgi>.

- Fyfe, L., Armstrong, F., and Stewart, J. 1998. Inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic acid the development of synergistic antimicrobial combinations. *Int. J. Antimicrobial Agents* 9: 195-199.
- Garrido, D., Jodral, M., and Pozo, R. 1992. Mold flora and aflatoxin-produced strain of *Aspergillus flavus* in spices and herbs. *J. Food Prot.* 55: (6) 451-452
- Gould, G.W. 1995. *New Method Of Food Preservation*, 1<sup>st</sup> ed. Blackie Academic and Professional. London.
- Gould, G.W. 1996. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *J. Food Prot.* supplement 82-86.
- Hodgson, I., Stewart, J. and Fyfe, L. 1998. Inhibition of bacteria and yeasts by oil of fennel and paraben : development of synergistic antimicrobial combinations. *J. Essent. Oil Res.* 10:293-297.
- Jay, J.M. 1996. *Modern Food Microbiology*, 5<sup>th</sup> ed. Van Nostrand Reinhold, New York.
- Kivanc, M., Akgul, A. and Dogan, A. 1991. Inhibitory and stimulatory effect of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. *Int. J. Food Microbiol.* 13: 81-86.
- Lachowicz, K.J., Jones, G.P., Briggs, D.R., Bienvenu, F.E., Wan, J., Wilcock, A., and Coventry. 1998. The synergistic preservative effects of essential oil of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. *Lett. Appl. Microbiol.* 26: 209-214.
- Manderfeld, M.M., Schafer, H.W., Davidson, P.M., and Zottola, E.A. 1997. Isolation and identification of antimicrobial furocoumarins from parsley. *J. Food. Prot.* 60:(1) 72-77.
- Meena, M.R. and Sethi, V. 1994. Antimicrobial activity of essential oils from spices. *J Food Sci. Technol.* 31 (1): 68-70.
- Paster, N., Menasherov, M., Ravid, U. and Juven, B. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J. Food Prot.* 58 (1): 81-85.
- Salmeron, J., Jordano, R. and Pozo, R. 1990. Antimycotic and antiaflatoxinogenic activity of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris*, L.). *J. Food Prot.* 53: (8)697-700.

- Saxena, A.P. and Vyas, K.M. 1986. Antimicrobial activity of seeds of ethnomedicinal plants. *J. Econ Tax. Bot.* 8 (2): 291-299.
- Thomann, R.J., Ehrich, J. and Bauermann, U. 1993. Distillation and use of essential oils from dill, celery, lovage, and parsley made in Germany. *Acta Horticulturae.* 333:101-111.
- Uda, Y., Matsuoka, H., Shima, H., Kumagami, H. And Maeda, Y. 1993. Antimicrobial activity of water-soluble products derived from radish mustard oil and identification of an active component in there. *Nippon Shokuhin Kogyo Gakkaishi* 40 (11): 801-806.
- Wilkins, K.M. and Board, R.G. 1989. Natural antimicrobial systems Ch 11. In Gould G.W. *Mechanisms of action of food preservation procedures.* Elsevier Applied Science, London.
- Zaika, L.L., Kissinger, J.C., and Wasserman, A.E. 1983. Inhibition of lactic acid bacteria by herbs. *J. Food Sci.* 48: 1455-1459.

### **Part III**

#### **Use of Black Cumin Essential Oil to Inhibit Spoilage and Pathogenic Microorganisms in Rainbow Trout**

## ABSTRACT

Fish is a highly perishable food even under refrigerated storage or on ice. Nutrients in fish promote the growth of both spoilage and pathogenic microorganisms. Since most antimicrobial products now available are organic acids, they are not very effective in neutral pH foods, such as fresh fish. The purpose of this study was to investigate the effectiveness of aqueous, ethanolic and petroleum ether extracts of black cumin (*Nigella sativa*) for control of growth and survival of common spoilage and pathogenic microorganisms in vitro and in application to fresh trout. In vitro, the ethanolic extract of black cumin seeds was found to be highly inhibitory (zone = 35-55 mm) against *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Rhodotorula*, moderately inhibitory (zone = 16-28mm) against *Escherichia coli* O:157:H7, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, and *Geotricum*, and only weakly or not inhibitory (zone < 12 mm) against *Aspergillus niger* by the paper disc agar diffusion method. Freshly harvested rainbow trout fillets were stored at 0°C (on ice) and at 4°C using five marinate treatments. Treatments evaluated were plain teriyakis marinate (control), and four marinates containing *Nigella sativa* (black cumin) and/or dimethyl dicarbonate (DMDC). Trout treated with marinate using black cumin oil had significantly lower aerobic counts (> 3 log / g), coliform counts, *Listeria* and yeasts and mold counts ( $p < 0.05$ ). DMDC marinated trout did not significantly differ from control marinate for all counts ( $p > 0.05$ ). Fresh trout treated with either ground seeds or the combination of black cumin oil and DMDC marinate exhibited moderate reduction (>1 log) in all counts ( $p < 0.05$ ). Since a marinate

containing black cumin essential oil was highly inhibitory to both spoilage and pathogenic microorganisms and also significantly ( $p < 0.0001$ ) increased shelf-life by almost two weeks, it may be a useful as a further- processing method for increasing shelf-life of fish.

## INTRODUCTION

Fish is a high value food, but because of rapid spoilage at refrigeration temperature or on ice, national distribution of regional aquaculture products is very difficult (Hobbs, 1987). The growth of some aquaculture products such as fresh trout has been limited because of rapid spoilage in retail stores (Draughon et al., 1999). Draughon et al. (1999) reported that approximately one third of the purchased fish from 29 different grocery stores was already spoiled at the time of purchase. The cause of whole fish spoilage might be due to at least four factors; bacterial activity, fish digestive enzymes, fish muscle enzymes, and feed enzymes that cause autolysis (Windsor and Thoma, 1974; Gram and Huss, 1996).

Common organic acids, such as: sorbate, are not effective in fresh fish because of the pH of the fish is approximately 7.0 which is far above the pKa of the organic acids (Kabara, 1982; Shibasaki, 1982). Other antimicrobials are slightly more effective but only for short periods of time and shelf-life is not extended. In addition, these antimicrobials cause off-flavor and off-color problems in fish (Hobbs, 1987).

Middle Eastern and Eastern European cooking traditionally have used a number of

spices which are not commonly used in the western world. One such spice is *Nigella sativa* or black cumin, which used in pickling and bakery products, not only for flavoring, but also traditionally for preservation purposes (Saxena and Vyas, 1986; Al-Jassir, 1992). The whole or crushed seeds of black cumin were used in bakery products, as a flavoring agent in vinegar, as a constituent of curry, as a substitute for pepper in cooking, and for cheese flavoring (Al-Jassir, 1992). Chemical composition or antibacterial activity of black cumin seeds was reported by Toppozada et al., 1965; El-Alfy et al., 1975; Hasan et al., 1989.

Value-added meat products such as marinated chicken breasts are becoming popular with consumers. The development of similar products with fish may allow application of inhibitory compounds during retail display if acceptable compounds can be identified. Since cumin is used as a spice for fish in some cultures, this study was undertaken to evaluate the antimicrobial activity of alcohol, water and oil extracts of *Nigella sativa* and the application of black cumin in a fish marinate.

## MATERIALS AND METHODS

### **Extraction of *Nigella sativa* (black cumin)**

Water extract was prepared by mixing seeds which were washed, dried, and ground (with a coffee mill) with equal volume of distilled water and shaking for 18-24 hs at refrigeration temperature (4°C). Samples were vacuum filtered using 0.7-cm filter paper (Whatman #1) and centrifuged at 5,000 rpm to obtain the water extract (Mahmoud

et al. 1993). Brucella broth (Difco Detroit, MI) was made using black cumin in water extract to reconstitute instead of distilled water then cold sterilize by filtering using 0.45 µm filter (Nalge Rochester, NY).

For oil extraction, aliquots of 50 gm of black cumin ground seeds (previously washed, dried, and ground) were extracted with 300 ml of petroleum ether or 300 ml of ethyl alcohol (absolute) continuously for 18-24 h (4°C). Samples were filtered by using 0.7 cm filter paper (Whatman #1) and vacuum. The filtered liquid was cold sterilized by filtering again using 0.45 µm filter apparatus (Nalge Rochester, NY). Solvents were evaporated from the clear liquid by using rotoevaporator (Buchi B-465 Brinkman Westbury, NY) to obtain the clear essential oil. The oil of black cumin was kept in sterilized vials and stored at 4°C for further use.

#### **Antimicrobial assay and MLC determination**

After aqueous, petroleum ether and alcohol extraction, inhibitory activity of extracts was tested by inoculating 0.1ml of culture from BHI broth of freshly grown culture (24 ± 2h). Culture tested included: *Escherichia coli* O157:H7 (ATCC 35150, apple cider), *Lactobacillus plantarum* (ATCC 14917), *Listeria monocytogenes* Scott A(4, meat isolate), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 13563), *Yersinia enterocolitica* (AYCC 228), *Aspergillus niger* (UT stock culture), *Geotricum candidum* (ATCC 34614), and *Rhodotorula* (ATCC 32765). Each culture was inoculated into 20 ml broth made with black cumin water extract, 20 ml broth with 2 ml petroleum ether extract and 2 ml tween

80, and 20 ml broth with 2 ml ethanol extract with 2 ml tween 80. All tubes were incubated at 32°C for 72 hs then plated each 24 hs on standard methods agar (SMA) (Difco, Detroit, MI) to determine growth. The plates were incubated at 32°C for 48 hs. Controls included broth containing tween 80.

Using the filter paper disc diffusion method (Meena and Sethi, V. 1994), inhibitory effect of black cumin oil was determined. Standard method agar (SMA) (Difco, Detroit, MI) with 100 ppm of 2,3,5-triphenyl tetrazolium chloride added (TTC) (Fisher Fair lawn NJ) was used to distinguish bacterial colonies and to clarify the zone of inhibition. SMA with no TTC added was used for the antifungal assay. Each plate was inoculated with bacteria (24 hs) and/or fungal culture (72 hs) directly from the inoculum and spread uniformly using a sterile loop. Each plate was undisturbed for 30 minutes to allow inoculum to be absorbed onto the medium surface. Sterile 6-mm diameter absorbent blank filter paper discs (Difco, Detroit, MI) were dipped in each black cumin extract and then placed on inoculated plates (approximately 24 µg). The plates were incubated at 32°C and observed daily, for 2-4 days. The action of the antimicrobial was estimated by measuring the distance of growth inhibition (zone of inhibition in mm). Placing discs saturated with each extraction solvent on each inoculated plate also tested the possible inhibitory effect of the ethanol and petroleum ether without black cumin. All plates were then incubated at 32°C for 2-4 days (observed daily) after which the zones of inhibition were measured. The antibiotics used for positive control were cephalothin (Difco, Detroit, MI) 30µg, amoxicillin (Difco, Detroit, MI) 20µg and clavulanic acid 10µg, nalidixic acid 30µg, tetracycline (Difco, Detroit, MI) 30µg, neomycin 5µg (Difco,

Detroit, MI), penicillin (BBL, cockeysville, MD) 10 iu, tetracycline (BBL, cockeysville, MD) 5 µg, bacitracin (BBL, cockeysville, MD), and chloramphenicol (BBL, cockeysville, MD) 30 µg.

The minimum lethal concentration (MLC) was determined using 2-fold serial broth dilution method (with 2 ml oil added to 20 ml Brucella broth (Difco, Detroit, MI) and 2 ml Tween 80 emulsifier up to .008 ml oil) for microorganisms that were inhibited by black cumin oil. All tubes were incubated at 32°C for 72 hs. The results were measured visually and by plating on SMA plates (incubated for 24-48 hs at 32°C) and recorded as (+) for growth or (-) for no growth. All tests were conducted in triplicates with three replications.

### **Microbial Analysis of Essential Oils**

Black cumin seeds were ground using a coffee mill. Ground seeds (10 g) was added to 90 ml of sterile peptone water and blended. Serial dilutions (0.1 ml) of the diluted seeds were spread on the surface of SMA and RoseBengal agar (RBA-O) (Difco Detroit, MI) by using a sterile bent glass rod. Extracts were also subjected to microbial analysis for quality control. Each extract (0.1 ml) was spread on the surface of SMA and RBA-O (Difco Detroit MI) agar by using a sterile bent glass rod. All SMA plates were incubated at 32°C for 48 hs and RBA-O plates at room temperature (~25°C for 5 days). All tests were conducted in triplicates.

## **Fish samples**

Freshly harvested rainbow trout were purchased from a local trout farm. Fish were filleted and held on crushed ice during transportation ( $\approx 30$  minutes). All samples were over wrapped in an air permeable film as is typical of industry practice. All fillets were kept on crushed ice and used within 18-24 hs after harvest.

## **Fish preparation**

Five treatments were conducted using commercial Ebara Teriyaki sauce as a marinate. Treatments consisted of plain marinated fish as a control; marinated fish + 10% ground black cumin seeds (previously washed and dried); marinated fish + 200 ppm of Dimethyl Dicarbonate (DMDC) as a preservative; marinated fish + 100 ppm (DMDC) + 5% black cumin essential oil; marinated fish + 10% black cumin essential oil. Treated trout fillets were kept individually in a loosely closed sterile Seward Medical Stomacher Closure Bags (Tekmar; Cincinnati, OH) and stored at two different temperatures, one at 4°C (refrigerator) and one on crushed ice until the end of the experiment. All tests were conducted in triplicates.

## **Bacterial culture**

*Listeria monocytogenes* Scott A (4, meat isolate) was grown in Brucella broth (Difco; Detroit, MI) at 32°C. Separate cultures were subjected to three successive 24-hs transfers before use. Culture was kept refrigerated on tryptose soy agar slant during the experiment (up to two weeks) and was tested for viability and purity before each use. All

treatments were inoculated with *L. monocytogenes* culture at day two of the experiment, since all treatments were negative for *L. monocytogenes*.

### **Microbiological analysis**

Samples were taken at day 0, 3, 6, 9, 12, 15, 21, 28, 35, and 60 from all treatments. Serial dilutions were made using 25-g of treated trout samples in 225 ml of 0.1% peptone water (Difco, Detroit MI). Samples were mixed in sterile Seward Medical Stomacher Closure Bags (Tekmar; Cincinnati, OH) using a Model 400 Stomacher® Lab Blender (Steward, London, England). Serial dilutions in 0.1% peptone water were plated on four different agars. Standard method agar (SMA) (Difco; Detroit MI) was used for aerobic plate count, violet red bile agar (VRB) (BBL Cockeysville, MD) for coliform counts, RoseBengal agar (RBA-O) (Difco; Detroit MI) with Oxytetracyclin supplements (Sigma St. Louis, MO) for yeasts and mold counts, and modified oxford agar (MOX) using *Listeria* selective supplement (Oxoid; Hampshire, England) for isolation of *Listeria*. All counts were performed using the spread plate method of 0.1 ml of the initial dilution after enrichment for *Listeria*. All plates were incubated at 32°C for 24-48 hs, RBA-O plates were left at room temperature for 5 days. All plantings were conducted for all replications.

### **Experimental design and statistical design**

The design of this experiment was a randomized block design with repeated measure

treatment arrangement, blocked on replication. Data were analyzed using Proc Mixed (SAS versions 6.12; Cary, N.C).

## RESULTS AND DISCUSSION

### In vitro antimicrobial activity

Extracts were tested separately for their antimicrobial activity against the test microorganisms and these data are presented in Table 1. Water extracts of black cumin were not antimicrobial. However, both the alcohol and petroleum ether extracts were quite inhibitory. The analysis of data revealed that the antimicrobial activity of black cumin ethanolic extract was highly inhibitory (zone  $\geq 28$  mm) against *L. monocytogenes*, *S. aureus*, and *Y. enterocolitica*, moderately active (zone  $\geq 16$ -28 mm) against *E. coli*, *S. typhimurium*, *L. plantarum*, and *Geotricum*, mild or not inhibitory (zone  $< 12$ ) against *A. niger*, *P. aeruginosa* and *Rhodotorula*. The black cumin petroleum ether extract was highly active (zone  $\geq 28$  mm) against *L. monocytogenes* and *S. aureus*, moderately active (zone  $\geq 16$ -28 mm) against *Geotricum*, *Y. enterocolitica*, *L. plantarum*, and *Rhodotorula*, and weakly active (zone 12-14) against *A. niger*, *E. coli*, *S. typhimurium*, *P. aeruginosa*. The type and amount of the volatile compounds extracted by each solvent may explain the differences in the antimicrobial effect of the two extracts. Ethanol controls were slightly inhibitory to some microorganisms. However, petroleum ether was not inhibitory to any culture studied. These controls were

**Table. 1** Inhibition zones (mm<sup>1</sup>) of growth of tested microorganisms by black cumin essential oil.

|                    | <i>L.monocytogenes</i> | <i>L.plantarum</i> | <i>S.aureu</i>  | <i>E.coli</i>     | <i>S.typhimurium</i> | <i>Y.enterocolitica</i> | <i>P.aerogenosa</i> | <i>A.nlger</i>    | <i>O.candida</i> | <i>Rhodotula</i>  |
|--------------------|------------------------|--------------------|-----------------|-------------------|----------------------|-------------------------|---------------------|-------------------|------------------|-------------------|
| BC EE <sup>4</sup> | 53 <sup>a</sup>        | 17 <sup>b</sup>    | 60 <sup>b</sup> | 18 <sup>a</sup>   | 21 <sup>a</sup>      | 28 <sup>a</sup>         | 0 <sup>b</sup>      | 11 <sup>b</sup>   | 20 <sup>b</sup>  | 11 <sup>b</sup>   |
| BCPEE <sup>5</sup> | 53.3 <sup>a</sup>      | 23.3 <sup>a</sup>  | 87 <sup>a</sup> | 12 <sup>b</sup>   | 14 <sup>b</sup>      | 22 <sup>b</sup>         | 14 <sup>a</sup>     | 12.3 <sup>a</sup> | 23 <sup>a</sup>  | 25.7 <sup>a</sup> |
| BCWE <sup>6</sup>  | 0 <sup>c</sup>         | 0 <sup>d</sup>     | 0 <sup>d</sup>  | 0 <sup>d</sup>    | 0 <sup>d</sup>       | 0 <sup>c</sup>          | 0 <sup>b</sup>      | 0 <sup>c</sup>    | 0 <sup>c</sup>   | 0 <sup>c</sup>    |
| Ethanol            | 11.7 <sup>b</sup>      | 9 <sup>c</sup>     | 12 <sup>c</sup> | 10.3 <sup>c</sup> | 11 <sup>c</sup>      | 0 <sup>c</sup>          | 0 <sup>b</sup>      | 0 <sup>c</sup>    | 0 <sup>c</sup>   | 0 <sup>c</sup>    |
| Pot.ether          | 0 <sup>2c</sup>        | 0 <sup>d</sup>     | 0 <sup>d</sup>  | 0 <sup>d</sup>    | 0 <sup>d</sup>       | 0 <sup>c</sup>          | 0 <sup>b</sup>      | 0 <sup>c</sup>    | 0 <sup>c</sup>   | 0 <sup>c</sup>    |

1 The diameter of the filter paper discs (6mm) is included.

2. No inhibition (<6 mm diameter).

3. Least square means in columns followed by different letters (a-i), are significantly different ( $p < 0.001$ ).

4. Black cumin ethanolic extract.

5. Black cumin Pot. Ether extract.

6. BC seeds water extract.

studied even though essentially all solvent was removed from extracts by the rotatoevaporator evaporation.

Ela and his co-workers (1996), reported that a black cumin preparation was strongly active against *Staphylococcus aureus*, moderately active against *A. niger*, and inactive (no inhibition) against *Escherichia coli*. Agarwal et al.(1979) stated that black cumin essential oil exhibited more antibacterial activity against gram-positive than gram-negative bacteria in a limited study. Topozada and his co-workers (1964) reported that black cumin oil was inhibitory to 7 strain of *S. aureus*. Other research has shown black cumin essential oil may have variable antibacterial, antifungal and antihelminthic activity (Ferdous et al., 1992, Mahmoud, 1993; Kandil et al., 1994). Hanafy and Hatem (1991) reported that ether extract of black cumin inhibited *S. aureus*, *E. coli*, *P. aeruginosa*, but not *S. typhimurium*.

Work by Rathee et al. (1982) disagrees with Hanafy and Hatem (1991), that the petroleum ether black cumin oil has a very strong antimicrobial properties against *S. typhimurium* and *P. aeruginosa*. One difficulty in evaluating older research literature on black cumin is that concentration tested or type of extract is not always given. Some studies have used selective or differential media for plating microorganisms exposed to black cumin extracts. Because of bacterial injuries, use of a selective / differential medium may give erroneously low recovery of bacteria.

Our findings revealed that, the black cumin cold aqueous extract does not have inhibitory activity against any microorganism. On the contrary, the aqueous extract of black cumin seeds actually increased growth. Since extracts were filter sterilized, we do

not know why growth increased. Mahmoud (1993) also showed that aqueous extract of black cumin seeds had no antimicrobial activity with other microorganisms. Al-Jassir (1992) reported that the aerobic plate count (APC) of black cumin seeds was  $7 \times 10^7$  CFU/g and the yeast and mold counts were  $4 \times 10^2$  CFU/g. Conversely, Kandil et al., (1994) reported that, the aqueous extract of black cumin seeds has potent antibacterial activity against pathogenic bacteria.

Microbial counts of seeds used in our study were performed for quality control. Our black cumin seeds were found to be highly contaminated (APC was 5.9 Log CFU/g, yeast and mold count 2.2 Log CFU/g), but both extracted oils were sterile (APC < 2 Log CFU/ml). The high levels of contaminants in black cumin seeds have also been shown by Al-Jassir (1992). These data are similar to the previous results by Al-Jassir (1992); Garrido et al. (1992); Charalambous (1994) and Jay (1996).

MLC values for black cumin extracts are shown in Table 2. The importance of determining MLC values is shown by the confusing data reported above. If concentrations or dilutions of a possible antimicrobial are not provided, the terms strongly or weakly inhibitory become meaningless. Data in Table 2 (MLC) demonstrate that ethanolic extract of black cumin are only weakly inhibitory to *L. plantarum*, *E. coli* and *P. aeruginosa*. However, the petroleum extract was strongly inhibitory to all culture tested except *Salmonella*. Both the ethanolic and petroleum extract give black cumin essential oil, but they are not the same.

**Table 2: Minimum Lethal Concentration (MLC) of black cumin essential oils for tested bacteria**

|       | <i>L.monocytos</i> | <i>L.plantarum</i> | <i>S.aureus</i> | <i>E.coli</i> | <i>S.typhi</i> | <i>Y.enterocoli</i> | <i>P.aeruginosa</i> |
|-------|--------------------|--------------------|-----------------|---------------|----------------|---------------------|---------------------|
|       | MLC (ppm)          |                    |                 |               |                |                     |                     |
| BCEE  | 6250               | >100000            | 3000            | >100000       | 50000          | 50000               | >100000             |
| BCPEE | 12500              | 50000              | <800            | 50000         | >100000        | 50000               | >100000             |

MIC could not be determined because visual and instrumental detection of growth was impaired due to broth colloidal suspension and turbidity.

### **Antimicrobial activity in fish meat**

Aerobic plate counts for each of the five antimicrobial treatments of rainbow trout at 4°C are shown in Figure 1. Initially all treatments had approximately 4.5 log CFU/g. APC did not change significantly at 4°C until after approximately 6 days of storage. APC of fillets treated with ethanol extract of black cumin (NS oil) began to decline after day 6, and final counts even after 60 days were lower than the original levels found in trout. This was an extraordinary finding. Although no formal sensory evaluation was done at this time, the smell of trout marinated in the black cumin oil treatment remained fresh and pleasant even after 60 days at 4°C with no spoiled or fishy odors detected. APC of control fish and DMDC treated fish increased rapidly after day 6 to greater than 8.0 log CFU/g and fish was considered spoiled after 15 days at 4°C. Treatments containing only black cumin seeds or 5% oil combined with DMDC had reduced APC but counts exceeded 6.0 log CFU/g.

At 0°C, changes in APC of trout fillets for each treatment were less dramatic (Figure 2) but followed the same trends. APC of trout fillets treated with black cumin oil at 0°C decreased slightly and continuously throughout the 60 day storage period. Even at 0°C, APC of control marinated trout and trout treated with DMDC or black cumin seeds increased gradually to a maximum of approximately 6.5 log CFU/g. Odor and smell of marinated fish treated with NS oil was pleasant and not spoiled even after 60 days at 0°C.

Coliform counts for each of the five antimicrobial treatments of marinated rainbow trout at 4 and 0°C are shown in Figures 3 and 4. Counts were initially very low

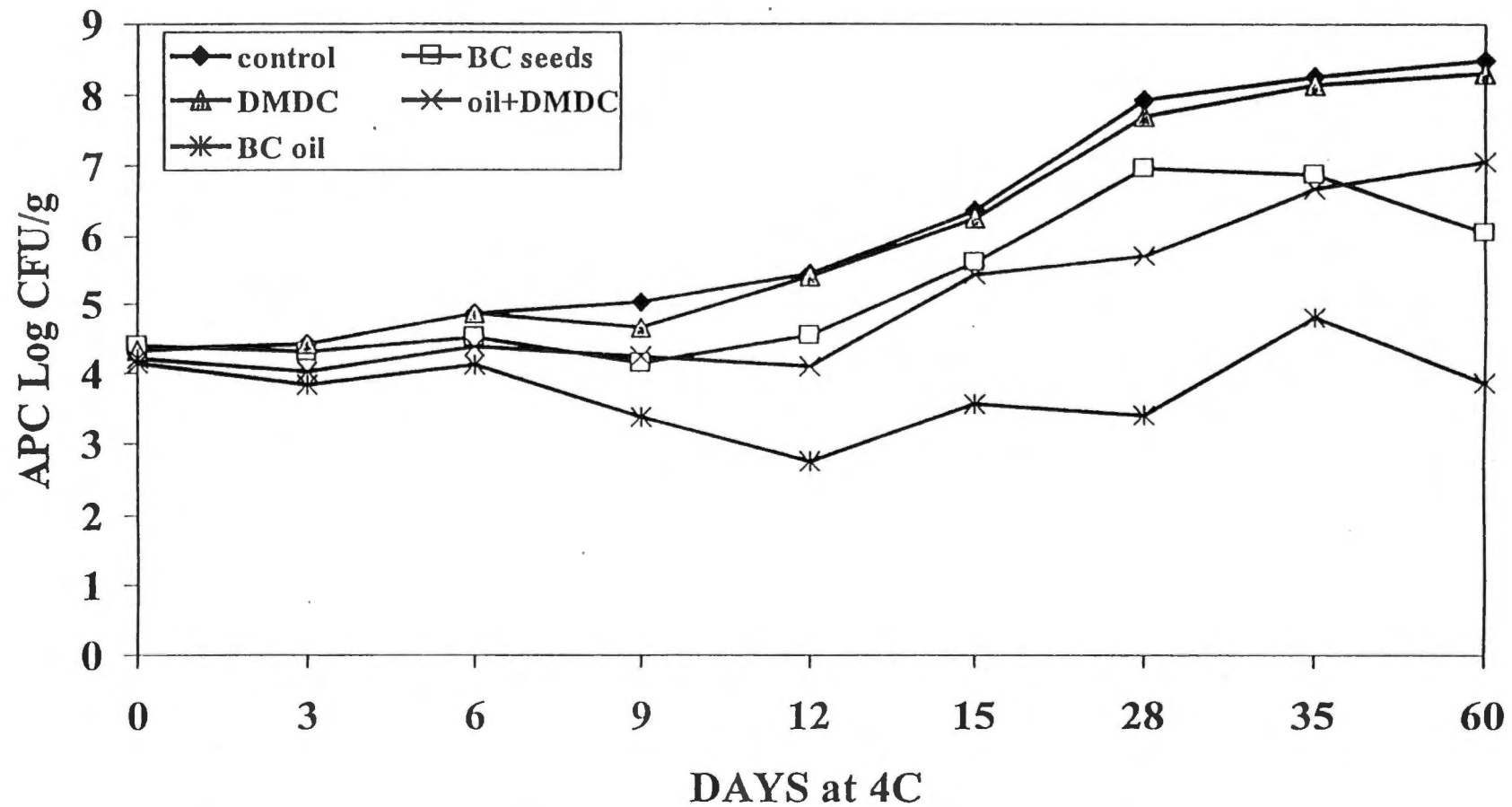


Figure 1. Change in aerobic plate counts (APC) of five treated trout fillet during storage period (60 days) at 4°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

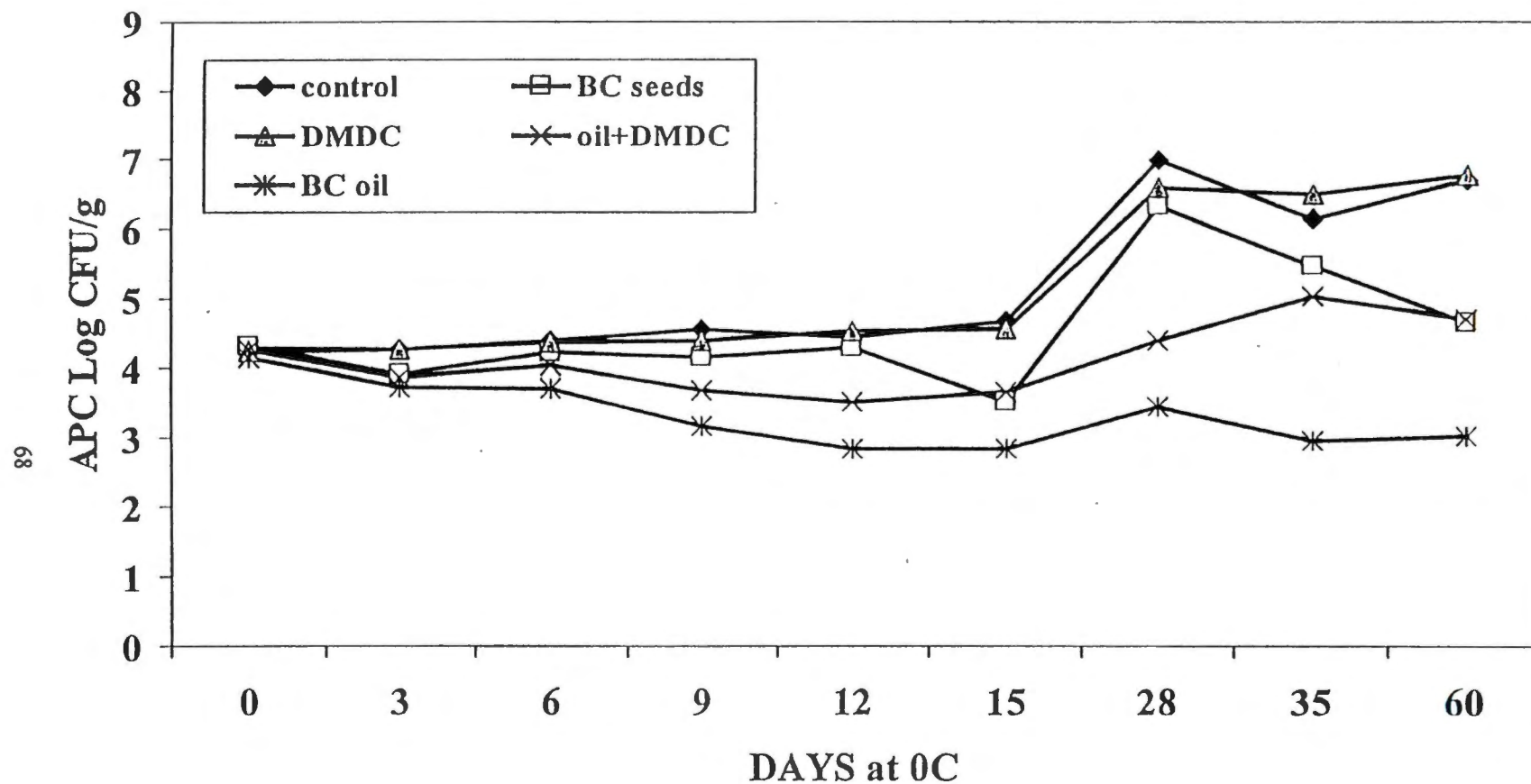


Figure 2. Change in aerobic plate counts (APC) of five treated trout fillet during storage period (60 days) at 0°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

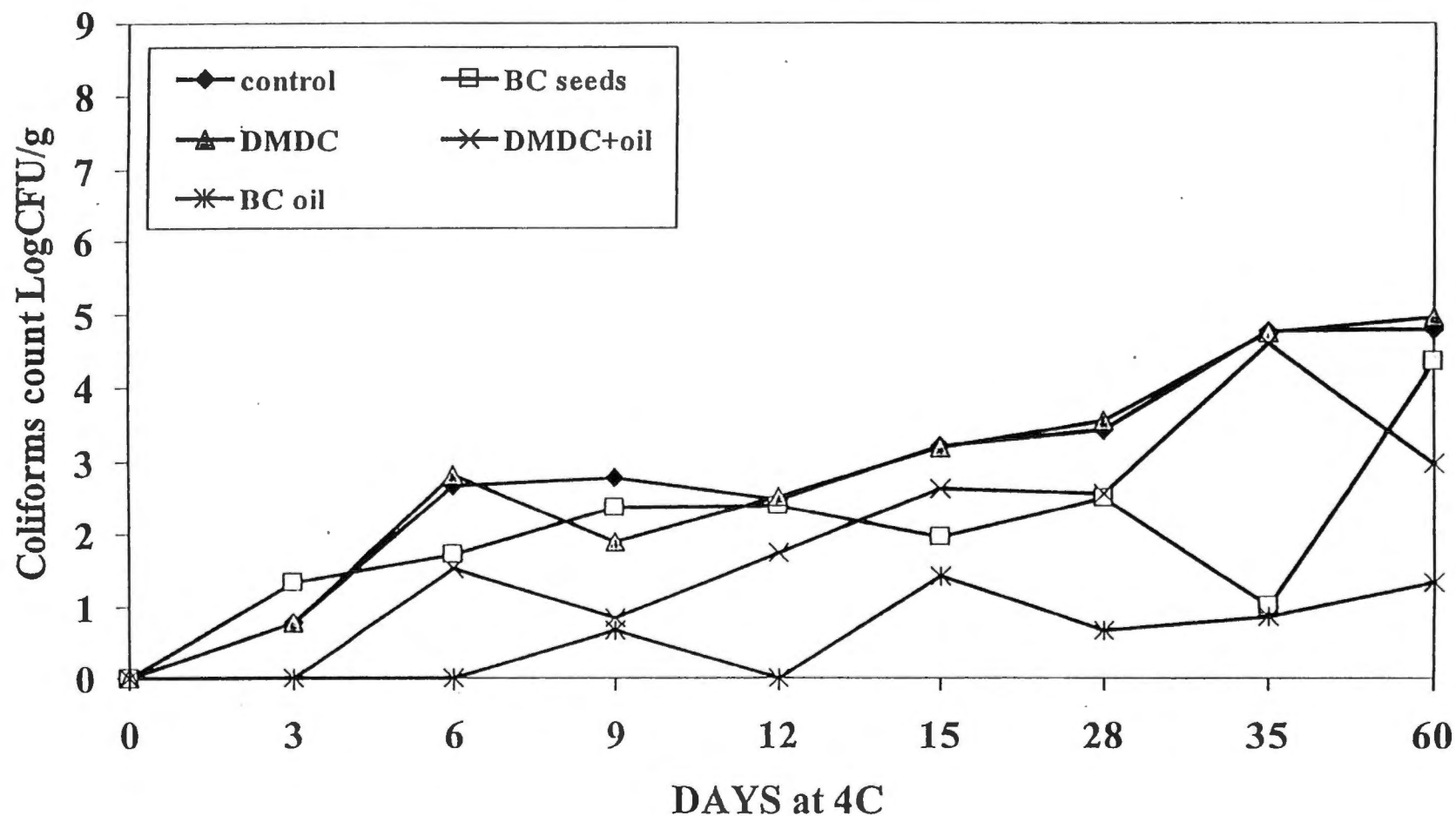


Figure 3. Change in coliform counts of five treated trout fillet during storage period (60 days) at 4°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

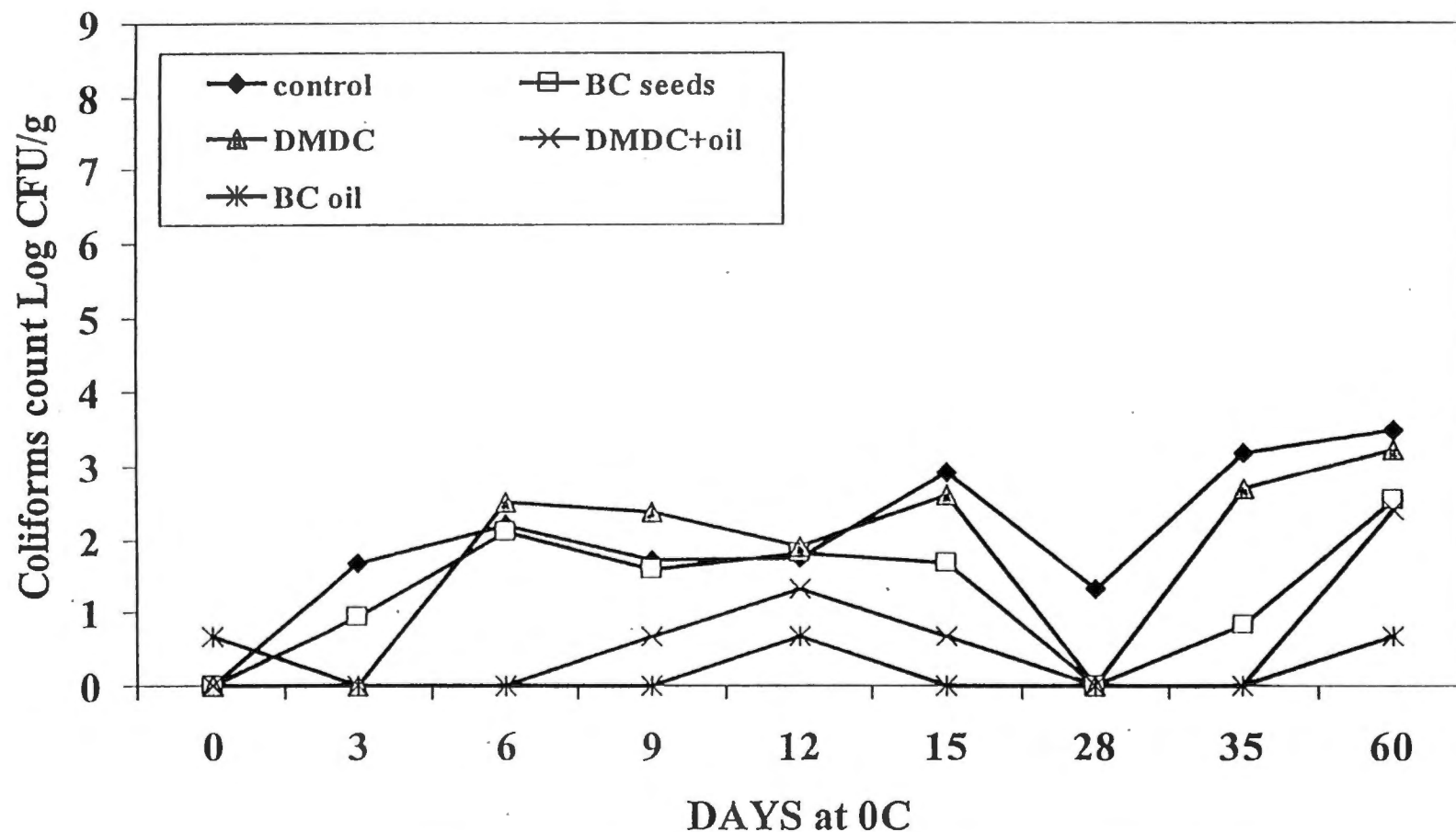


Figure 4. Change in coliform counts of five treated trout fillet during storage period (60 days) at 0°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

in all treatments but increased gradually after the third day. However, the trout marinates containing black cumin oil had non detectable levels of coliform at almost all sampling days except for day 60, at which they were less than 2.0 log CFU/g. Therefore, the antimicrobial activity shown by the black cumin ethanol extract against *E. coli* and other gram negative bacteria in the disk diffusion assay and MLC (Tables 1 & 2) carried over to trout in the fish marinate.

*Listeria monocytogenes* was not detected in any sample tested at the beginning of this study; therefore, *L. monocytogenes* was inoculated into a set of fish treatments starting on day 2 of storage at both 4 and 0°C. A recent publication by Draughon et al. (1999) showed that *L. monocytogenes* and other *Listeria spp.* were frequently found in trout purchased at retail stores. However, they could not find the *L. monocytogenes* in fresh product at the trout farm. Since this indicates that contamination of trout is occurring at the retail level or during transport to the grocery store or fish market, methods which reduce this occurrence would be useful. *L. monocytogenes* counts for each of the five antimicrobial treatments of rainbow trout at 4°C and 0°C are shown in Figures 5 and 6. Initially all treatments were inoculated with approximately 3.0 log CFU/g, however, trout treated with marinate having any type of black cumin (10% oil, seeds, or 5% oil with DMDC) had reduced counts immediately after inoculation due to the antimicrobial activity of the black cumin. After six days of storage at 0°C, *L. monocytogenes* could not be detected in any treatment containing black cumin. At 4°C, *L. monocytogenes* was not detected in the black cumin oil treatment and was kept below 2.0 log CFU/g in other black cumin treatments. *L. monocytogenes* levels in control or

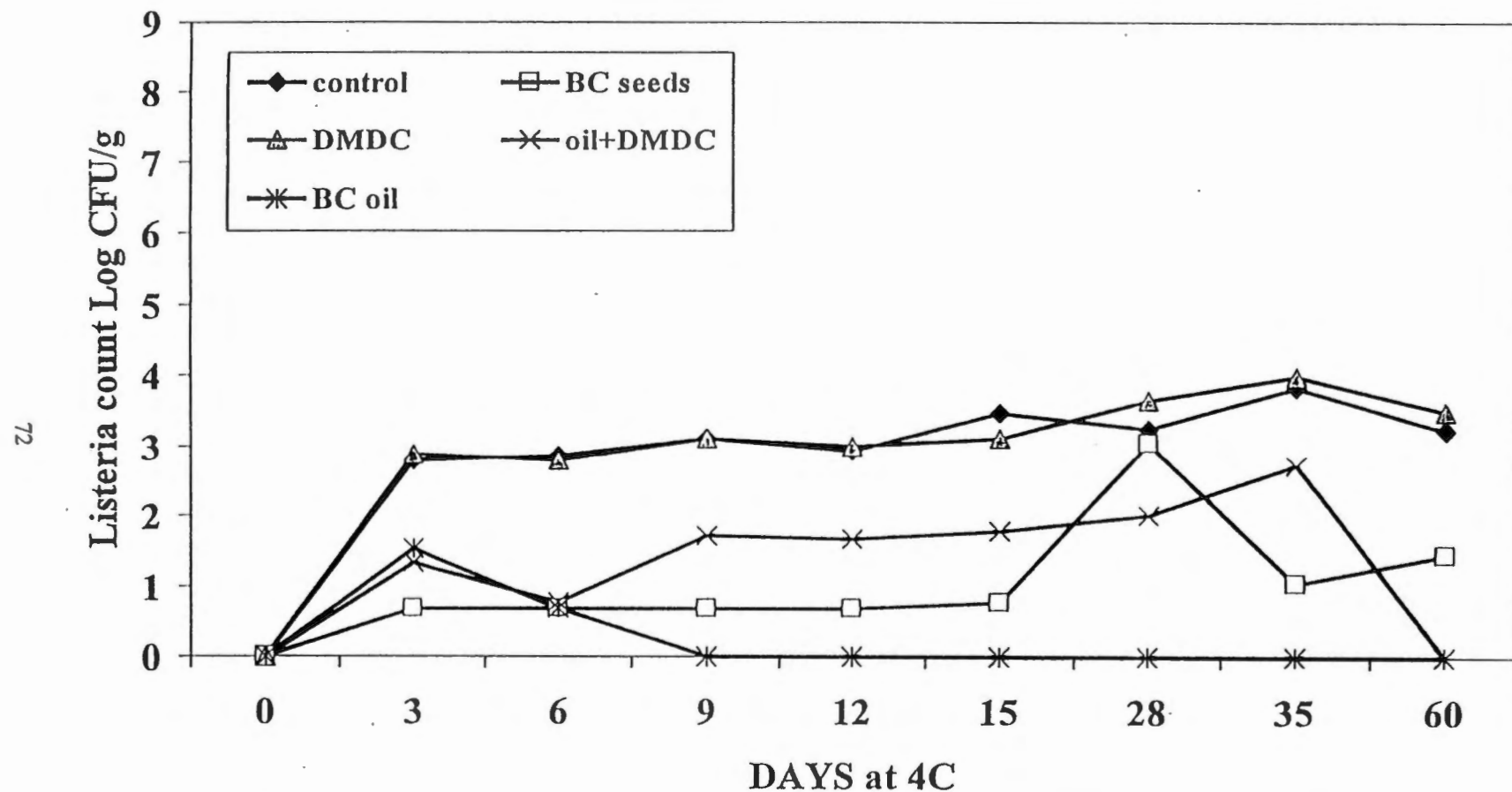


Figure 5. Change in *L. monocytogenes* counts of five treated trout fillet during storage period (60 days) at 4°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

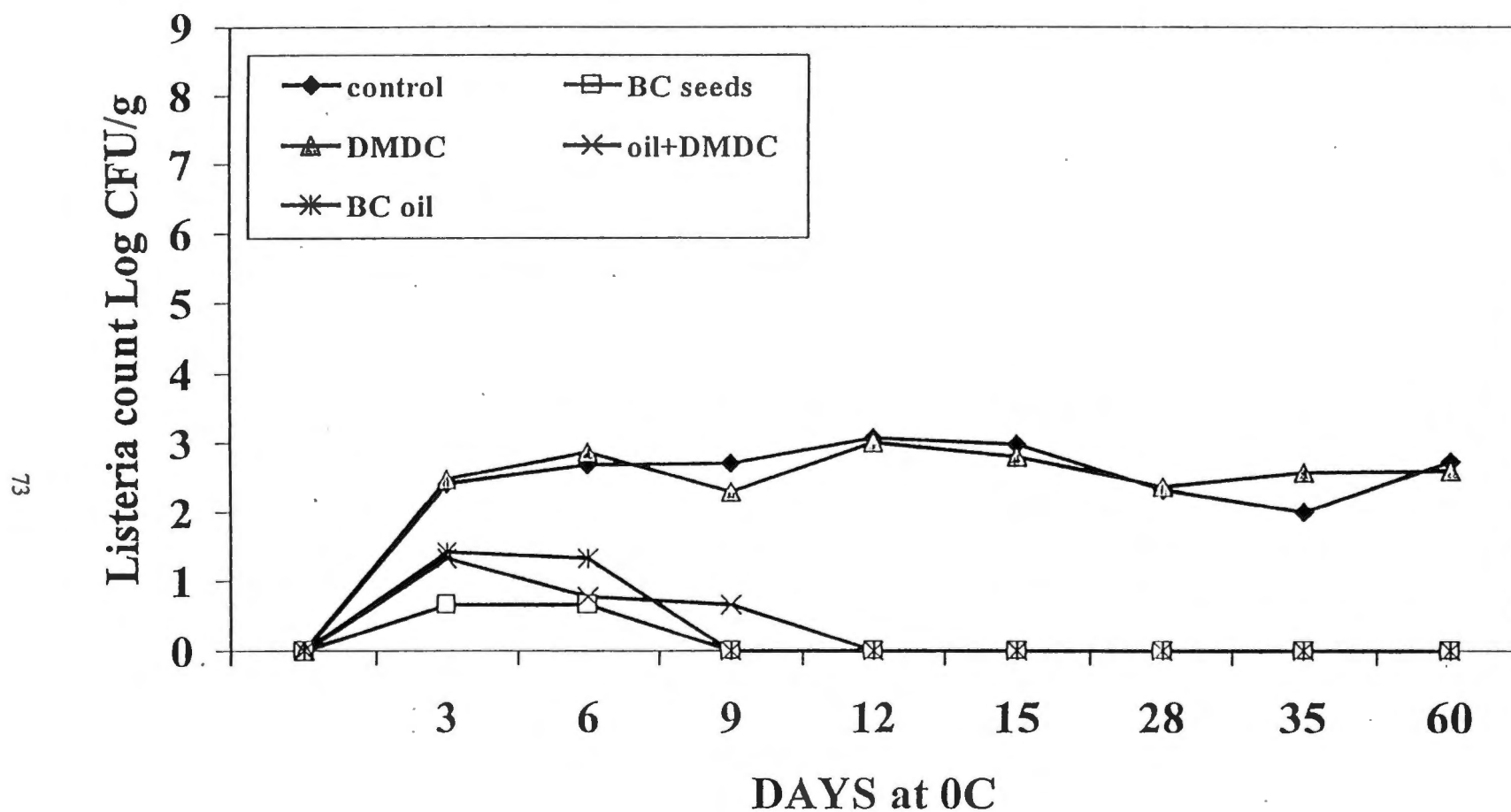


Figure 6. Change in *L. monocytogenes* counts of five treated trout fillet during storage period (60 days) at 0°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

DMDC did not change significantly at 4 or 0°C in trout during storage.

Yeasts and mold counts in each of the five treatments for marinated trout increased over time for all treatments but increased only slightly with trout marinates containing black cumin oil (Figures 7 & 8). Yeasts and molds increased rapidly to greater than 6.0 log CFU/g for controls, DMDC and seed treatments. One problem with black cumin seed treatments was that seeds contained high levels of yeasts and molds. However, it was obvious from the data that only treatments containing oil of black cumin effectively inhibited yeasts and molds during at least part of the storage period.

Windsor and Thoma (1974) stated that preservation of fish by icing or freezing is an effective method of preservation but it is usually not economic for industrial fish. The disadvantages of using chemicals preservative in foods, are off-color, off-flavor, texture problems, side effects when used in a high dose (allergy problem and toxicity for human and experimental animals) and decreased nutritional value, which explains why these chemicals are not generally used (Davidson and Branen, 1993; Jay, 1996; Doyle et al., 1997).

Spices and herbs have the advantage of being considered “natural” chemicals but may not be useful in some foods since the volatile or flavoring compounds may contribute undesirable flavor attributes to the food product. Organic acids are somewhat useful but also contribute to off flavors and off colors in fish (Hubbs, 1987; Huss, 1992). Therefore, the use of essential oils such as black cumin as an antimicrobial in fish would be limited to products, which can be prepared as a value-added product in the form of marinates, or other new flavor formulations.

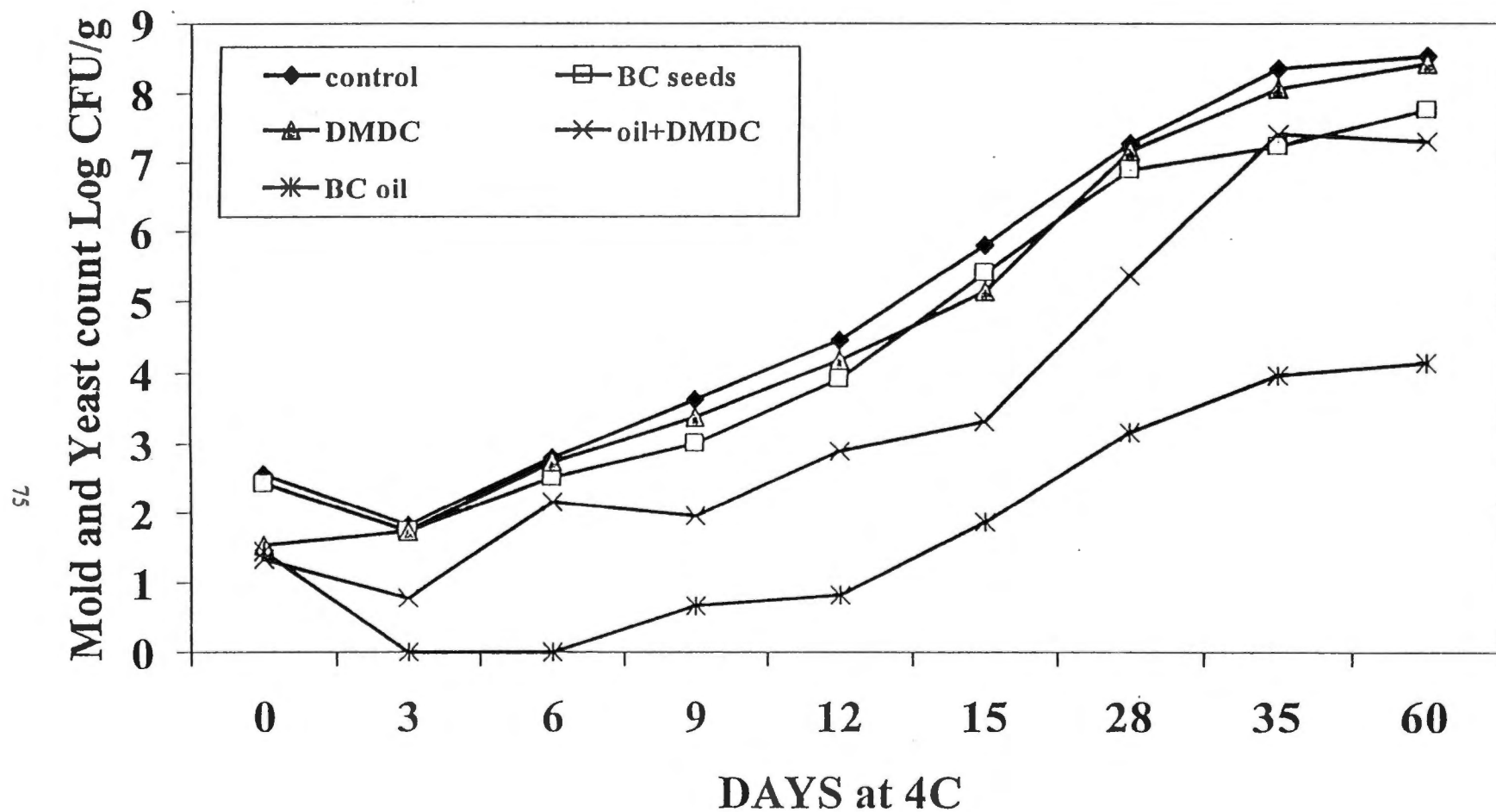


Figure 7. Change in mold and yeast counts of five treated trout fillet during storage period (60 days) at 4°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

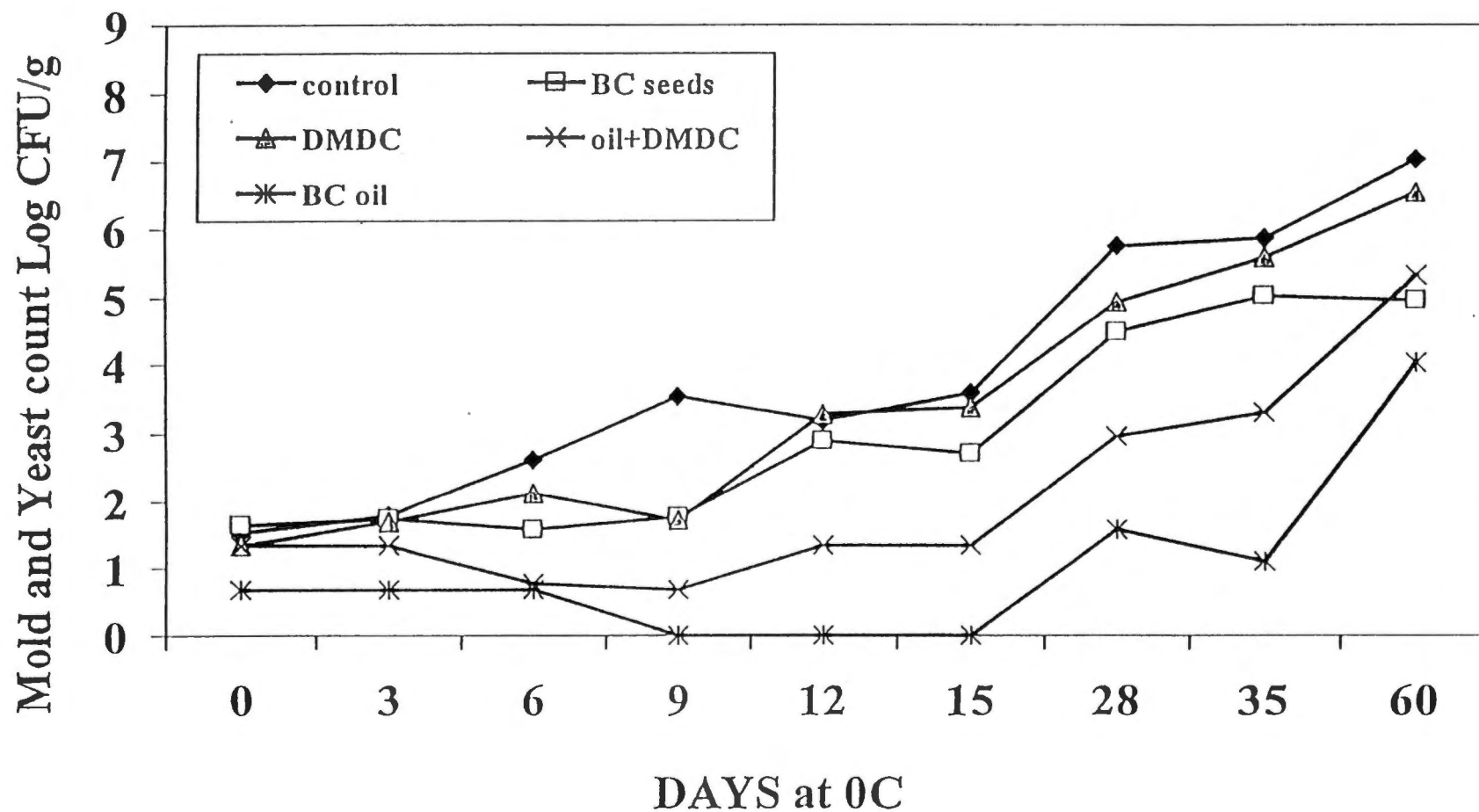


Figure 8. Change in mold and yeast counts of five treated trout fillet during storage period (60 days) at 0°C. Treatments are plain marinated trout (control), marinated trout with 10% black cumin ground seeds (BC seeds), marinated trout with 200 ppm DMDC (DMDC), marinated trout + 5% BC oil with 100 ppm DMDC (oil +DMDC) and marinated trout with 10% BC oil (BC oil).

Our study presents some interesting data, which may have a significant impact on fish quality. Since these data show that *L. monocytogenes* and aerobic bacteria found on fish are not only inhibited by black cumin essential oil in a marinate formulations but are also reduced below initial levels and to non-detectable levels on ice, this type of antimicrobial formulation containing black cumin would provide a novel method for increasing both safety and shelf-life of fish while at the same time increasing value. Other herb or spice extracts may offer similar benefits and should be investigated.

## REFERENCES

- Agarwal, R., Kharya, M.D., Shrivastava, R. 1979. Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. . Ind. J. Exper. Biol. 17: (11), 1264- 1265.
- Al-Jassir, M.S. 1992. Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chem. 45: 239-242.
- Charalambous, G. 1994. Spices, herbs and edible fungi, Ch 3, 12, 19, 20. Elsevier Science B.V. Amsterdam.
- Davidson, P.M. and Branen, A.L. 1993. Antimicrobials in foods, Ch. 13 in D.E.Conner (ed.) Naturally occurring compounds. Marcel Dekker, Inc, New York.
- Doyle, M.P., Beuchat, L.R., and Montville, T.J. 1997. Food Microbiology Fundamentals and Frontiers. 1<sup>st</sup> ed. ASM Press Washington D.C.
- Draghon, F.A., Anthony, B., Denton, M.E. 1999. *Listeria* species in fresh Rainbow trout purchased from retail markets. Dairy Food Env. San. 19: (2) 90-94.
- El-Alfy, T.S., El-Fataty, H.M., and Toama, M.A. 1975. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. Phamazie 30: (2) 109-111.
- Ela, M.A., El-Shaer, N.S., and Ghanem, N.B. 1996. Antimicrobial evaluation and

- chromatographic analysis of some essential and fixed oils. *Pharmazie* 51: (12) 993-995.
- Ferdous, A.J., Islam, S.N., Ahsan, M., and Hasan, C.M. 1992. In vitro antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug-resistant isolates of *Shigella* spp. and isolates of *Vibrio cholera* and *Escherichia coli*. *Phytotherapy Res.* 6: 137-140.
- Garrido, D., Jodral, M., and Pozo, R. 1992. Mold flora and aflatoxin-produced strain of *Aspergillus flavus* in spices and herbs. *J. Food Prot.* 55: (6) 451-452
- Gram, L. and Huss, H.H.. 1996. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol* 33: 121-137.
- Hanafy, M.S.M. and Hatem, M.E. 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J. Ethnopharma.* 34: 275-278.
- Hasan, C.M., Ahsan, M., and Islam, S.M. 1989. In vitro antibacterial screening of the oils of *Nigella sativa* seeds. *Bangladesh J. Bot.* 18: 171-174.
- Hobbs, G. 1987. Microbiology of fish. *Essays in Agricultural and Food Microbiology.* John Wiley and Sons Ltd.
- Huss, H.H.. 1992. Development and use of HACCP concept in fish processing. *Int. J. Food Microbiol.* 15:33-44.
- Jay, J.M. 1996. *Modern Food Microbiology*, 5<sup>th</sup> ed. Van Nostrand Reinhold, New York.
- Kabara, J.J. 1982. A new preservation system for food. *J. Food Safety* 4: 13-25.
- Kandil, O., Radwan, N.M., Hassan, A.B., Amer, A.M., El-Benna, H.A., and Amer, M.M. 1994. Antimicrobial activity of *Nigella sativa* and *Zingiber officinale*. *Vet. Med.J. Giza* 42: (1) 159-168.
- Mahmoud, H.M.A. 1993. Inhibitory action of black cumin (*Nigella Sativa*) against *Listeria monocytogenes*. *Alex. J. Agric. Res.* 38 (1):123-134.
- Meena, M.R. and Sethi, V. 1994. Antimicrobial activity of essential oils from spices. *J Food Sci. Technol.* 31 (1): 68-70.
- Rathee, P.S., Mishra, S.H., and Kaushal, R. 1982. Antimicrobial activity of essential oil, fixed oil and unsaponifiable matter of *Nigella sativa* Linn. *Ind. J Pharm. Sci.* 44: (1) 8-10.

- Saxena, A.P. and Vyas, K.M. 1986. Antimicrobial activity of seeds of ethnomedicinal plants. J. Econ Tax. Bot. 8 (2): 291-299.
- Sayed, M.D. 1980. Traditional medicine in health care. J. Ethnopharma 2: 19-22.
- Shibasaki, I. 1982. Food preservation with non traditional antimicrobial agents. J. Food Safety 4: 35-58.
- Terrell, F.R., Morris, J.R., Johnson, M.G., Gbur, E.E., and Makus, D.J. 1993. Yeast inhibition in grape juice containing sulfur dioxide, sorbic acid, and dimethyldicarbonate. J Food Sci. 58: (5) 1132-1134.
- Toppozada, H.H., Mazloun, H.A., and El-Dakhakhny. 1964. The antibacterial properties of the *Nigella sativa* L. seeds. Active principle with some clinical applications. J.Egypt. Med.Assoc. 48: 178-202.
- Windsor, M.L. and Thoma, T. 1974. Chemical preservation of industrial fish: New preservative mixture. J. Sci. Food Agric. 25: 993-1005.

**APPENDIX**  
**Statistical Analyses**

## **Part II: Antimicrobial Activity of Essential Oils from Herbs and Spices to Selected Pathogenic and Saprophytic Microorganisms**

Statistical analysis was conducted using SAS version 6.12 (SAS Institute; Cary, NC) to determine differences in antimicrobial activity of tested spice and herb essential oils in comparison to controls. This experiment was performed as a completely randomized block by replications (3). Data were fit to a completely randomized design, with factorial treatment arrangement, and analyzed using PROC MIXED.

### SAS program for antimicrobial effect of herbs and spices study:

```
data H&S;
input treat $10. rep @;
org='L.mon ' ; input ize @; output;
org='L.pla' ; input ize @; output;
org='S.aur' ; input ize @; output;
org='E.coli'; input ize @; output;
org='S.typ' ; input ize @; output;
org='Y.ent' ; input ize @; output;
org='P.ero' ; input ize @; output;
org='A.nig' ; input ize @; output;
org='Geo'   ; input ize @; output;
org='Rhod'  ; input ize @; output;
cards;
data one; set one;
logzone=log(ize);
run;
%macro domix(var);
proc mixed data=one;
title1 "ANOVA for variable &var";
class org treat rep;
model &var= treat | org /predicted;
lsmeans org | treat/pdiff;
make 'predicted' out=rrr noprint;
make 'lsmeans' out=mmm noprint;
make 'diffs' out=ppp noprint;
run;
```

```

%include 'a:pdmix612.sas';
title1 "LSD Mean separation for &var";
%pdmix612 (ppp,mmm);
proc univariate plot normal data=rrr;
title1 "Check on normality for &var";
var _resid_ ;
run;
%mend;
%domix(izone );
%domix(logzone );

```

### **Part III: Use of Black Cumin Essential Oil to Inhibit Spoilage and Pathogenic Microorganisms in Rainbow Trout.**

The design of this experiment was a randomized block design with repeated measure treatment arrangement, blocked on replication. Data were analyzed using Proc Mixed (SAS versions 6.12; Cary, N.C).

#### SAS program for in vitro antimicrobial effect of black cumin essential oil:

```

data BC;
input treat $10. rep @;
org='L.mon' ; input izone @; output;
org='L.pla' ; input izone @; output;
org='S.aur' ; input izone @; output;
org='E.coli' ; input izone @; output;
org='S.typ' ; input izone @; output;
org='Y.ent' ; input izone @; output;
org='P.ero' ; input izone @; output;
org='A.nig' ; input izone @; output;
org='Geo' ; input izone @; output;
org='Rhod' ; input izone @; output;
cards;
data one; set one;
logzone=log(izone);
run;

```

```

%macro domix(var);
  proc mixed data=one;
  title1 "ANOVA for variable &var";
  class org treat rep;
  model &var= treat | org /predicted;
  lsmeans org | treat/pdiff;
  make 'predicted' out=rrr noprint;
  make 'lsmeans' out=mmm noprint;
  make 'diffs' out=ppp noprint;
  run;
%include 'a:pdmix612.sas';
title1 "LSD Mean separation for &var";
%pdmix612 (ppp,mmm);
  proc univariate plot normal data=rrr;
  title1 "Check on normality for &var";
  var _resid_ ;
run;
%mend;
%domix(izone );
%domix(logzone );

```

### SAS program for in vivo antimicrobial effect of black cummin essential oil:

```

data fish;
input treat rep temp $ day pca coli mox rose;
cards;
%macro domix(var);
  proc mixed data=one;
  title1 "ANOVA for variable &var";
  class day temp treat rep;
  model &var= temp | treat | day /predicted;
  random rep rep*temp*treat;
  lsmeans day | temp | treat/pdiff;
  make 'predicted' out=rrr noprint;
  make 'lsmeans' out=mmm noprint;
  make 'diffs' out=ppp noprint;
  run;
%include 'a:pdmix612.sas';
title1 "LSD Mean separation for &var";
%pdmix612 (ppp,mmm);
  proc univariate plot normal data=rrr;
  title1 "Check on normality for &var";

```

```
var _resid_ ;  
run;  
%mend;  
%domix(pca );  
%domix(coli);  
%domix(mox );  
%domix(rose);
```

Pdmix 612.sas\* was provided courtesy of Dr. Arnold Saxton

\* Saxton, A.M. 1988. A macro for converting mean separation output to letter grouping in PROC MIXED. In SAS 1988. Proceeding of the 23rd annual SAS Group International Conference. Pp. 1243-1246.

## VITA

Mona Elgayyar was born in Cairo, Egypt on December 15, 1969. She graduated from El-Namozagia High School in Cairo, Egypt in July, 1986. Afterwards, she enrolled in the College of Veterinary Medicine at Suez Canal University in Ismailia, Egypt. After she graduated in 1992, she worked for a year as a teaching assistant in the College of Veterinary Medicine at Tanta University, Egypt. In 1993, she joined the Animal Health Research Institute in Giza, Egypt as a research assistant. In August 1996, she started her graduate study for a Master's Degree in the Department of Food Science and Technology. There she pursued a Master of Science degree, with concentration in Food Microbiology.

8237 3339

10\*21\*99

10

MAB



9  
FEDERAL  
DEPARTMENT OF  
COMMERCE  
U.S. DEPARTMENT OF  
COMMERCE