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Student Research - Inhibition of Microorganisms by Citrus Peel Oils

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INHIBITION OF MICROORGANISMS BY CITRUS PEEL OILS

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Abstract

Oils of citrus peels contain a chemical which has exhibited insecticide properties. Citrus oils might also have natural antibiotic or antifungicide properties which could prove beneficial to mankind. The purpose of this project was to analyze the possible inhibition of microorganisms by oils of citrus peels.

Five citrus fruit peels were tested for bacterial or fungal inhibition on *Escherichia coli, Bacillus cereus* and *Aspergillus niger.* Oils of lemon peel showed an inhibition zone on the *A. niger* in the first trial. In the second experiment, all of the citrus peel oils showed an inhibition zone on the *A. niger.* The bacteria showed no inhibition by the oils.

Introduction

Even before the discovery of penicillin, the pharmaceutical industry sought natural agents found in both plants and animals to combat infection. Antibiotics, compounds produced by microorganisms which inhibit the growth of other microorganisms, are powerful chemotherapeutic agents. However, many of these have produced toxic or undesirable side-effects in clinical use (Ross, 1983). Identifying plant or animal products which exhibit antibiotic and antifungal properties and yet are harmless to humans and higher animals might produce benefits to mankind.

Oils of citrus fruit peels have been shown to contain limonene, a highly effective insecticide (Taylor and Vickery, 1974). This research project attempts to determine whether oils of citrus fruit peels have antifungal or antibacterial properties.

Methods

The citrus fruits used in this experiment were obtained at a local market in Albany, Oregon. Five citrus fruits were used: lime, lemon, orange, tangerine and grapefruit. The fruits were refrigerated until use to preserve freshness.

Two types of bacteria and one fungus were selected to test for inhibition. These were: *Escherichia coli,* Gram negative bacteria; *Bacillus cereus,* Gram positive bacteria; and *Aspergillus niger,* a fungus.

Six petri dishes were each divided into four quadrants. Prepared nutrient agar from Carolina Biological Supply was poured into the plates using sterile techniques. Two plates each were inoculated with cultures of *E. coli, B. cereus* and *A. niger.*

After a 24-hour incubation at 34 degrees Celsius, the plates were treated with oil of citrus from the five citrus types. The fruits were washed in water before

the peels were removed. The oil was extracted from the peels by bending the peel and applying hand pressure. Sterile, color-coded filter paper discs, 6 mm in diameter, were used to absorb the oil. The discs were handled by sterile tweezers dipped into alcohol and then flamed. One oiled disc from each peel type was placed on a petri dish quadrant of each of the 24-hour cultures. Distilled water was placed on one disc as the control.

The plates were returned to the incubator at 34 degrees Celsius and were checked at regular 24-hour intervals.

Results

In the first experiment, oil of lemon peel showed a 15 mm inhibition zone on *A. niger* after 24 hours. The disc was included in the measurements. None of the other fruit peel oils demonstrated an inhibition of the microorganisms. After 96 hours, there was no change on any of the plates.

The experiment was repeated a second time using a fresh supply of fruit which produced a larger quantity of oil from the peels. This resulted in better absorption on the filter paper discs. More dramatic results were thus obtained in the second experiment. All five of the citrus oil types showed an inhibition zone on the *A. niger.* These zones were: lime (20 mm); lemon (10 mm); orange (20 mm); tangerine (15 mm) and grapefruit (20 mm). There was no inhibition of the bacteria by the oils. The inhibition of the fungus was noted after 24 hours and no further change had occurred after 72 and 96 hours.

The distilled water controls produced no inhibition of the microorganisms.

Discussion

Research has been reported in the literature concerning the insecticide properties of oils from citrus peels. This has been attributed to limonene (Ross, 1983). Limonene, $C_{10}H_{16}$, is related to isoprene. It is a colorless liquid derived from lemon, bergamot, caraway, orange and other oils including peppermint and spearmint. It is commonly used as a flavoring agent and fragrance (Hawley, 1977). The limonene content of citrus oils has been found to be about 98 percent and the pure limonene can be separated from the oil by steam distillation, although such distillation was not necessary before the oil could be used as an insecticide (Linstromberg and Baumgarten, 1983).

Both experiments conducted in this project demonstated an inhibition of *A. niger* by oils of citrus peels. Some of the possible variables in the two experiments include the following: The citrus oils from some fruit peels were more difficult to extract, notably the lime, because of its thin peel. Thus, saturation of the filter paper discs may not have been uniform among the fruit types. In the first experiment, the fruit had been stored in the refrigerator before use, which had a drying effect. The oils were thus harder to extract.

The fruits were washed before use. Chemical sprays, such as pesticides, which might have been used on the fruit in the orchard are an unlikely source of the inhibition.

Further studies which could be included in this investigation might be to test the citrus oils on other types of fungus. Isolating the limonene from the peel by steam distillation should also be attempted and the limonene tested for inhibition of fungus.

In conclusion, the results of this investigation indicate that five types of citrus peel oils exhibit antifungal properties.

Literature Cited

Ross, F.C. 1983. *Introductory Microbiology,* Charles Merrill Pub., Columbus, Ohio.

Taylor, W.E. and B. Vickery. 1974. Ghana Journal of Agricultural Science 7, 61-62.

Hawley, G. 1977. *The Condensed Chemical Dictionary,* 9th Ed., Litton Educational Pub., New York.

Linstromberg, W.W. and H. Baumgarten. 1983. *Organic Experiments.* 5th Ed., D.C. Heath Pub., Lexington, MA.

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Editor's Note: Additional Bibliography

Greany, P.D., S.C. Styer, P.L. Davis, P.E. Shaw, and D.L. Chambers. 1983. Biochemical Resistance of Citrus to Fruit Flies. Demonstration and Elucidation of Resistance to the Caribbean Fruit Fly, *Anastrepha suspensa.* Entolmol. Exp. Appl. 34:40-50.

Hink, W.F. and B.J. Fee. 1986. Toxicity of D-limonene, the Major Component of Citrus Peel Oil, to All Life Stages of the Cat Flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). Journal of Medical Entomology 23:400-404.

Sheppard, Craig. 1987. *The Orange Peel Murder Mystery.* Science of Food and Agriculture 5(2):9-12.

Sheppard. D.C. 1984. Toxicity of Citrus Peel Liquids to the House Fly and Red Imported Fire Ant. Journal of Agricultural Entomology 1(2):95-100.