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ANTAGONISITICS EFFECTS OF SELECTED ESSENTIAL OILS ON THE GROWTH OF MICROORGANISMS COLLECTED FROM DIFFERENT SURFACES

THESIS

TORRYE D. HOOPER

2007

ANTAGONISITICS EFFECTS OF SELECTED ESSENTIAL OILS ON THE GROWTH OF MICROORGANISMS COLLECTED FROM DIFFERENT SURFACES

THESIS

Presented in Partial Fulfillment of the Requirements for

the Master of Science Degree in the Graduate School

of Texas Southern University

By

Torrye Denise Hooper, B.S.

Texas Southern University

2007

Approved By

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By

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Texas Southern University, 2007

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Several fungi and bacteria were collected from different surface areas such as exterior of buildings, bark of trees, door hedges, floors, windows, soil and water pipes. The microorganisms were then isolated in pure cultures; light microscope (LM), stereomicroscope (SM), and scanning electron microscope (SEM) were used to identify four gram-positive and four gram negative bacteria. Fungi *Aspergillus flavus, Rhizopus sp., Geotrichium sp, Fusarium sp., Periconia sp., Aspergillus niger, Trichoderma, Penicillium sp., and Botrytis sp.*, were also identified using the different microscopes. The antifungal activity of the essential oils of selected seeds in the Apiaceae family was evaluated using the diffusion bioassay. The selected oil within the family Apiaceae included seeds of anise (*Pimpinella foeniculum*), caraway (*Carum carvi*), carrot (*Daucus carota*), celery (*Apium graveolens*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare*).

Oils that are alsotested for the antifungal activity but outside the Family Apiaceae included grapefruit (*Citrus paradise*), anise star (*Illicium parviflorum*), and yellow mustard (*Brassic hirta*). Different amounts of each tested oil were used, 0.5μl, 1.0μl, and 2.0μl, oils were applied into small drilled holes in the potato dextrose agar and nutrient agar in each petri dish plate. Each plate was inoculated with one of the tested fungi or bacteria. Every treatment was replicated three times for consistency of results. All plates were incubated for 24-72 hours, 27°C for fungi and 35° for bacteria cultures. Results indicated that some of the tested oils possessed some antimicrobial properties, as it was evident from the inhibition zones surrounding the area where the chemicals were applied. The most effective oil for fungi was grapefruit, which inhibited the growth of seven different fungi with *Aspergillus niger* having the highest inhibition. The least effective essential oil was celery and anise oil for fungi. Caraway was the most effective essential oil against bacteria, and the least effective was anise against bacteria. The most widely effective essential oil for fungi and bacteria was the grapefruit.

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CHAPTER 1

INTRODUCTION

Microorganisms are organisms requiring magnification to see and resolve their structures. Bacteria are prokaryotes that are almost always microscopic while a number of eukaryotes are microscopic including most protist and a number of fungi. Microorganisms, like other living organisms have the ability to grow and reproduce. However, unlike many other living organisms, microorganisms have adapted to living in all types of environment including harsh environments. An adaptive characteristic of microorganisms is their ability to attach and grow on inert surfaces [1,2]. Surface areas of microbial attachment include household faucets, membrane distillation systems, food processing environments, soil, monuments, buildings, and hospital devices [1-10,29]. Microorganisms also have the ability to attach themselves to living organisms including humans, they have been found to live on the surface areas of the skin, nose, throat, mouth, intestinal tract, and other body parts [1,9,13,17]. The activities of microorganisms play a vital role in the environment because of their interactions with the physical and chemical agents that they are exposed to, and are great to study because they reproduce rapidly and are great in number.

Research indicates that in order for microorganisms to survive on inert surfaces that might be exposed to inhospitable conditions such as U.V. light, desiccation, heat,

cold, and shear surfaces, they must adapt by existing as adherent populations. These microorganisms appear to be protected in antagonistic environment by growing as colonies encased in an extracellular matrix of carbohydrates or exopolysaccharide. Microbial colonization is often the first step in pathogenesis. Microorganisms may attach themselves to various surfaces in hospitals, kitchens, restaurants, outdoor playgrounds and many other surfaces. The effects of microbial formation can lead to respiratory, neurological, and immune dysfunction in humans, but can also lead to corrosion, deterioration and alteration of monuments [1-13].

Once these microorganisms begin to colonize or localize as a group, they make up a biofilm formation. This process begins as microorganisms make a simple layer, one cell deep, and begin to produce slime. This slime protects them from being washed away or drying out and also slows down antibiotics and other toxins that might come in. Research further indicates that after several hours, the microorganisms make protein messages. When these chemical messages become concentrated the microbes start to pile on top of each other making three-dimensional structures called biofilm. A biofilm is an assemblage or collection of microbial cells that is associated with a surface and enclosed in a matrix of primarily polysaccharide material. The matrix, which encloses or surrounds the biofilm, may include mineral crystals, corrosion particles, clay, or blood components depending on the environment. Biofilms form on a variety of surfaces, which include living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems. Not only do the variety of surfaces provide a habitat for the biofilm, but they also provide nutrients for growth of cells and stimulate regrowth of incompletely killed biofilm organisms [1-17].

Biofilm resistance to antibiotics and disinfectants is a major threat to public health. Many strategies to control biofilm formation have been proposed, however, one strategy has been to use natural products when substitutions are not effective. Naturally occurring biologically active compounds from plants are regarded as safe. These plant extracts are more acceptable and less hazardous than synthetic compounds. This means that essential oils can be used as an alternative anti fungal and anti bacterial treatment [15-19]. The purpose of this research was to determine the efficacy of a group of essential oils to treat microorganisms such as bacteria and fungi. Laboratory research included collection, isolation and identification of the microorganisms which was colonized on different surfaces, as well as identifying selected Essential Oils for their antimicrobial activities.

Samples were collected from the following surface areas: exterior of buildings, bark of trees, door hedges, floors, windows, and bathrooms. Once samples were collected they were placed into empty sterile petri dishes. Samples were then incubated onto sterile nutrient agar and potato dextrose agar plates. The unknown samples were then incubated for 24-72 hours in a 37°C for the bacteria, and 27°C for the fungi. Pure cultures of isolated fungi and bacteria were then established. Grams test was used to determine the gram-negative and gram-positive bacteria. Selected essential oils of different plant seeds were tested for their antimicrobial activity.

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CHAPTER 2

RELATED LITERATURE

Microorganisms or microbes are organisms that are microscopic. Microorganisms can be bacteria, protist, and fungi. Microbial organisms are present in high populations in soil, liquid, food, medical devices, monuments and even body parts. Although microbes are cells that must be magnified in order to see them, when cultured on solid media that allow their growth and multiplication, they form visible colonies consisting of millions of cells. Microorganisms are the cause of many infectious diseases. The organisms involved include bacteria causing diseases such as plague, tuberculosis, and anthrax; protozoa causing diseases such as malaria, sleeping sickness, and toxoplasmosis; and also fungi causing diseases such as ringworm, candidiasis or histoplasmosis [2].

Hospitals are considered important for the containment of antimicrobial resistance. The combination of seriously ill patients, the intensive use of antibiotics, and cross contamination has resulted in nosocomial infections with highly resistant bacterial pathogens. Infections caused by antibiotic resistant microorganisms are associated with higher mortality rates and higher costs than are antibiotic sensitive bacterial infections. The prevalence of resistance for nearly all important microorganisms/antibiotic combinations is generally higher among isolates from patients in intensive care units than

that among non-ICU inpatients. *Enterobacteria* and *Pseudomonas aeruginosa* have emerged as major causes of nosocomial infections and account for approximately 30%-50% of all bloodstream infections. Bacterial colonization is often the first step in the pathogenesis of nosocomial infections. Different mechanisms may lead to the colonization of hospital patients with resistant strains. First, these strains may enter the hospital upon the admission of patients already colonized with resistant strains. Secondly, during the hospitalization, susceptible bacteria may develop resistance due to genetic mutations or through the transfer of resistance genes. Thirdly, resistance may emerge through the induction of genes that are already present in susceptible bacterial subpopulations. After discharge, patients may remain colonized with resistance bacteria acquired in the hospital, and these may subsequently spread into the community [1-13].

Infections associated with intravascular devices represent a serious mortality in hospitals. Nonsterile insertion techniques, prolonged use of intravascular (IV) catheters, administration of irritating infusates, and other similar factors may predispose patients to catheter infections. Bacterial colonization of IV catheters is a key step of this process and precedes the development of clinical catheter infection. The interaction of bacteria with other surfaces depends on multiple factors, including intrinsic catheter surface features such as hydrophobicity, presence of trace elements, microbial traits such as glycocalyx or pilus production.. The presence of fibrin, plasma products, and other materials circulating in the host may also participate in this interplay. Other studies have shown that bacteria, especially coagulase negative staphylococci, erode the catheter surface with microcolony formation on catheters [1-14]. Monuments exposed to the open air over a long period of time deteriorate due to various causes. The activities of microorganisms play a key role

because of their interactions with physical and chemical agents. Heterotrophic bacteria have been found on decaying building stone and it has been suggested that they are active in the decaying process as a result of mineralizing activities. It is likely that most stones contain sufficient organic matter from soil, airborne particles to maintain the growth and activity of microorganisms. Heterotrophic bacteria appear in weathered rocks, forming bacterial associations with the ability to create biofilms that retain moisture. Studies have also shown that the fungus *Penicillium* enhance and accelerate the biodeteriorative processes, and the colonization from the bacteria *Thiobacilli* is responsible for certain types of damage to limestone by the transformation of calcium carbonate into calcium sulphate [1-5].

Microscopic filamentous fungi are ubiquitous microorganisms with a great capacity to colonize many kinds of substrates and to develop under extreme environmental conditions. Moulds and other airborne particles in the indoor and outdoor environment have been recognized as possible causative agents of various diseases in humans including the airway infections, irritation of respiratory mucous membranes, acute and chronic damage of respiratory organs and mycotoxicoses. One of the mould sources in the indoor environment is damp building material of the dwellings. Studies in the U.S. have showed that the proportion of dwellings with mould growth varies between 20% and 40%. Mould growth on damp building materials mostly depend on water activity. Materials that are attached by mould growth are usually colonized by *Penicillium chrysogenum*, and *Aspergillus versicolor* followed by *Aspergillus fumigatus*, *Aspergillus niger*, and *Eurotium spp.* [1-15].

amount of water treatment plants and the water from associated distribution systems are not meeting biostability criteria because of biofilm formation. These biofilms are multiplying in the crevices as well as in the interior wall of distribution pipes [6].

Silicone gastrostomy devices (tubes and buttons) are used extensively for long term feeding and administration of special diets and medications. It is estimated that over 250,000 gastrostomy are performed annually in the United States. In many of these patients, particularly children, the gastrostomy catheters or the skin level devices remain in situ for lengthy periods. The following organisms have been found previously associated with gastrostomy: *Candida tropicalis, Torulopsis glabrata, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus* and *Lactobacillus sp.* Microbial colonization has been linked to tube deterioration, fungal attachment, and gastrostomy site wound infection [1-7].

Biofilm formation causes membrane biofouling, which results in loss of productivity and creates several operational problems for membrane plants. Biofouling inevitably occurs during the production of potable or demineralized water with the application of microfiltration, nanofiltration or reverse osmosis. Microorganisms present in raw water find favorable conditions for growth in these plants and form a biofilm, which covers the membrane surface. This biofilm contaminates drinking water with pathogenic bacteria [1-8]. The oral cavity is a reservoir for colonization and infection of systemic organs by pathogenic biofilm formation. It is understood that aging, tooth eruption, hormonal changes, active disease, oral hygiene, and other factors have an influence on biofilm formation and bacterial accumulation in the oral cavity. Oral biofilm is produce by the sequential attachment of a number of bacteria, and is dependent

on both species and surface attachment. These attaching biofilm are able to accumulate on the surface. However, biofilm is known to evade antimicrobial challenges involving antibiotics or host immune defenses by multiple mechanisms and it has been shown that antimicrobial agents fail by not yet being able to fully penetrate it. Furthermore, the biofilm community may increase in the oral environment, presenting considerable hygiene and host defense problems in elderly people [1-9].

In the United States, the Water Quality Association reported that there are at least 325 manufactures of Point of Use (POU) home water treatment devices and that 41% of all American homes used such devices in 2000. These devices are designed to remove a wide variety of contaminant in drinking water, including heavy metals, pesticides, chlorine and particulates. The types of systems used include passage through activated carbon, distillation, reverse osmosis, ultra filtration, and porcelain filters. In some designs, the treatment packages consist of several processes in series to achieve better removal and control of water contaminants. A wide variety of bacteria biofilm can be present in drinking water. Most of these bacteria do not have public health risk if ingested but many are opportunistic pathogens and it has been suggested that they may pose a potential health risk. These bacteria may increase in numbers and grow within the distribution systems or household taps, and in water treatment devices. The proliferation of bacteria depends upon the concentration of disinfectant in the distribution system, temperature, season and the amount of organic matter [1-10].

Fungi biofilm play an important role in biodeterioration of materials. They are implicated in health hazards and are used in many industrial and biotechnological processes as well as being important members of natural biofilm communities from

habitats such as streams and rivers. Many industrial processes experience considerable problems associated with extensive microbial growth, this biofilm formation is detrimental to the system causing significant losses. These problems are also encountered in the photoprocessing industry. Photoprocessors are fully automated and consist of a series of flowing water tanks with PVC walls. The first tanks contain chemicals, which develop and fix photographs while the remaining tanks are for rinsing. Biofilm growth occurs within the rinsing tanks due to the prevailing physical conditions, pH7 and operating temperature between 30 and 35°C. Detachment of the biofilm causes dirt and streaks on the prints; it can also affect transport characteristics within the machines and plug lines [1-11].

Copper tubing is widely in domestic plumbing systems. It is normally considered to be corrosion resistant, but there have been many reports of in service failures. The presence of copper in potable waters has risks for public health. Copper has been reported to induce nausea, vomiting, diarrhea and stomach cramps with acute exposures. Copper in water also causes aesthetic problems including discoloration, staining of bathroom fittings and a metallic, bitter taste upon consumption. These causes are often noticed experiencing water stagnation and low chlorine residuals, suggesting microbial involvement. Biofilms are commonly found on surfaces within drinking water distribution system including copper. Biofilm has previously been implicated in the pitting of corrosion of cooper and copper release in hot and cold water systems. Research has demonstrated that the exposure of new copper tubes to extracts obtained from blue water corrosion can similarly initiate copper corrosion. This can be related to the chemical and microbiological composition of the extracts [1-12].

Antibiotics and disinfectants are generally used to treat infections and microbial growth. The toxicity to humans and other animals from these agents are considered to be low, however, prolonged use of these substances can have a negative impact on health. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam distillation and by expression. The main constituents of essential oils mono and sesquiterpenes, including carbohydrates, alcohols, ethers, aldehydes, and keytones, are responsible for the fragrant and biological properties of aromatic and medicinal plants. Essential oils have been isolated from different parts of plants and also are used for similar purposes. Essential oils cover a broad spectrum of microbial activities. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant, and anticancerogenic properties. Others are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects, and plants [1-19].

Many plant essential oils, which are mixtures of numerous organic chemicals, contain compounds that inhibit microbial growth. In an experiment, six essential oils were screened for the ability to inhibit the growth of E.*coli*. Essential oils derived from C. *camphora* and M. *alternifolia* slightly inhibited the growth of E.*coli*. Z. *officinale* essential oil had no effect on growth, and essential oils derived C. *camphora* and M. *alternifolia* slightly inhibited the growth of E. *coli*. C. *zeylanicum* and C. *cassia* essential oils substantially reduced the growth rate of E.coli. C. *nardus* had a minimal effect on growth after 2 hours, but growth inhibition was evident after 15 hours. The effects of cinnamaldehyde, eugenol and citronellon on the growth of E.*coli*, P. *aeruginosa*, P. *putida*, and P. *fluorescens* were evaluated as well. Cinnamaldehyde

significantly inhibited the growth of all four species that were tested. Although eugenol strongly inhibited the growth of E.coli, the growth of the three Pseudomonas spp. was reduced by only 40%. The growth of all four species was moderately inhibited by citronellol after 2 hours [1-16].

Infected farm animals and foods of animal origin contaminated with enteric pathogens are often identified as primary sources of food borne disease in humans. Salmonella and Escherichia coli are two major food borne pathogens. Many essential oil components are generally recognized as safe by the food and drug administration of the US and have been used as artificial flavorings and preservatives, in the manufacture of perfume, and in over the counter for mulations of medicines. Essential oils have long been found as plants chemical defense against insects, fungi, and other invaders. In the study of *Salmonella typhimurium*, *Escherichia coli* 0157:H7 and *Escherichia coli K88*, cinnamon oil was the most effective in inhibiting the growth of all three pathogens followed by thymol, carvacrol, clove oil and geraniol. Thymol, cinnamon oil and carvacol have demonstrated a broad spectrum of antimicrobial activities [18].

Essential oils have been an interest with antifungal activity, which are more acceptable, ecological safe and less hazardous than disinfecting synthetic compounds that are used in air cleaning . In a study of essential oils against moulds from damp buildings, the most abundant fungi isolated from wall scrapes were species of *Aspergillus* and *Penicillium* . Essential oils of thyme, which were used in this study exhibited strong antifungal activity against isolated microfungi. In addition, essential oil of *Thymus*

spathuilifolius, suppressed the growth of *Trichophyton* spp., *Fusarium* spp., *Penicillium*., *Rhizopus* spp., *Alternaria* spp., and *Aspergillus* spp. [15].

Fungi are significant destroyers of food during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Storage fungi are commonly controlled by synthetic chemicals, however most of the fungicides create several side effects in the forms of carcinogenicity, tetraogenecity, and residual toxicity. Plant products posses the potential to be of best interest in pest management. In a study against *Aspergillus flavus*, which is known to be a dominant, mycotoxin producing storage fungus, cymbopogon oil was an effective post harvest fungi toxicant of this fungus [17].

CHAPTER 3

DESIGN OF THE STUDY

There were approximately five steps involved in the design of this research study, those steps included: collection of the unknown samples from different surface areas, isolation of the unknown microorganisms, identification of the unknown microorganisms using Stereomicroscopy, Light Microscopy, and Scanning Electron Microscopy (SEM), gram testing, and bioassay testing. The collection of the samples taken from different surface areas included: soil near the door of Nabrit Science Center, piping from water fountain of 2nd floor of NSC, walls at Herman Park, brick outside of an apartment building, outside window of Nabrit Science Center, outside building of new wing of the Nabrit Science Center, utility door outside of education building, brick from utility building behind Nabrit Science Center, bark of tree off tiger walk in front of Nabrit Science Center, 1st floor backdoor hedge of Nabrit Science Center, vent from womens bathroom on the second floor, floor of Nabrit Science Center near the middle exit and the 1st floor wall of Nabrit Science Center. Samples were collected from different surface areas.

To collect the unknown samples, a sterile razor blade was used to scrape the surface areas to where the unknown samples might have been colonizing. As the samples

were being collected from the different surface areas, they were being placed into empty sterile petri dishes. After the samples were collected and placed into the petri dishes they were brought back to the laboratory and preserved in the refrigerator for later research use. Once a stock of the samples were collected and preserved, each unknown sample was placed onto two different agar plates, nutrient agar and potato dextrose. Nutrient agar provides growth for bacteria and Potato Dextrose provides growth for fungi. After the unknown samples were placed on both agar plates they were sealed with parafilm to prevent dehydration. The unknown samples were incubated for 24-72 hours in 35˚C for the bacteria and 27˚ C for the fungi. At this point, there was no identification determined of the unknown samples. After the cultures were grown, numerous of different organisms were growing on each agar plate, therefore isolation of the organisms had to be performed.

To begin the process of isolation, a flamed inoculating loop was taken and streaked from each different bacteria and fungi onto a nutrient agar plate for bacteria, and a potato dextrose agar plate for fungi. It is important to note that the inoculating loop was sterilized to prevent contamination of other organisms, but let to cool down so that it would not destroy the isolating organism. Once the unknown samples were isolated, they were incubated for 24-42 hours, fungi at a temperature of 27˚C and bacteria at 35˚C. After the pure cultures were grown, identification was established. Arrangement of spores, hyphae, and mycelium were analyzed and studied to determine the fungi using Stereomicroscopy, Light Microscopy, and Scanning Electron Microscopy (SEM). Gram stain was used to study the morphology characteristics of bacteria.

To analyze fungi with Light Microscopy a sample of the unknown fungi attached to the agar, was taken with tweezers and placed onto a sterile glass slide, which had 2 to 3 drops of water. A cover slip was then added to the mounted sample of fungi and the sample was then analyzed for identification. When analyzing fungi with Stereomicroscopy, the fungi samples remained inside of the petri dishes, but the cover of the petri dish was removed to analyze and capture images of fungi. Fungi samples were examined with 20, 40, and 100x oil immersion objectives.

Preparing fungi for SEM identification began by taking a small piece of the fungi that was attached to the agar with tweezers. The sample was then mounted on an aluminum stub with double-sided sticky tape. After being mounted, the unknown sample was then air dried for 1-2 days. Sputtering of gold/palladium was deposited into the sample after it was mounted. Samples were then observed with SEM using accelerated voltage of 20kV. Images were captured with a digital image analysis and processing software.

Gram staining was performed for bacteria samples. The materials that were used were crystal violet ammonium oxalate solution, decolorizing alcohol, grams iodine-solution and counterstain-safranin. For the smear preparation the following steps were taken: first – the inoculating loop was sterilized in a bunsen burner flame and placed in a loopful of distilled water onto a clean slide; second – the inoculating loop was sterilized again and was allowed to cool for 10-15 seconds, then a small amount of the bacterial culture was removed; third – by using a circular motion, the culture material with distilled water was mixed and then spread over the center portion of the slide; fourth – the smeared bacteria was air dried. The following steps were performed for the staining of

bacteria: first – flooding of the smear with crystal violet for one minute, and the smear was washed with water for five seconds; second – the smear was covered with grams iodine solution for one minute, and washed with water; third – decolorization occurred by flooding the bacteria smear with alcohol for several seconds, pouring off the excess and repeating the decolorization twice and finally rinsing with water; fourth – blotting occurred with a lent free paper of the bacteria smear ; fifth – examination under oil immersion was performed of the bacteria. Gram positive bacteria retained the initial stain and appeared to be blue to purple or black. Gram-negative organisms decolorized with alcohol and retained the second stain, they appeared red to reddish pink.

Bioassay was utilized to test several essential oils of Family Apiaceae, Rutaceae, Illicium, and Brassicaceae for their antimicrobial activity. The essential oils that were used to test for antimicrobial activities included: Oils of seeds from the Family Apiaceae included Anise (*Pimpinella foeniculum*), Caraway (*Carum carvi*), Carrot (*Daucus carota*), Celery (*Apium graveolens*), Coriander (*Coriandrum sativum*), Cumin (*Cuminum cyminum*), Dill (*Anethum graveolens*), Fennel Sweet (*Foeniculum vulgare*), and Parsley (*Petroselinum crispum*); Grapefruit oil (*Citrus paradise*) from the Family Rutaceae; Anise star (*Illicium parviflorum*) from the Family Illicium and Mustard (*Brassica hirta*) from the Family Brassicaceae. The diffusion bioassay of drill hole cavity was utilized to determine the antimicrobial activity of each essential oil against each isolated bacterial and fungal species. First, on the outside bottom of the Nutrient agar and Potato Dextrose agar plate, the plate was divided from the center into seven sections, with one section left for control. Once the plates were divided into sections, each section was labeled accordingly for each essential oil. A drill hole was punctured with a micropipette inside

of the agar plates of each divided section for later use of the essential oils. The bacteria was then inoculated onto the nutrient agar plate with 3-4 drops of bacterial inoculum suspended in sterile distilled water for an even distribution, and each fungus was inoculated directly onto potato dextrose agar plates. Each essential oil was applied to the drill hole cavity on the nutrient agar and potato dextrose agar by a micropipette. Each treatment was replicated three times and three doses were used for each essential oil, 0.5μl, 1μl, and 2μl. Nutrient agar plates were incubated for at 35°C for 24-72 hours and potato dextrose agar plates were incubated for 27°C for 24-72 hours. All bioassay treatments were performed at the same time, in one day for consistency, however the entire bioassay was replicated three times for consistency of the results. Samples were then examined, and growth inhibition zones were measured in millimeters (mm).

CHAPTER 4

RESULTS

Several bacteria and fungi were isolated and identified from different surface areas. *Aspergillus flavus* and *Fusarium sp.* were isolated on the outside surface of the TSU library building. *Rhizopus sp.* was isolated from the bark of a tree, and the floor of Nabrit Science Center. *Geotrichium sp.* and gram-positive bacteria were revealed on the brick outside of an apartment building. *Periconia sp.* was found attached and colonized to the 1st floor back door hedge of Nabrit Science Center. *Aspergillus niger* and *Penicillium sp.* illustrated adherence to a vent inside of the Nabrit Science Center. *Aspergillus sp.* showed adherence and colonization on the 1st floor wall of Nabrit Science Center. *Trichoderma sp.* presented adherence and colonization on the outside window of Nabrit Science center. *Botrytis sp.* and gram-positive bacteria showed colonization on the walls at Herman Park. Gram positive bacteria was found adhering and colonizing in soil near the front door of Nabrit Science Center, and piping from a water fountain. Gram-negative bacteria illustrated adherence and colonization on the outside building of the new wing of Nabrit Science Center, utility door of education building and brick from utility building behind Nabrit Science Center.

Stereomicroscopy , Light Microscopy , and SEM led to the identification of different types of organisms that were collected. The various microscopy techniques allowed detailed studies of fungi and bacteria species. During the observation,

differences in morphology and sporulation were observed. The different types of microscopes revealed several different growth characteristics of fungi ranging from branching hyphae to colony forming hyphae, and precise characteristics of structures. Figures 1-21 reveal the morphology characteristics of the isolated microorganisms.

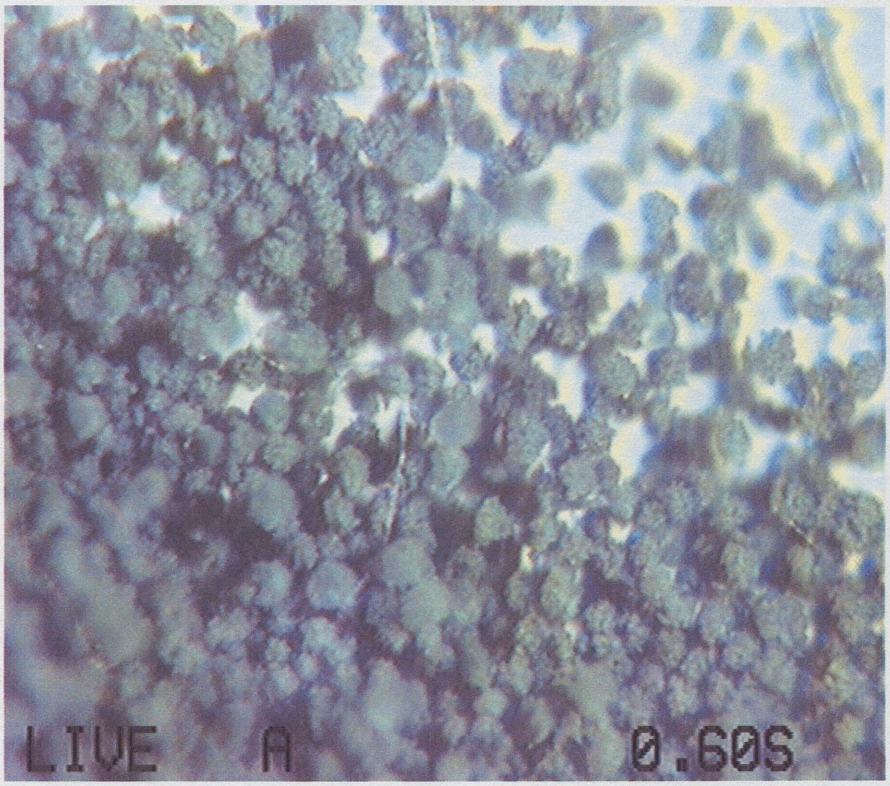


Figure 1. Stereomicroscopy Micrograph of spores from the fungi *Aspergillus flavus*

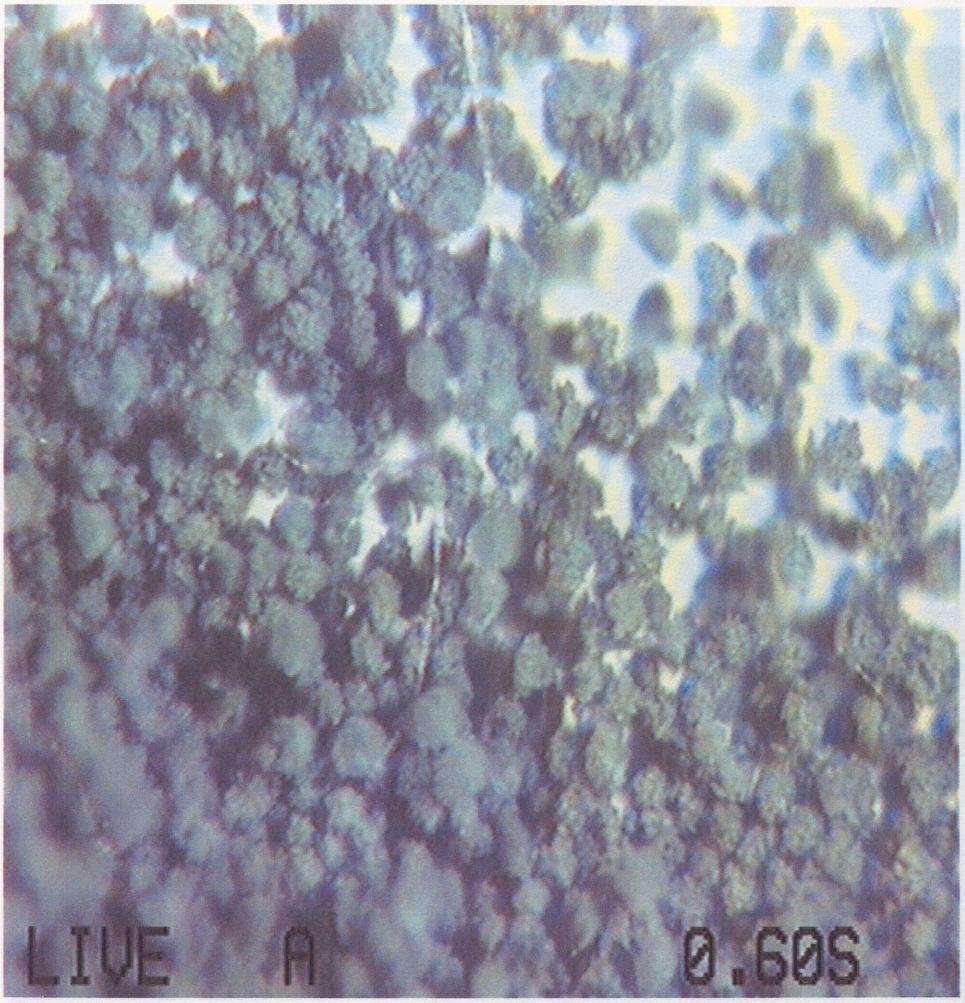


Figure 2. Stereomicroscopy Micrograph of the fungi *Aspergillus flavus*

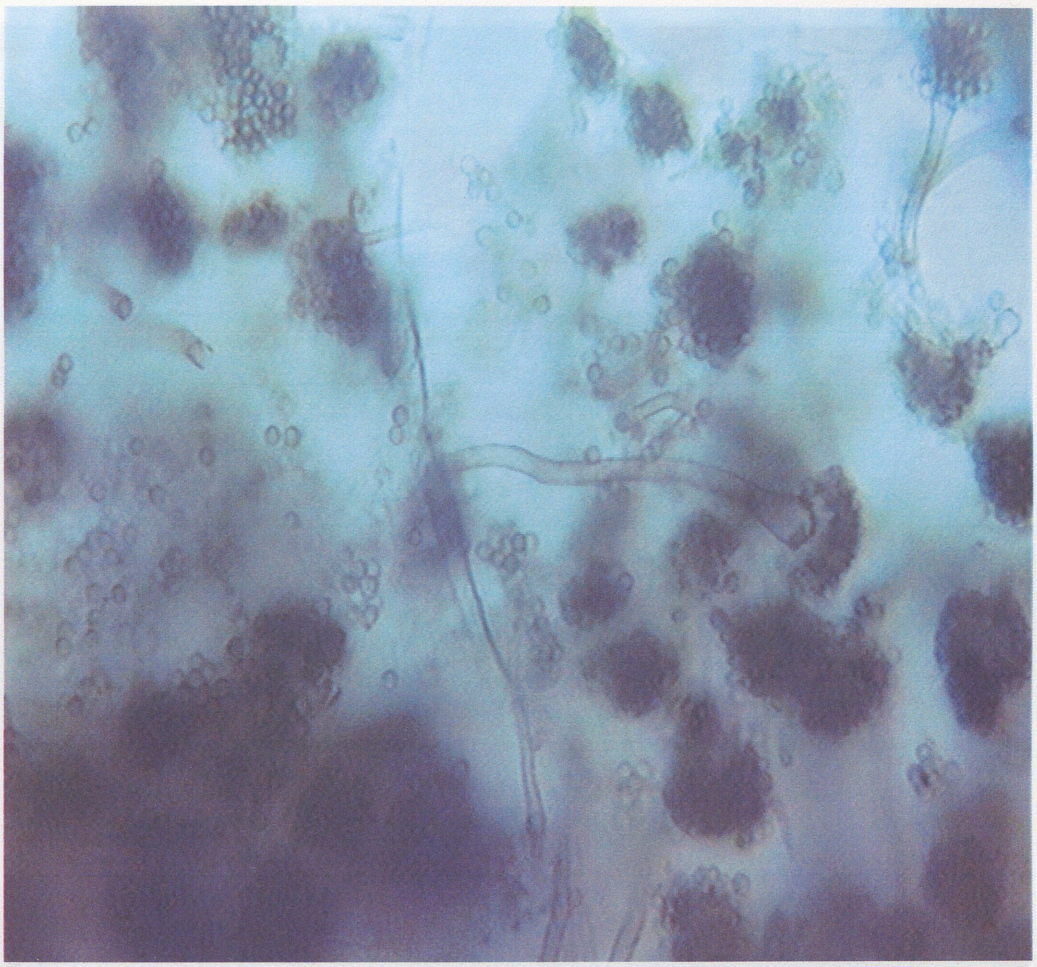


Figure 3. Light Microscopy Micrograph of the fungi *Aspergillus sp*

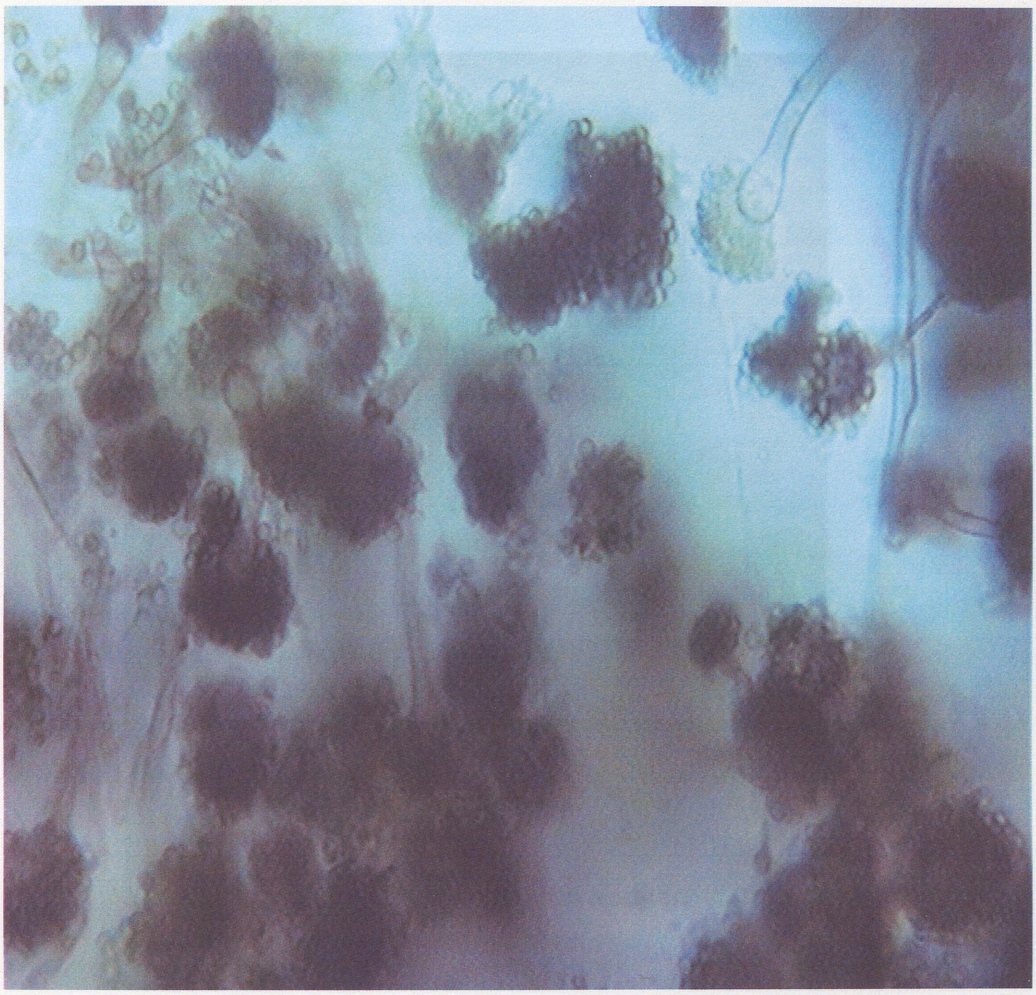


Figure 4. Light Microscopy Micrograph of the fungi *Aspergillus sp.*

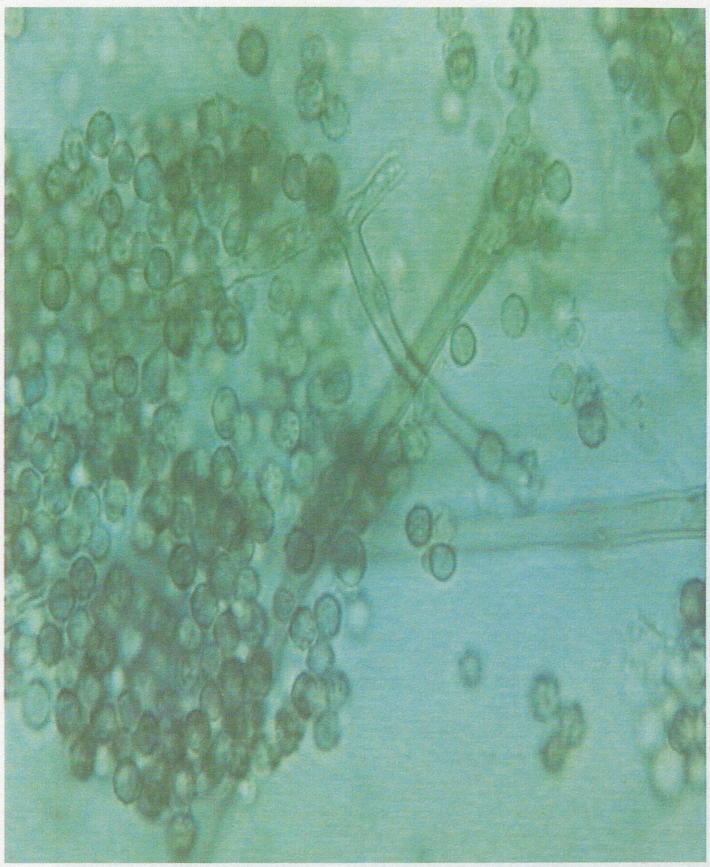


Figure 5. Light Microscopy Micrograph of fungi spores

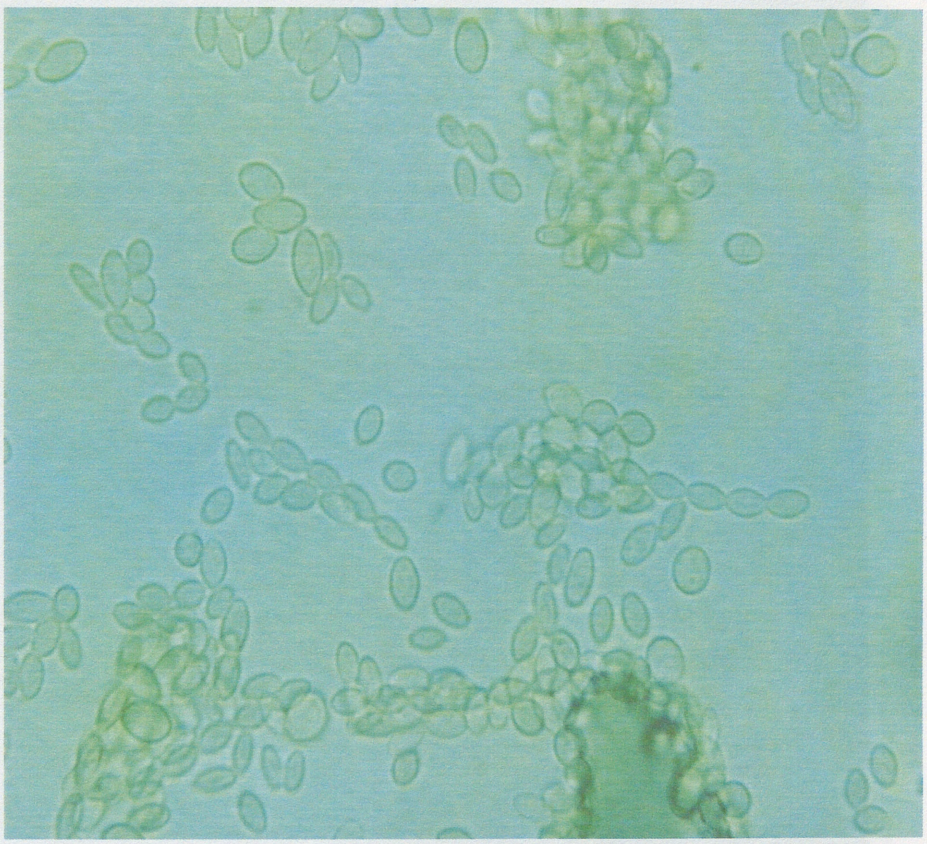


Figure 6. Light Microscopy Micrograph of fungi spores in chains.

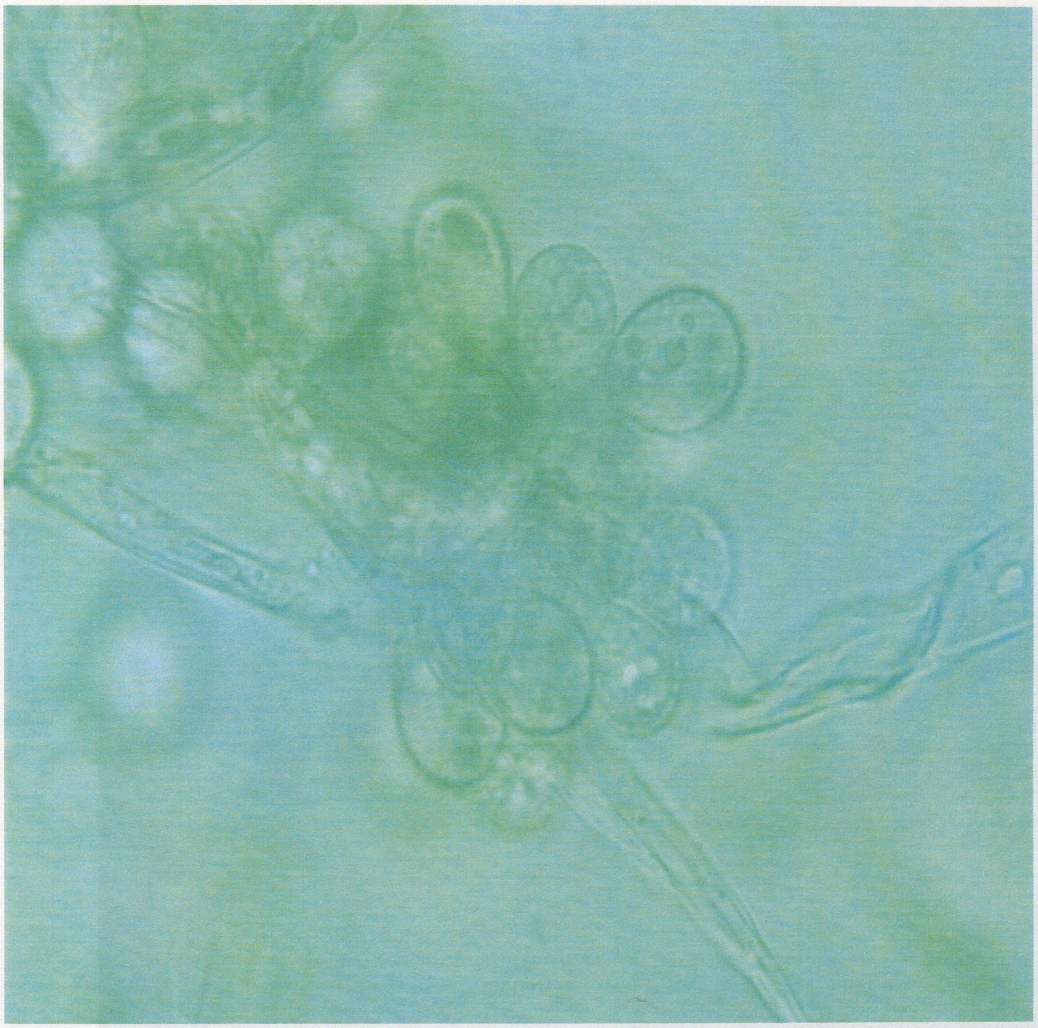


Figure 7. Light Microscopy Micrograph of fungi cluster spores on a sporophore

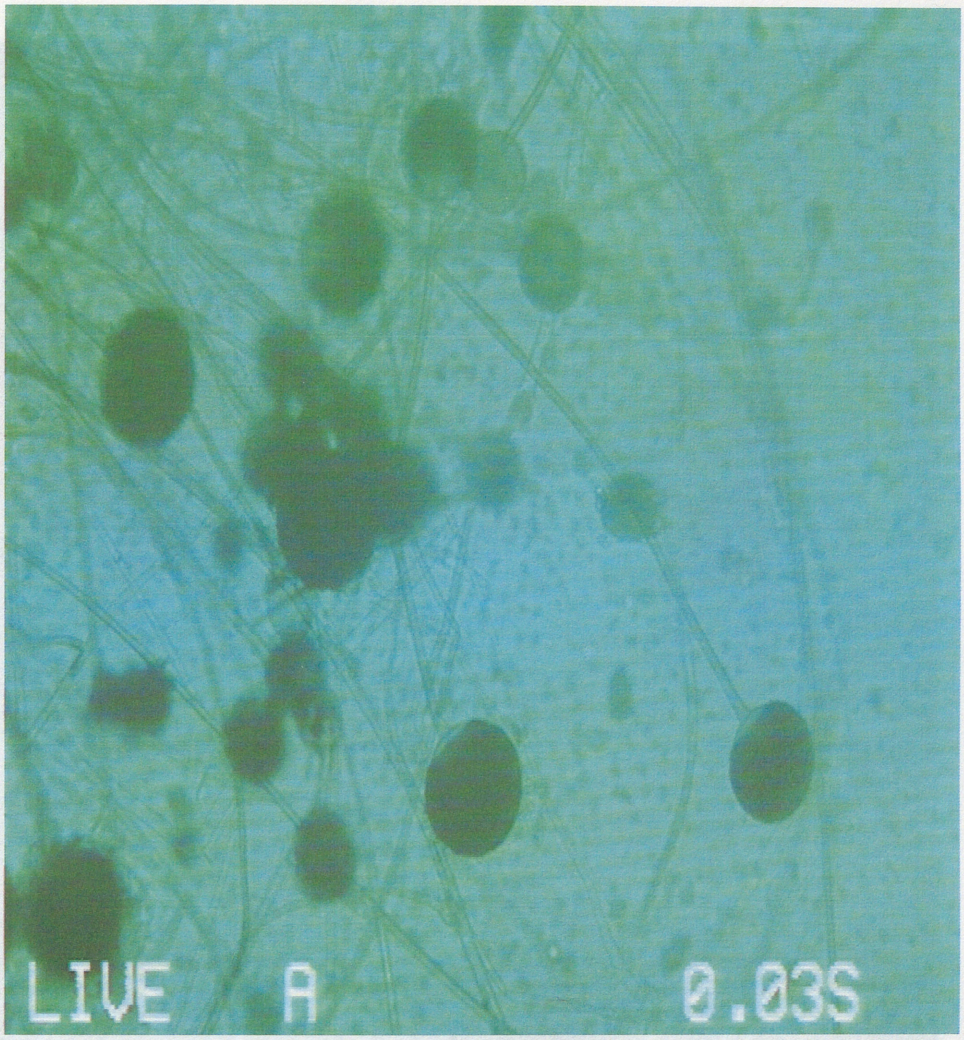


Figure 8. Light Microscopy Micrograph of sporangium of the fungi *Rhizopus sp.*

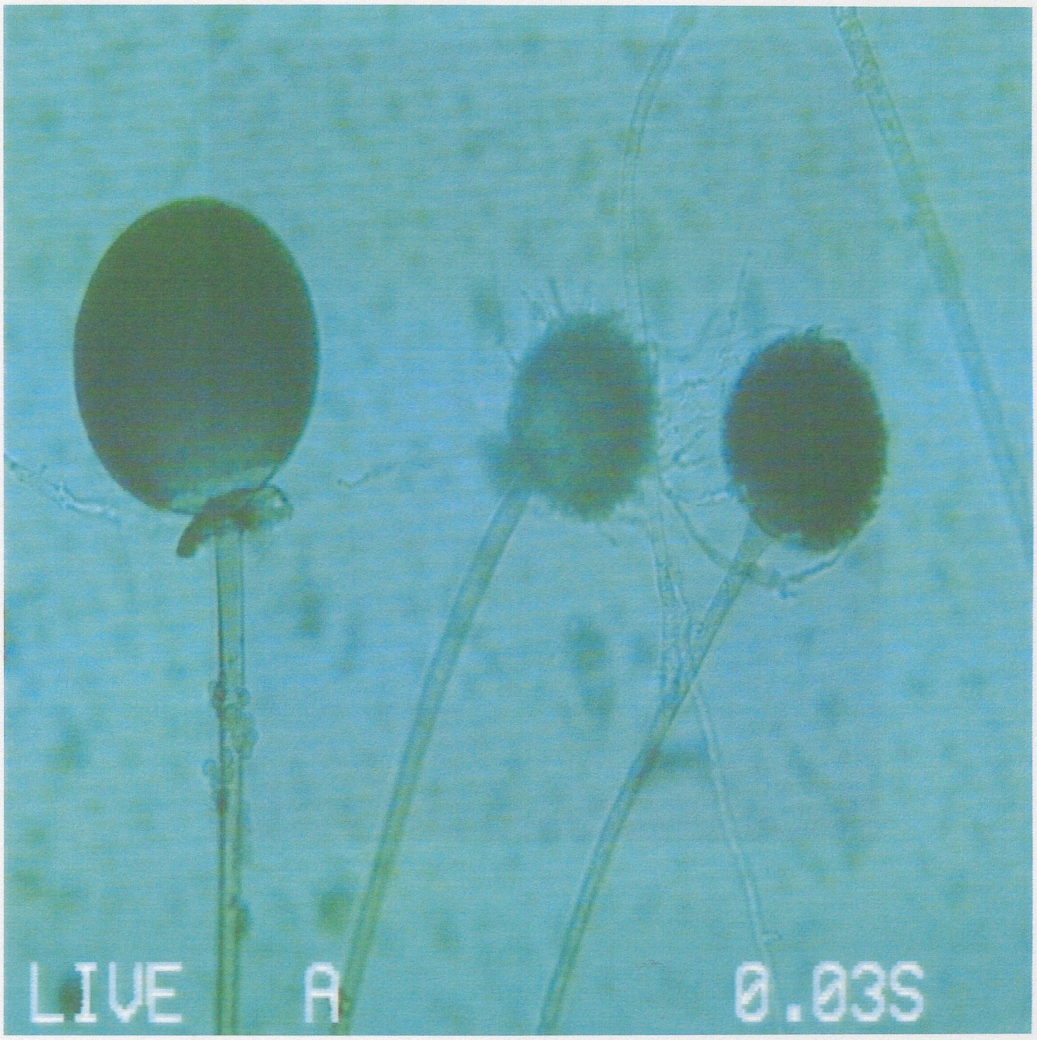


Figure 9. Light Microscopy of the fungi *Rhizopus sp* exhibiting sporangium

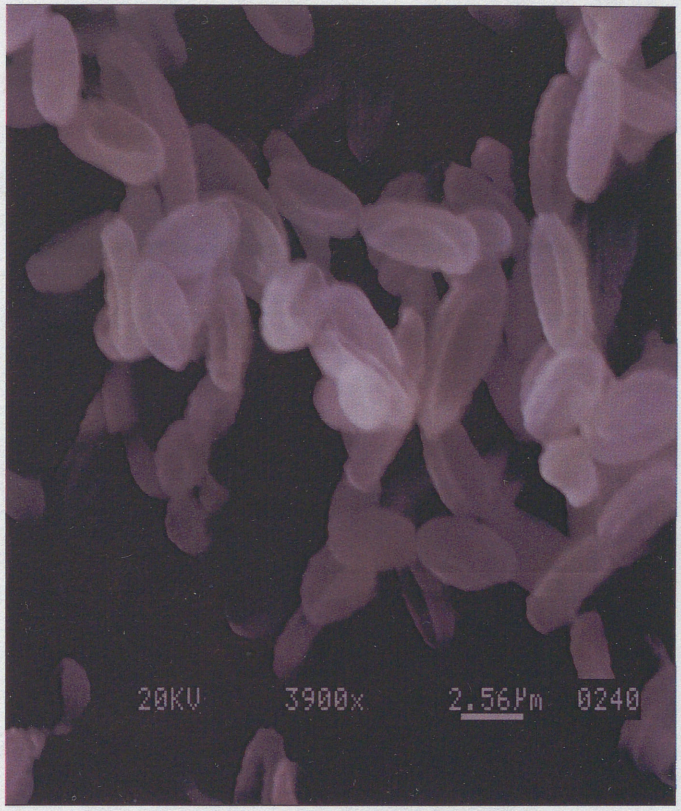


Figure 10. SEM Micrograph of fungi spores in chains

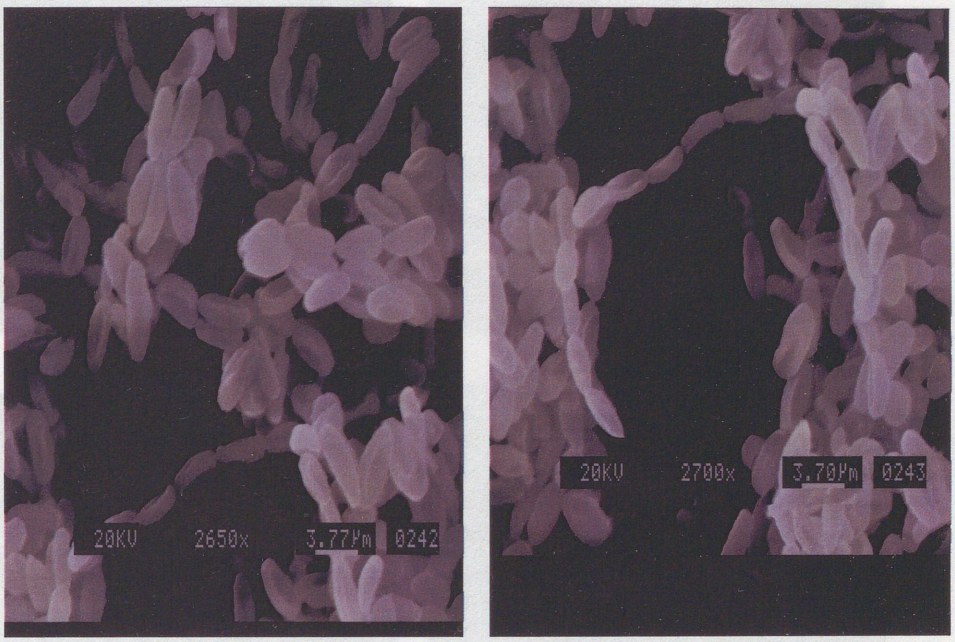


Figure 11. SEM Micrograph of fungi spores in chains

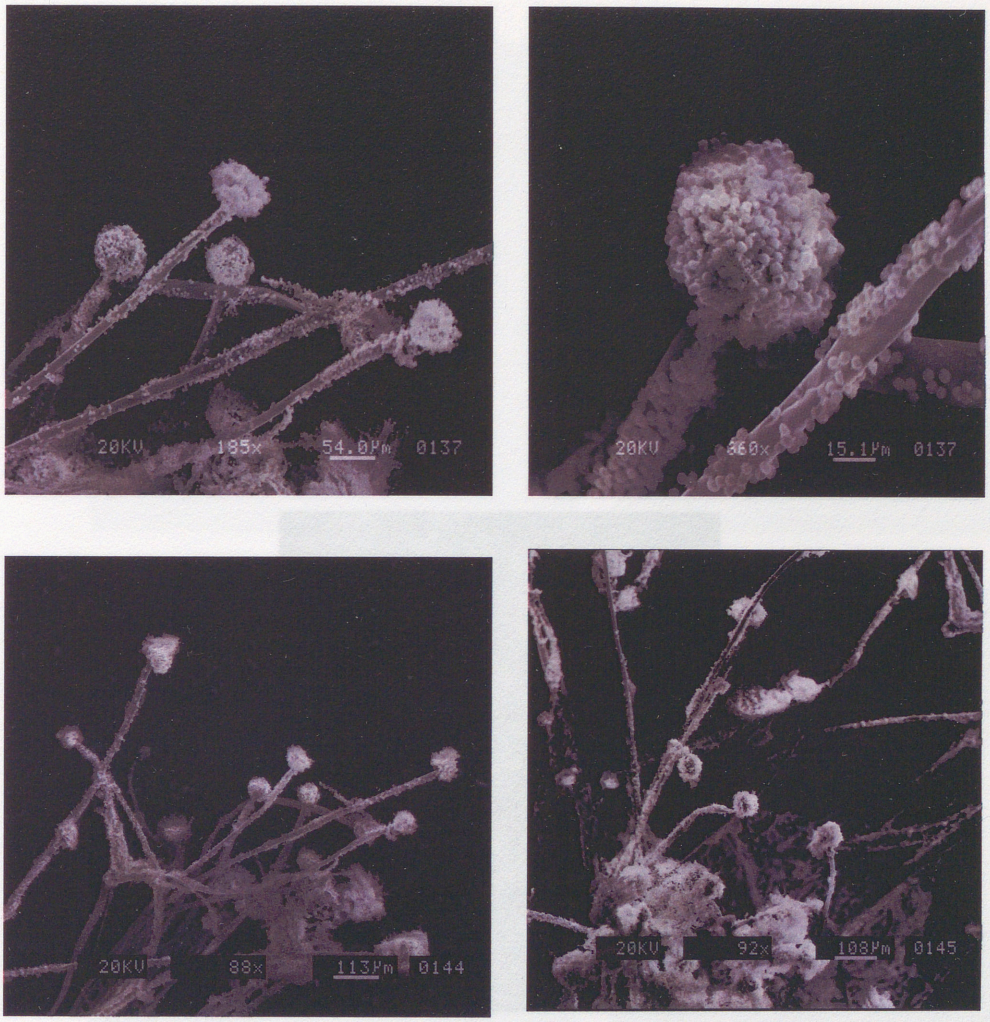


Figure 12. SEM Micrograph of fungi spores in chains

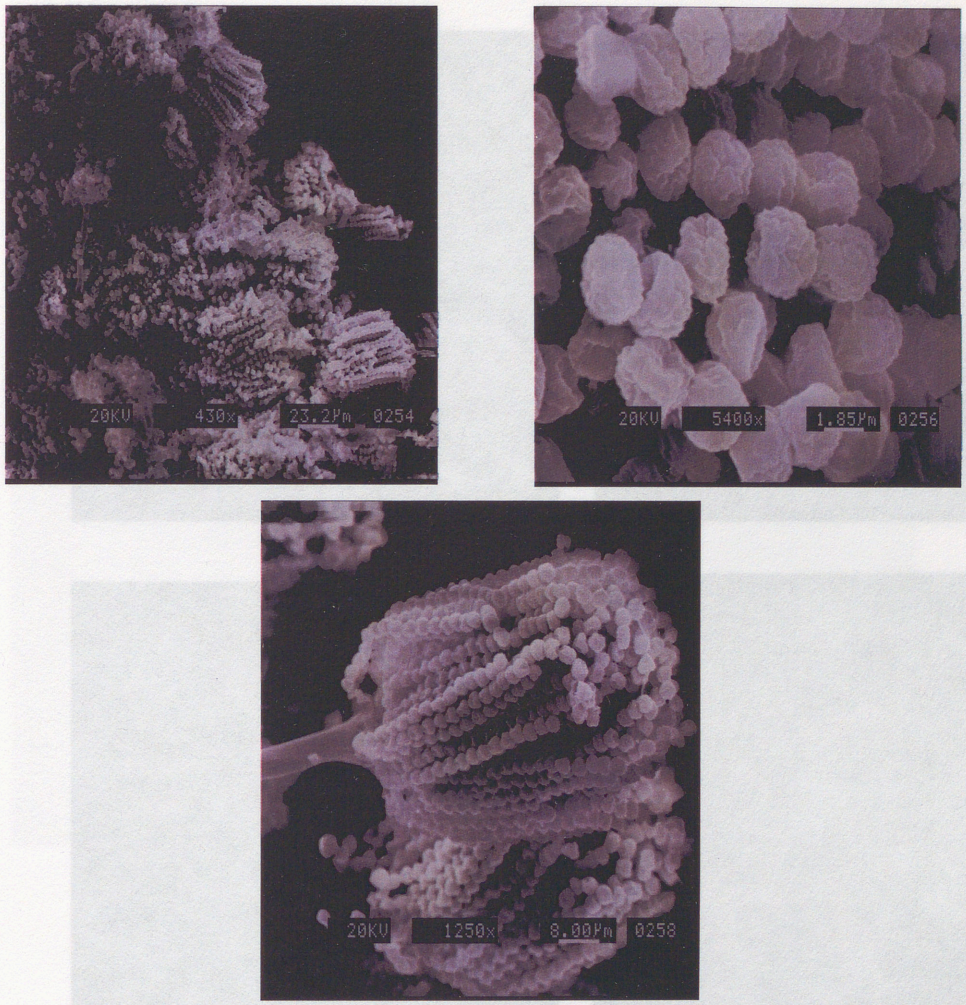


Figure 13. SEM Micrograph of the fungi *Aspergillus sp* showing the magnification of spores on a sporophore.

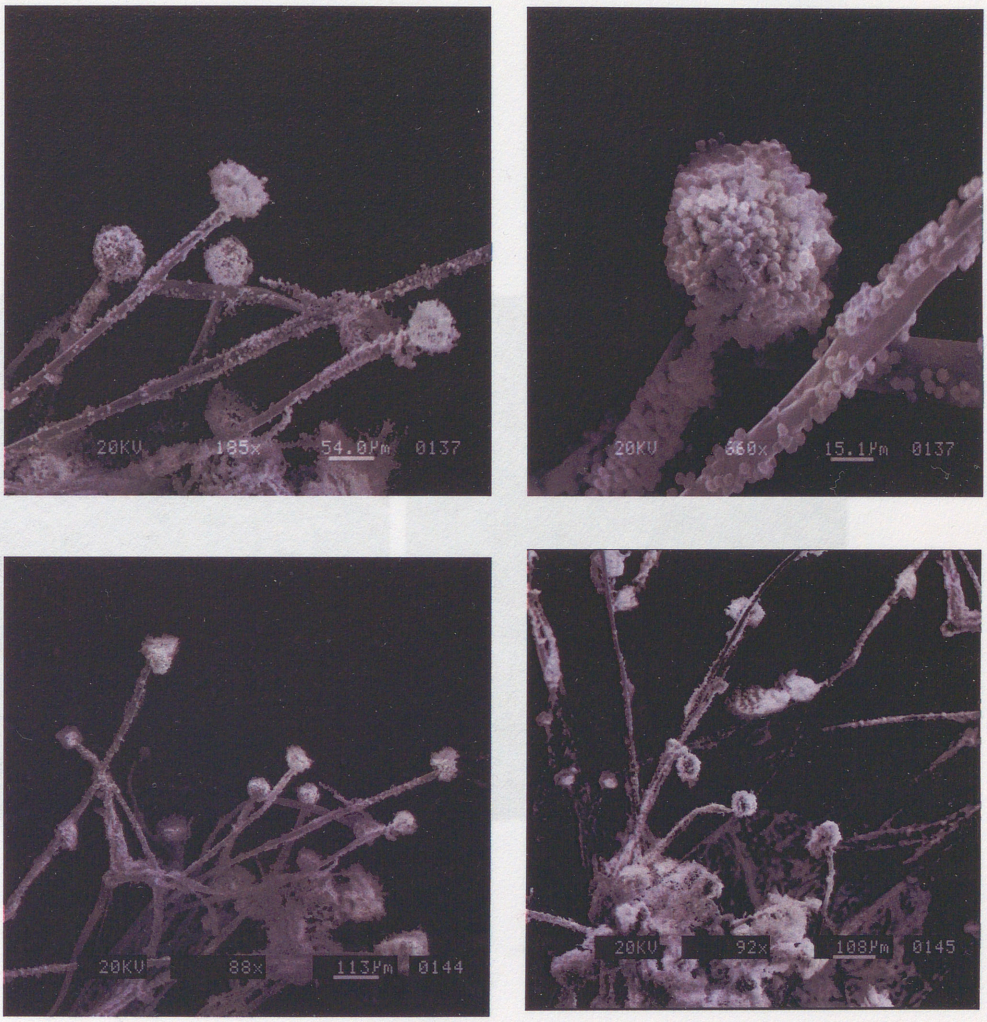


Figure 14. SEM Micrograph of the fungi *Aspergillus sp* spores on a sporophore

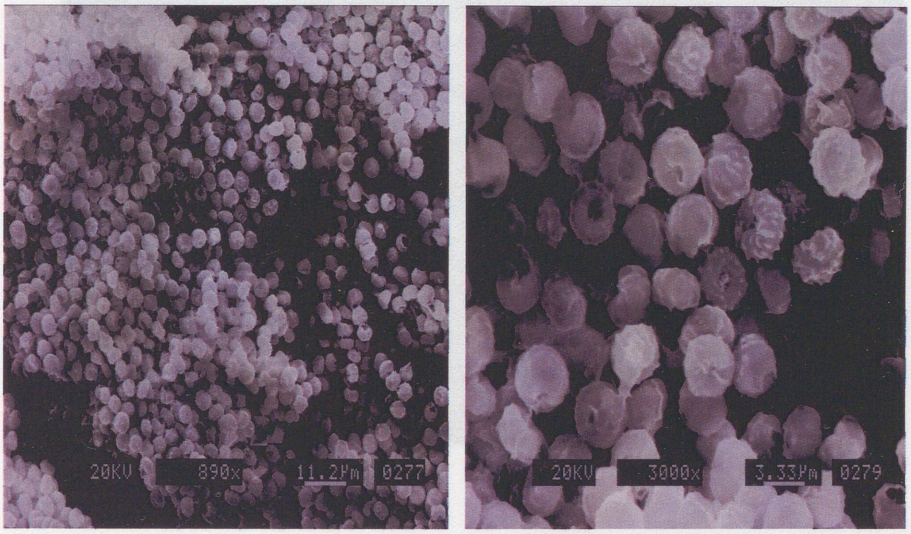


Figure 15. SEM Micrograph of fungi spores

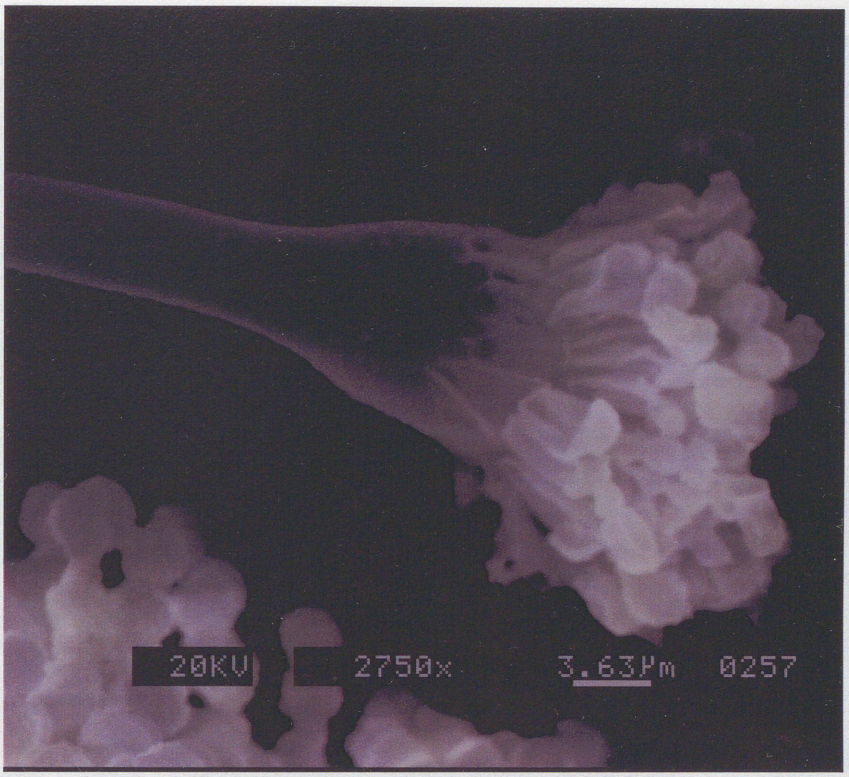


Figure 16. SEM Micrograph of sporophore from the fungi *Penicillum*

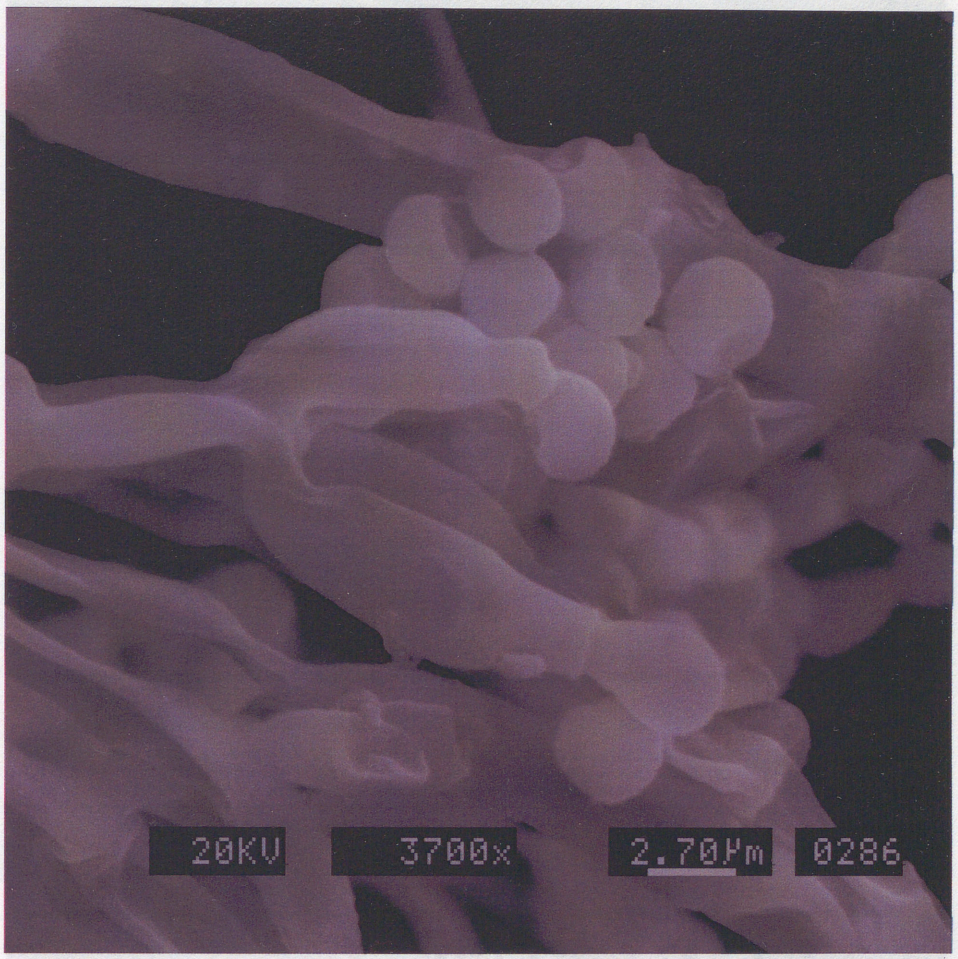


Figure 17. SEM Micrograph of spores from fungi *Penicillium*

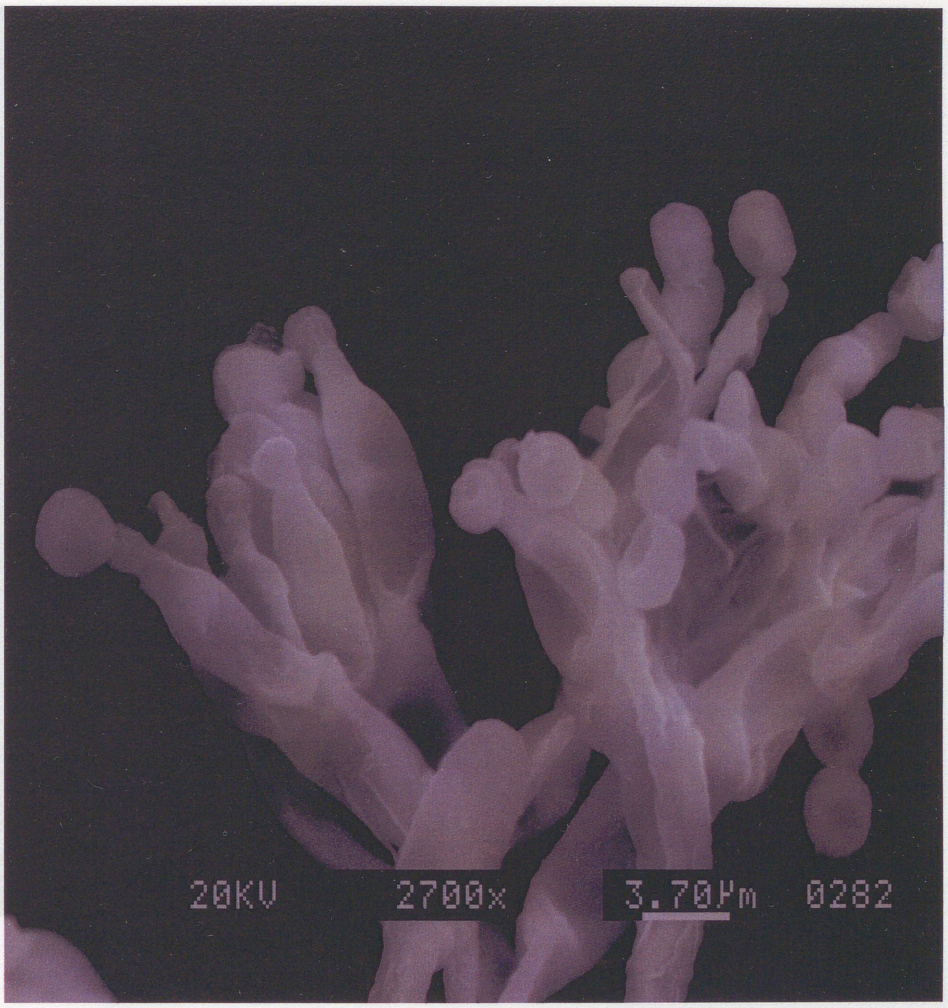


Figure 18. SEM Micrograph of the fungi *Penicillum*

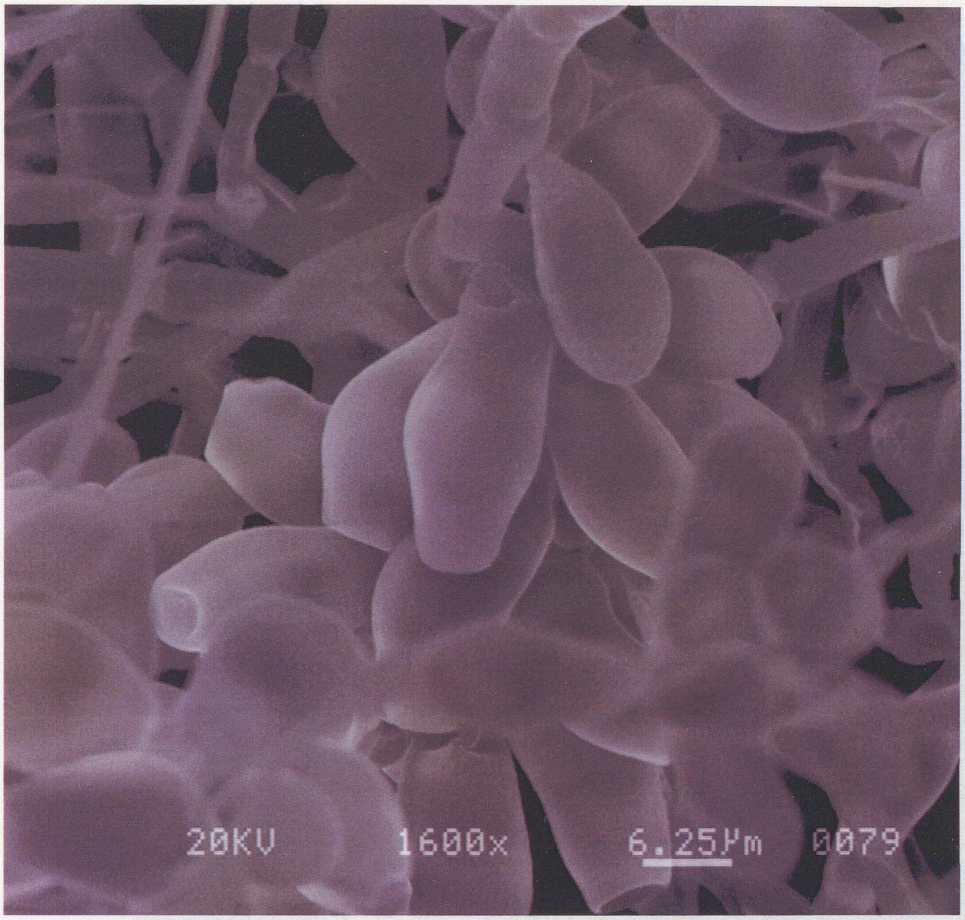


Figure 19. SEM Micrograph of spores from the fungi *Culvalaria sp*.

Figure 20. SEM Micrograph of the Anise seed

Figure 21. SEM Micrograph of outer surface oil secreting structures of the Anise seed

Results of the Bioassay Testing Essential Oils for Their Antimicrobial Activity

A Bioassay was utilized to test several essential oils of Family Apiaceae, Rutaceae, Illicium, and Brassicaceae for their antimicrobial activity. The essential oils that were used to test for antimicrobial activities included: Oils of seeds from the Family Apiaceae which included Anise (*Pimpinella foeniculum*), Caraway (*Carum carvi*), Carrot (*Daucus carota*), Celery (*Apium graveolens*), Coriander (*Coriandrum sativum*), Cumin (*Cuminum cyminum*), Dill (*Anethum graveolens*), Fennel Sweet (*Foeniculum vulgare*), and Parsley (*Petroselinum crispum*); Grapefruit oil (*Citrus paradise*) from the Family Rutaceae; Anise star (*Illicium parviflorum*) from the Family Illicium, and Mustard oil (*Brassica hirta*) from the Family Brassicaceae. The bioassay of drill hole cavity was utilized to determine the antimicrobial activity of each essential oil against each isolated fungi and bacteria species. The following figures display the tested oil zones of inhibition measured in millimeters. (Figures 22-26)

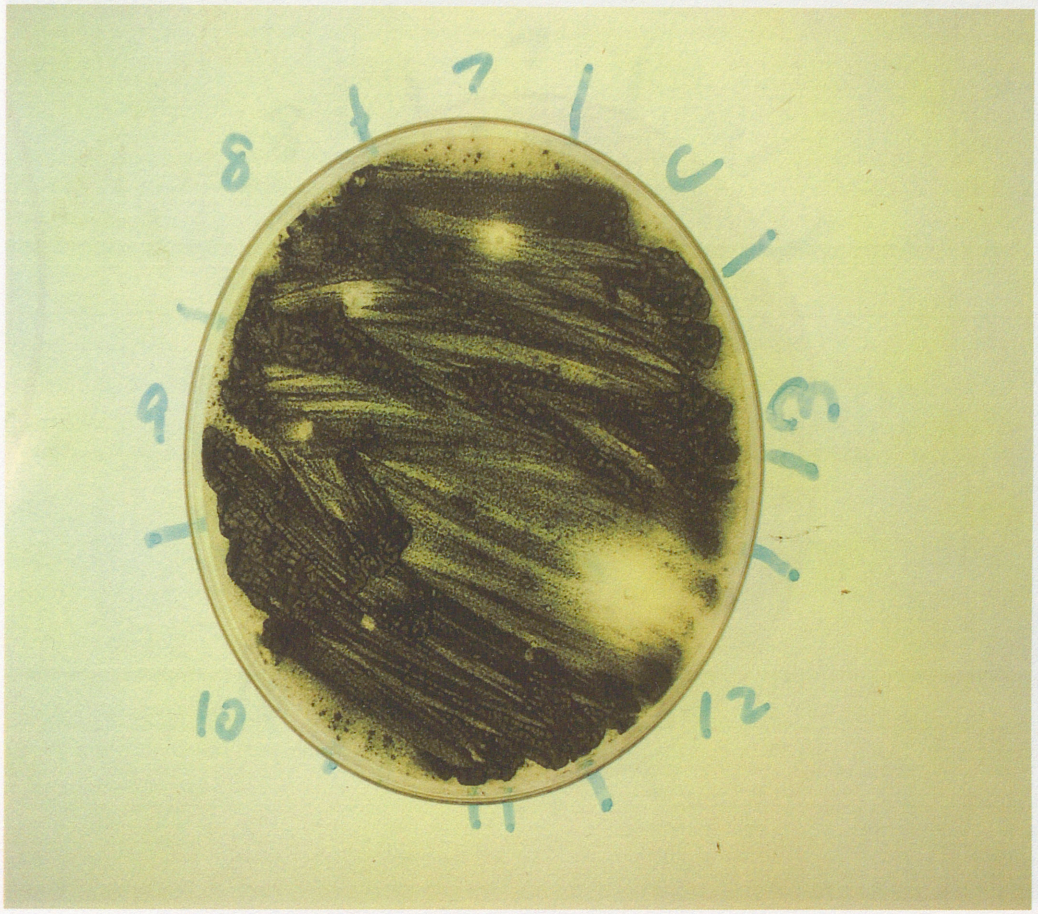


Figure 22. Micrograph of the fungi *Aspergillus sp* The zones that were inhibited included 7 (cumin), 8 (dill), and 12 (grapefruit). (Volume of oil used was 2.0μl.)

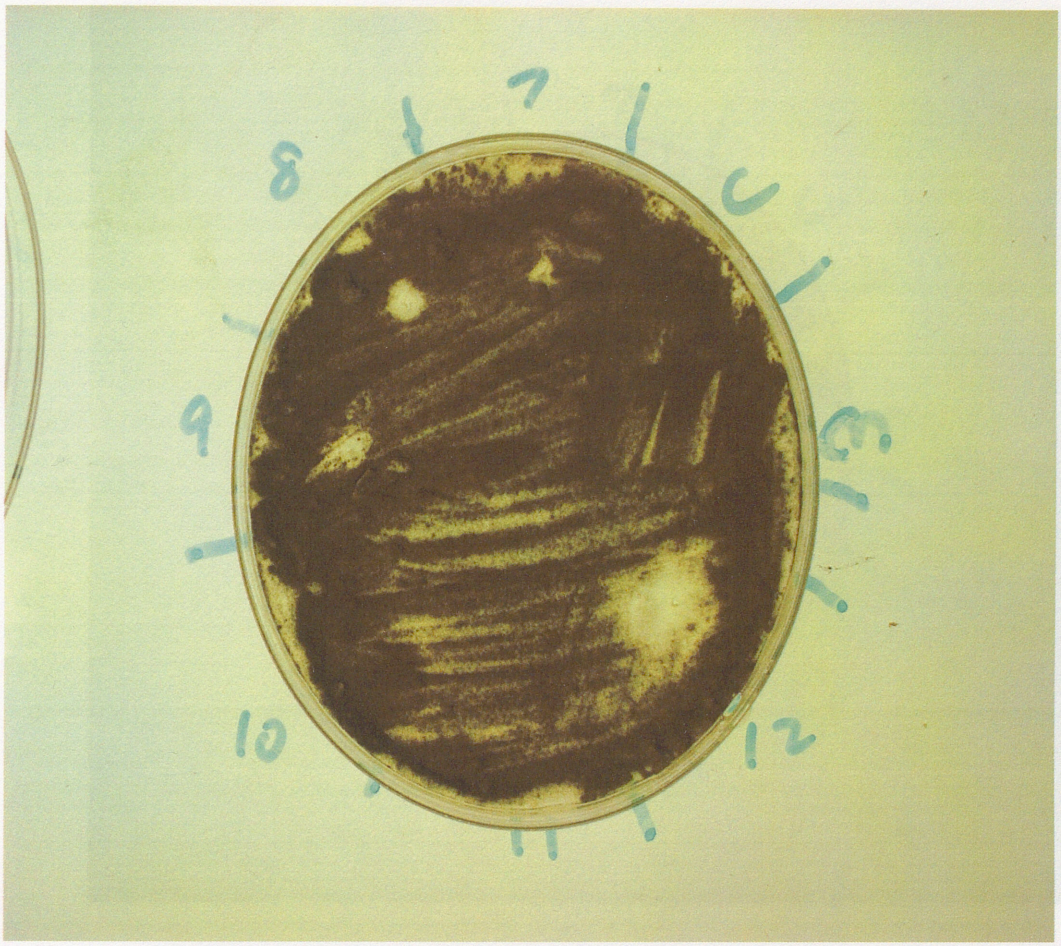


Figure 23. Micrograph of the fungi *Botrytis sp* Zones that were inhibited included 7 (cumin), 8 (dill), 9 (fennel) and 12 (grapefruit). (Volume of oil used was 2.0µl)

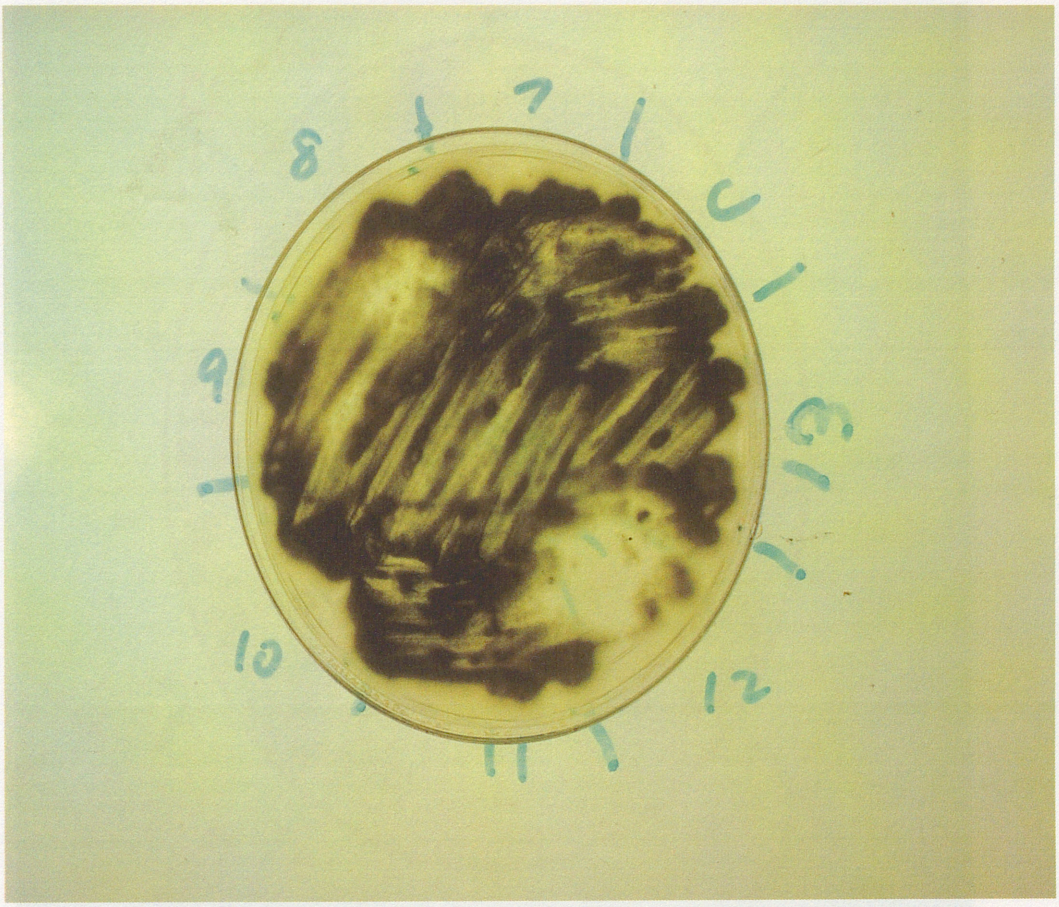


Figure 24. Micrograph of the fungi *Geotrichium sp* displaying the zones of inhibition from the tested oils. Zone 8 (dill oil) and 12 (grapefruit oil) were the most effective. (Volume of oil used was 2μl)

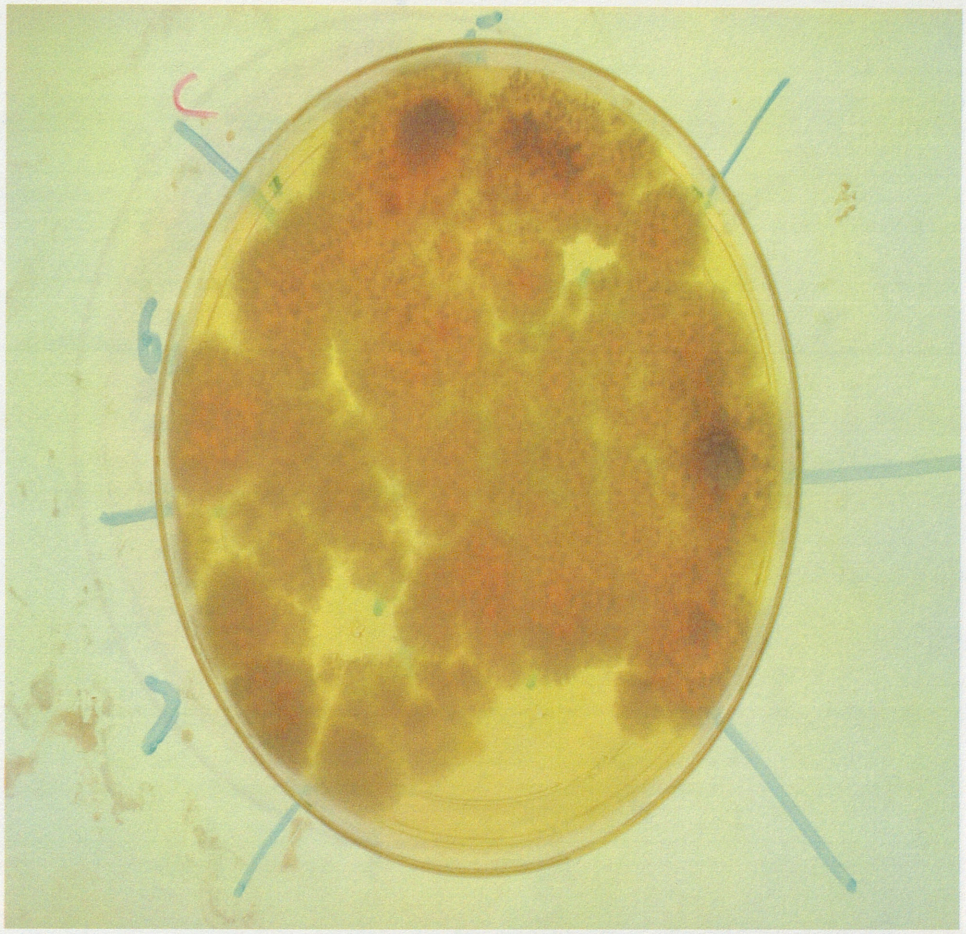


Figure 25. Micrograph of the Fungi *Trichoderma sp* displaying the zones of inhibition of the tested oils. Zones 6, 7, and 8 exhibit inhibition of the tested oils coriander, cumin, and dill, respectively. (Volume of oil used was 2.0μ)

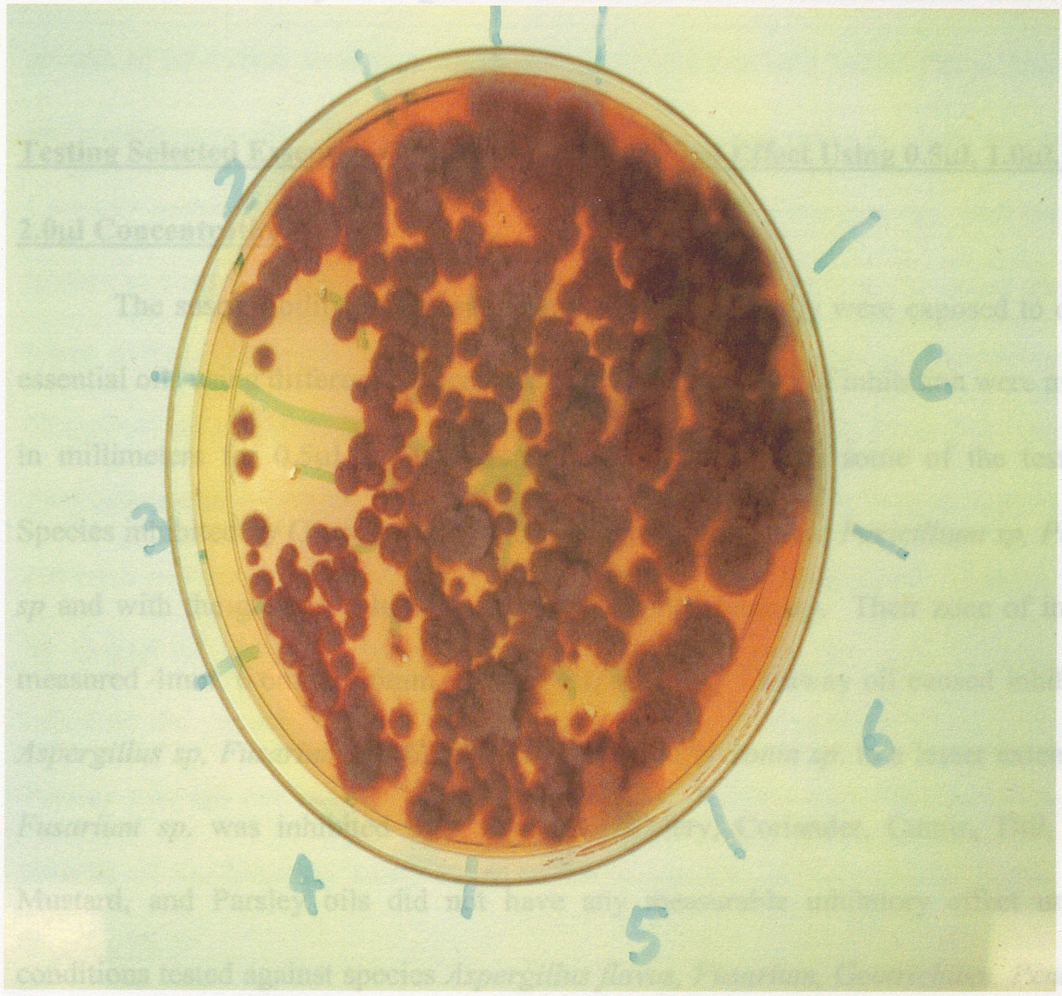


Figure 26. Micrograph of the Fungi *Trichoderma sp* displaying the zones of inhibition of the tested oils. Zones 2, 3, 4, and 5 exhibit inhibition of the tested oils which included, anise star, caraway, carrot, and celery respectively. (Volume of oil used was 2.0μ)

Results of the Bioassay Testing Selected Essential Oils for Their Antimicrobial Activity

Testing Selected Essential Oils for Their Antifungal Effect Using 0.5µl, 1.0µl, and 2.0µl Concentration

The susceptibility of different fungi varied when they were exposed to different essential oils using different concentrations. When the zones of inhibition were measured in millimeters for 0.5µl an inhibition effect was seen with some of the tested oils. Species inhibited by Grapefruit oil included *Aspergillus flavus, Penicillium sp, Periconia sp* and with the greatest inhibition seen with *Geotrichium sp*. Their zone of inhibition measured 4mm, 6.6mm, 7.6mm, 8.6mm respectively. Caraway oil caused inhibition in *Aspergillus sp, Fusarium sp, Geotrichium sp, and Periconia sp*. to a lesser extent. Only *Fusarium sp*. was inhibited by Carrot oil. Celery, Coriander, Cumin, Dill, Fennel, Mustard, and Parsley oils did not have any measurable inhibitory effect under the conditions tested against species *Aspergillus flavus, Fusarium, Geotrichium, Penicillium,* and *Periconia sp*. Species *Trichoderma* was inhibited by three essential oils, which included fennel, mustard and parsley with Fennel oil having the highest inhibition effect. Anise, Anise star, Caraway, carrot, Celery, Coriander, Cumin, Dill, and Grapefruit did not have any measurable inhibitory effect against species *Rhizopus Botrytis, Aspergillus niger* and *Aspergillus sp* under the conditions tested. Results of the bioassay using 0.5µl of the tested oils are revealed in Tables 1-2 and Figures 27-28.

When using 1.0µl, three of the tested fungi exhibited excellent response to the inhibition of growth from the essential oils, those fungi included *Geotrichium, Fusarium,* and *Aspergillus niger*. Their measurements of inhibition zones 23mm, 17.6mm, and

15mm respectively. When *Fusarium* was exposed to essential oil of carrot seed its growth of inhibition zone was minimized to a minimum of 3.3mm. Fungi that were not inhibited by any oils included *Rhizopus, Botrytis* and *Aspergillus sp*. Species inhibited by Caraway included *Aspergillus flavus., Geotrichium sp., Periconia sp.*, with the greatest inhibition seen with *Fusarium sp*. Carrot oil caused inhibition in *Fusarium* species to a lesser extent. Essential oils Dill and Mustard inhibited species of *Geotrichium*, while Grapefruit oil inhibited *Aspergillus flavus, Geotrichium* species, *Penicillum* species, and *Periconia* species. Anise, Anise star, Celery, Coriander, Cumin, Fennel, and Parsley did not have any measurable inhibitory effect under the conditions tested. Species inhibited by Grapefruit included *Aspergilllus niger*. Anise, Anise star, and Caraway oils all inhibited the growth of *Trichoderma* species. Celery, Corainder, Cumin, Fennel, and Parsley oils did not have any measurable inhibitory effect under the conditions tested. Results of the bioassay using 1.0µl of the tested oils are revealed in Tables 3-4 and Figures 29-30.

When using 2.0µl, *Aspergillus niger* was the most effective when it was exposed to the essential oil grapefruit, its inhibition zone measured 53.3mm, *Aspergillus niger* also had the lowest inhibitory effect when it was treated with mustard, its inhibition zone measured 2mm. Fungi that were not inhibited by any essential oils when using 2.0µl included *Rhizopus, Botrytis* and *Aspergillus sp*. Species inhibited by Grapefruit oil included *Aspergillus flavus, Fusarium* species, *Geotrichium* , *Penicillium* , *Periconia, Trichoderma, and Aspergillus niger* species, their measurements included 20mm, 7.3mm, 25mm, 25mm, 10mm, 4.3mm, 4.3mm, and 53.3mm respectively. Caraway oil was able to stop growth activity of fungi *Aspergillus flavus, Fusarium* species, *Geotrichium*

species, and *Periconia* species. Celery oil prevented growth activity of *Fusarium* species, as Mustard oil inhibited the growth of *Geotrichium* species. Essential oils that showed minimum inhibition on the isolated fungi included Anise, Carrot, Coriander, Cumin, Dill, Fennel, and Parsley. Oils that showed minimum inhibitory effects on the isolated species *Trichoderma* and *Aspergillus niger* included Anise, Anise star, Carrot, Parsley, Cumin, and Mustard. The oils that did not show any measurable inhibitory effects include Coriander, Dill, and Fennel. Results of the bioassay using 2.0µl of the tested oils are revealed in Tables 5-6 and Figures 31-32.

Results of the Bioassay Testing Selected Essential Oils for Their Antifungal Activity

Table 1. The effects of the essential oils on the growth of isolated fungi.

Oils	Fungi				
	Aspergillus flavus	*Fusarium sp.*	*Geotrichium sp.*	*Penicillium sp.*	*Periconia sp.*
Anise	-	-	-	-	-
Anise star	-	-	-	-	-
Caraway	5	5	2.6	-	2.6
Carrot	-	2.6	-	-	-
Celery	-	-	-	-	-
Coriander	-	-	-	-	-
Cumin	-	-	-	-	-
Dill	-	-	3	-	-
Fennel	-	-	-	-	-
Mustard	-	-	-	-	-
Parsley	-	-	-	-	-
Grapefruit	4	-	8.6	6.6	7.6

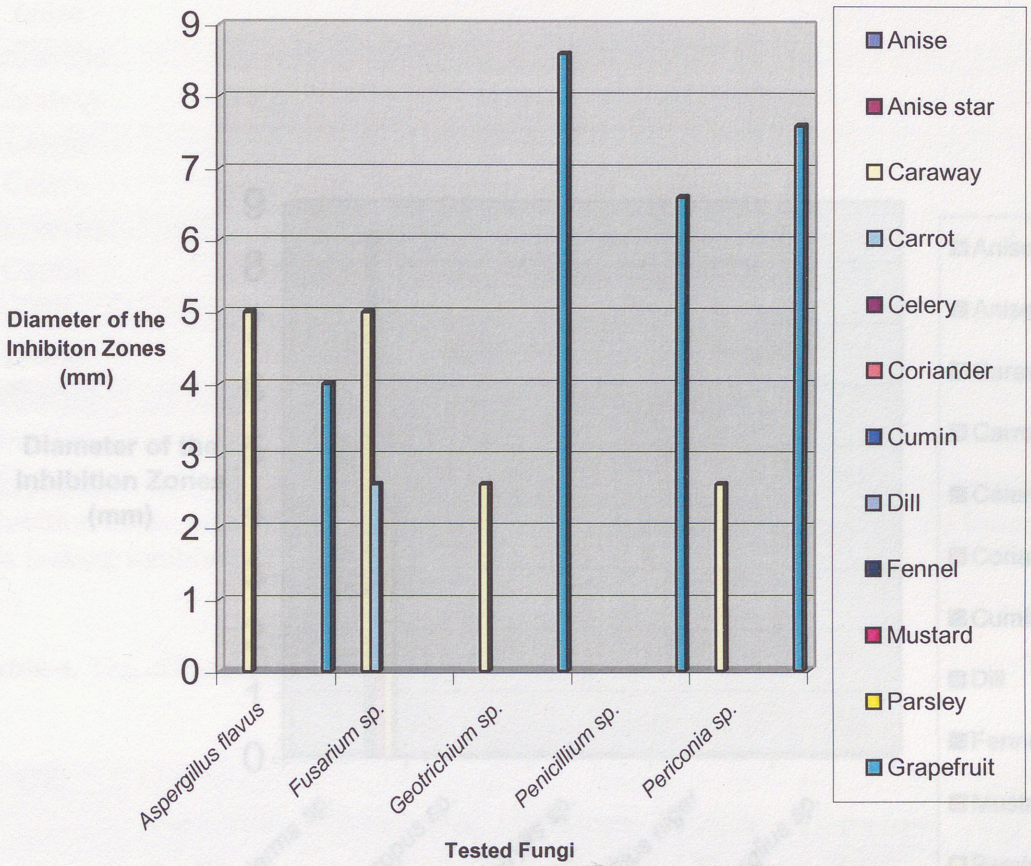
Inhibition zones measured in millimeters (mm); Volume of oil used 0.5µl. (-) Essential oils lacking inhibitory effect on the tested organism.

Table 2. The effects of the selected oils on the growth of isolated fungi.

Oils	Fungi				
	Trichoderma sp.	*Rhizopus sp.*	*Botrytis sp.*	*Aspergillus niger*	*Aspergillus sp.*
Anise	-	-	-	-	-
Anise star	-	-	-	-	-
Caraway	-	-	-	-	-
Carrot	-	-	-	-	-
Celery	-	-	-	-	-
Coriander	-	-	-	-	-
Cumin	-	-	-	-	-
Dill	-	-	-	-	-
Fennel	8.5	-	-	-	-
Mustard	3.5	-	-	-	-
Parsley	4	-	-	-	-
Grapefruit	-	-	-	-	-

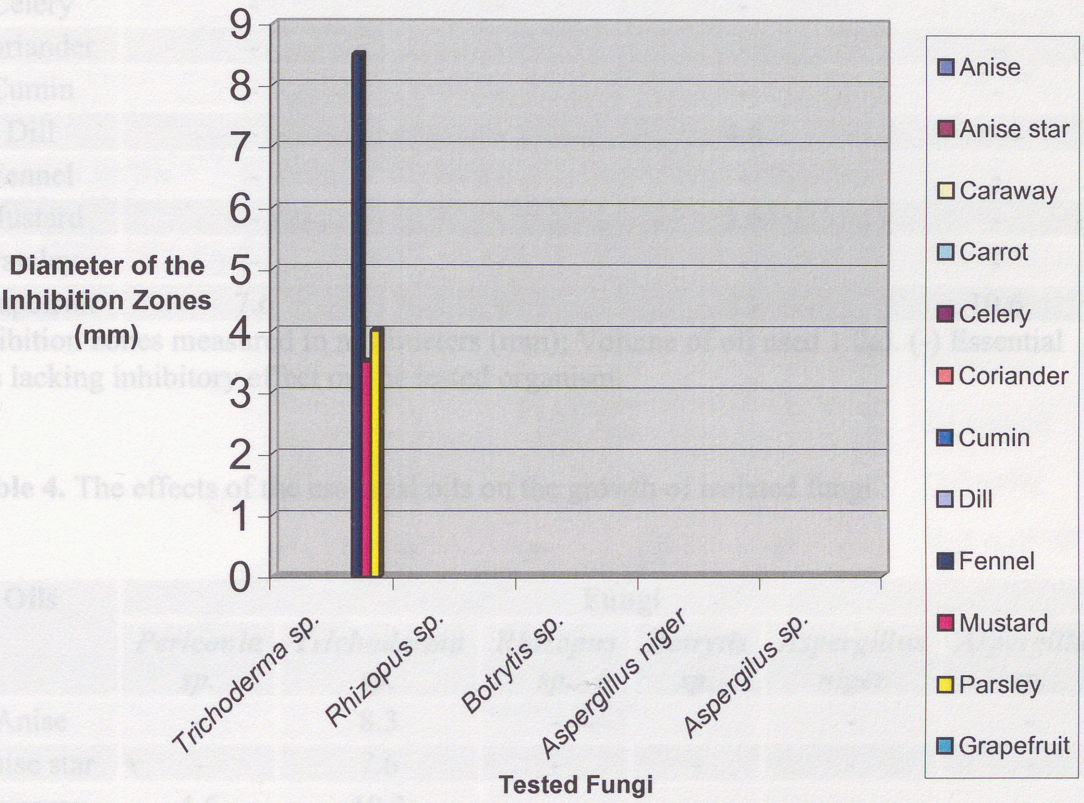
Inhibition zones measured in millimeters (mm); Volume of oil used 0.5µl. (-) Essential oils lacking inhibitory effect on the tested organism.

Figure 27. The Effect of Selected Essential Oils On The Growth of Isolated Fungi.



An inhibition effect was seen with Caraway, Carrot, and Grapefruit oils. Grapefruit oil had the highest inhibition against the isolated fungi *Penicillium sp.* Celery, Coriander, Cumin, Dill, Fennel, Mustard, and Parsley oils did not have any measurable inhibitory effect under the conditions tested. (Volume of oil used was 0.5μl.)

Figure 28. The Effect of Selected Essential Oils On The Growth of Isolated Fungi.



An inhibitory effect was seen with Fennel, Mustard, and Parsley oils. Species inhibited by all three essential oils included *Trichoderma species* only. Anise, Anise star, Caraway, carrot, Celery, Coriander, Cumin, Dill, and Grapefruit did not have any measurable inhibitory effect under the conditions tested. (Volume of oil used was 0.5μl)

Table 3. The effects of the essential oils on the growth of isolated fungi.

Oils	Fungi			
	Aspergillus flavus	*Fusarium sp.*	*Geotrichium sp.*	*Penicillium sp.*
Anise	-	-	-	-
Anise star	-	-	-	-
Caraway	6.6	17.6	4.6	-
Carrot	-	3.3	-	-
Celery	-	-	-	-
Coriander	-	-	-	-
Cumin	-	-	-	-
Dill	-	-	4.6	-
Fennel	-	-	-	-
Mustard	-	-	3.6	-
Parsley	-	-	-	-
Grapefruit	7.6	-	23	10.6

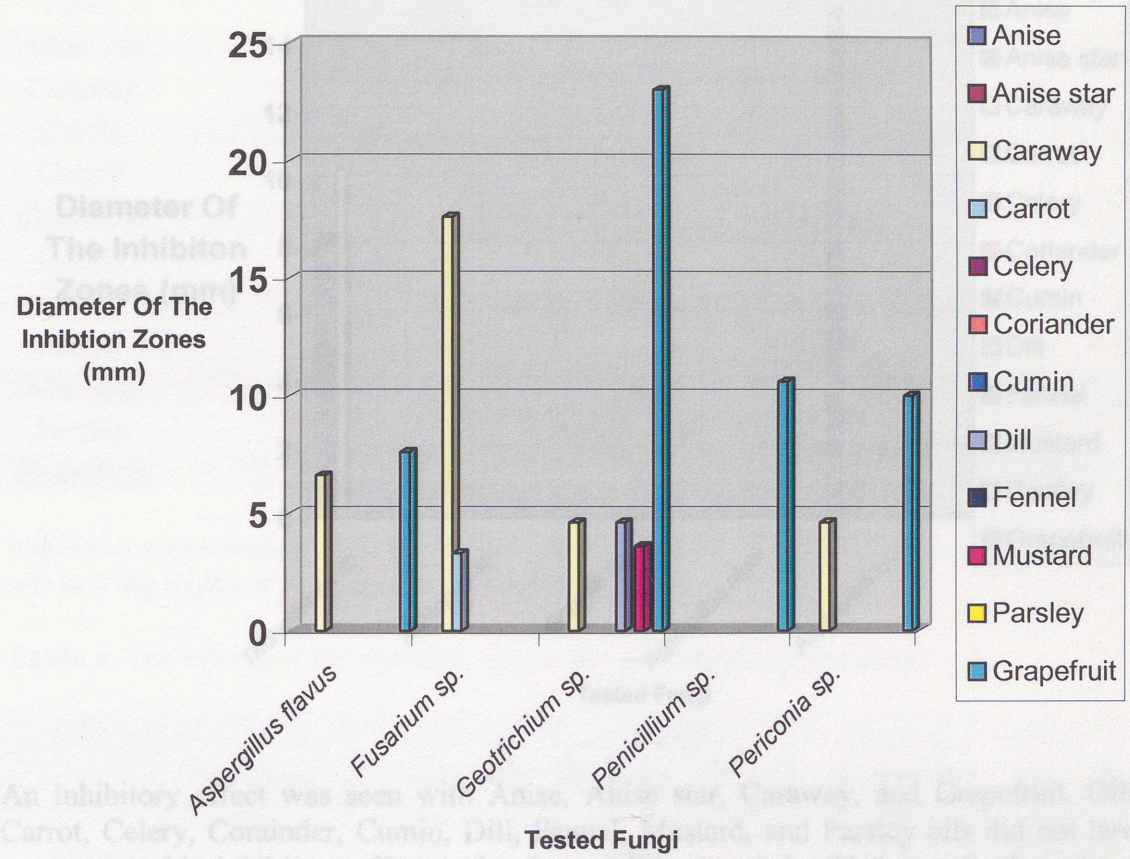
Inhibition zones measured in millimeters (mm); Volume of oil used 1.0µl. (-) Essential oils lacking inhibitory effect on the tested organism.

Table 4. The effects of the essential oils on the growth of isolated fungi.

Oils	Fungi					
	Periconia sp.	*Trichoderma sp.*	*Rhizopus sp.*	*Botrytis sp.*	*Aspergillus niger*	*Aspergillus sp.*
Anise	-	8.3	-	-	-	-
Anise star	-	7.6	-	-	-	-
Caraway	4.6	10.3	-	-	-	-
Carrot	-	-	-	-	-	-
Celery	-	-	-	-	-	-
Coriander	-	-	-	-	-	-
Cumin	-	-	-	-	-	-
Dill	-	-	-	-	-	-
Fennel	-	-	-	-	-	-
Mustard	-	-	-	-	-	-
Parsley	-	-	-	-	-	-
Grapefruit	10	-	-	-	15	-

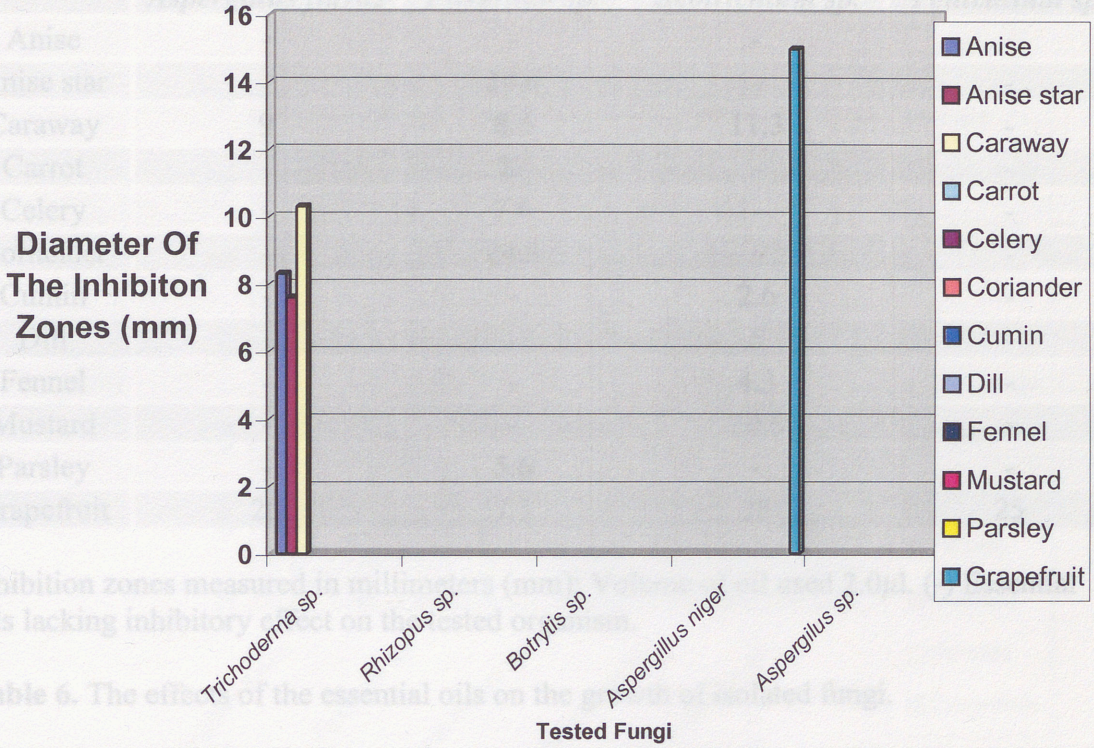
Inhibition zones measured in millimeters (mm); Volume of oil used 1.0µl. (-) Essential oils lacking inhibitory effect on the tested organism.

Figure 29. The Effect of Selected Essential Oils On The Growth of Isolated Fungi.



An inhibitory effect was seen with oils Caraway, Grapefruit, Carrot, Dill, and Mustard. Grapefruit had the highest inhibition on species *Geotrichium*. Anise, Anise star, Celery, Coriander, Cumin, Fennel, and Parsley did not have any measurable inhibitory effect under the conditions tested. (Volume of oil used was 1.0μl)

Figure 30. The Effect of Selected Essential Oils On The Growth Of Isolated Fungi



An inhibitory effect was seen with Anise, Anise star, Caraway, and Grapefruit. Oils. Carrot, Celery, Corainder, Cumin, Dill, Fennel, Mustard, and Parsley oils did not have any measurable inhibitory effect under the conditions tested. (Volume of oil used was 1.0μl.)

Table 5. The effects of the essential oils on the growth of isolated fungi.

Oils	Fungi			
	Aspergillus flavus	*Fusarium sp.*	*Geotrichium sp.*	*Penicillium sp.*
Anise	-	-	-	-
Anise star	-	24.6	-	-
Caraway	9	8.3	11.3	-
Carrot	-	27	-	-
Celery	-	7.6	-	-
Coriander	-	24.3	-	-
Cumin	-	-	2.6	-
Dill	-	-	5	-
Fennel	-	-	4.3	-
Mustard	-	-	20.6	-
Parsley	-	5.6	-	-
Grapefruit	20	7.3	25	25

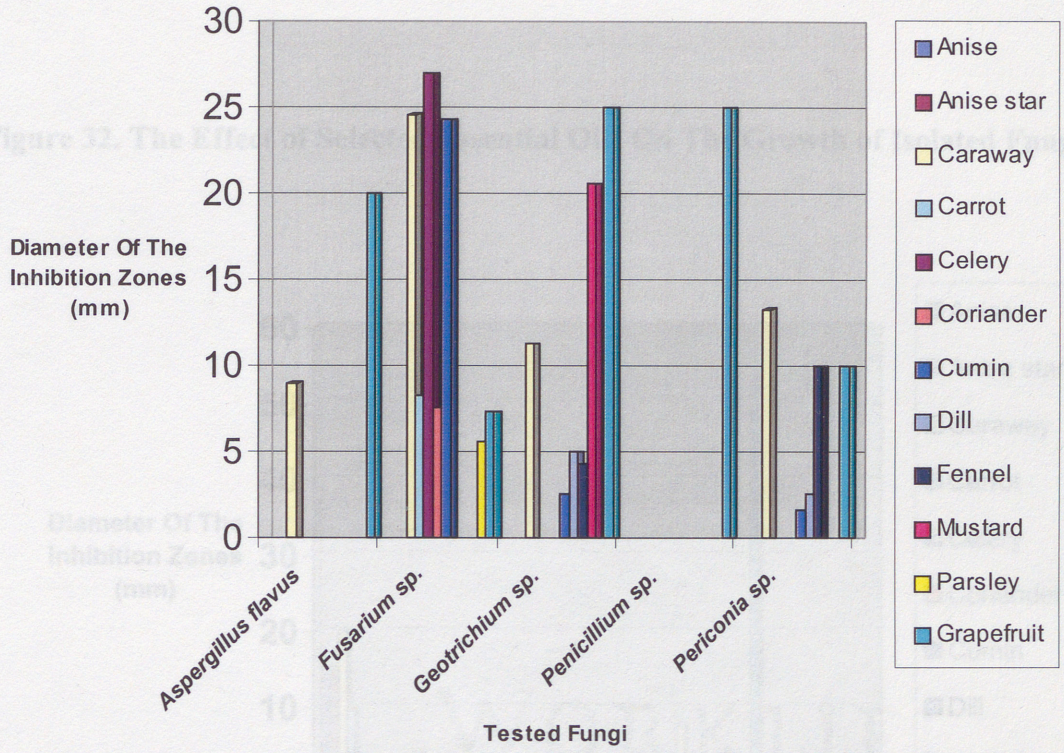
Inhibition zones measured in millimeters (mm); Volume of oil used 2.0μl. (-) Essential oils lacking inhibitory effect on the tested organism.

Table 6. The effects of the essential oils on the growth of isolated fungi.

Oils	Fungi					
	Periconia sp.	*Trichoderma sp.*	*Rhizopus sp.*	*Botrytis sp.*	*Aspergillus niger*	*Aspergillus sp.*
Anise	-	15	-	-	-	-
Anise star	-	15	-	-	-	-
Caraway	13.3	20	-	-	7.6	-
Carrot	-	9.6	-	-	-	-
Celery	-	-	-	-	-	-
Coriander	-	-	-	-	-	-
Cumin	1.6	-	-	-	3	-
Dill	2.6	-	-	-	-	-
Fennel	10	-	-	-	-	-
Mustard	-	-	-	-	2	-
Parsley	-	2.3	-	-	-	-
Grapefruit	10	4.3	-	-	53.3	-

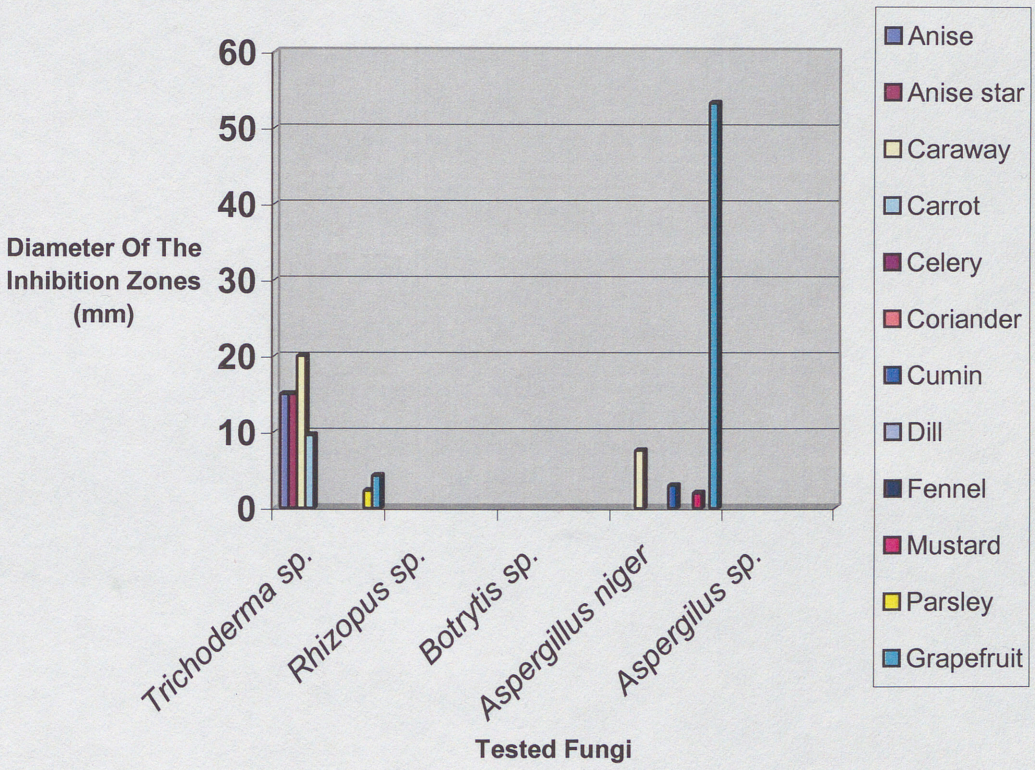
Inhibition zones measured in millimeters (mm); Volume of oil used 2.0μl. (-) Essential oils lacking inhibitory effect on the tested organism.

Figure 31. The Effect of Selected Essential Oils On The Growth of Isolated Fungi.



An inhibitory effect was seen with Anise, Caraway, Carrot, Celery, Coriander, Cumin, Dill, Fennel, Mustard, Parsley, and Grapefruit. Essential oil Anise star did not have any measurable inhibitory effect under the conditions tested. (Volume of oil used was 2.0μl)

Figure 32. The Effect of Selected Essential Oils On The Growth of Isolated Fungi.



An inhibitory effect was seen with Anise, Anise star, Caraway, Carrot, Parsley, Grapefruit, Cumin, Mustard. The oils that did not show any measurable inhibitory effects include Coriander, Dill, and Fennel. (Volume of oil used was 2.0μl)

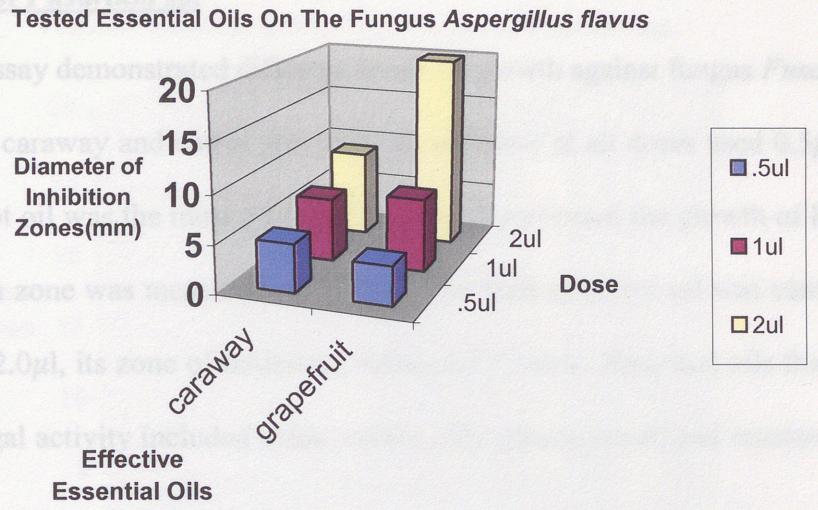
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Aspergillus flavus*

The bioassay demonstrated different levels of growth inhibition against the fungi *Aspergillus flavus*. Essential oils of grapefruit and caraway were the most effective against *Aspergillus flavus*. Grapefruit oil showed strongest antifungal activity because it inhibited the fungus growth the most around the diffusion hole cavity in the greatest amount. When using 2.0μl of grapefruit, the inhibition zone was measured 20mm. When using 2.0μl of caraway, its greatest inhibition zone measured 9mm. Essential oils that showed no inhibition activity included anise, anise star, carrot, celery, coriander, cumin, dill, fennel, mustard, and parsley. (Table 7, Figure 33)

Table 7. Results of the tested essential oils on the fungus *Aspergillus flavus* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
caraway	5	6.6	9
grapefruit	4	7.6	20

Figure 33.


Tested Essential Oils On The Fungus *Aspergillus flavus*
Diameter of Inhibition Zones(mm)
20
15
10
5
0
2ul
1ul
.5ul
Dose
.5ul
1ul
2ul
caraway
grapefruit
Effective Essential Oils

The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Fusarium sp.*

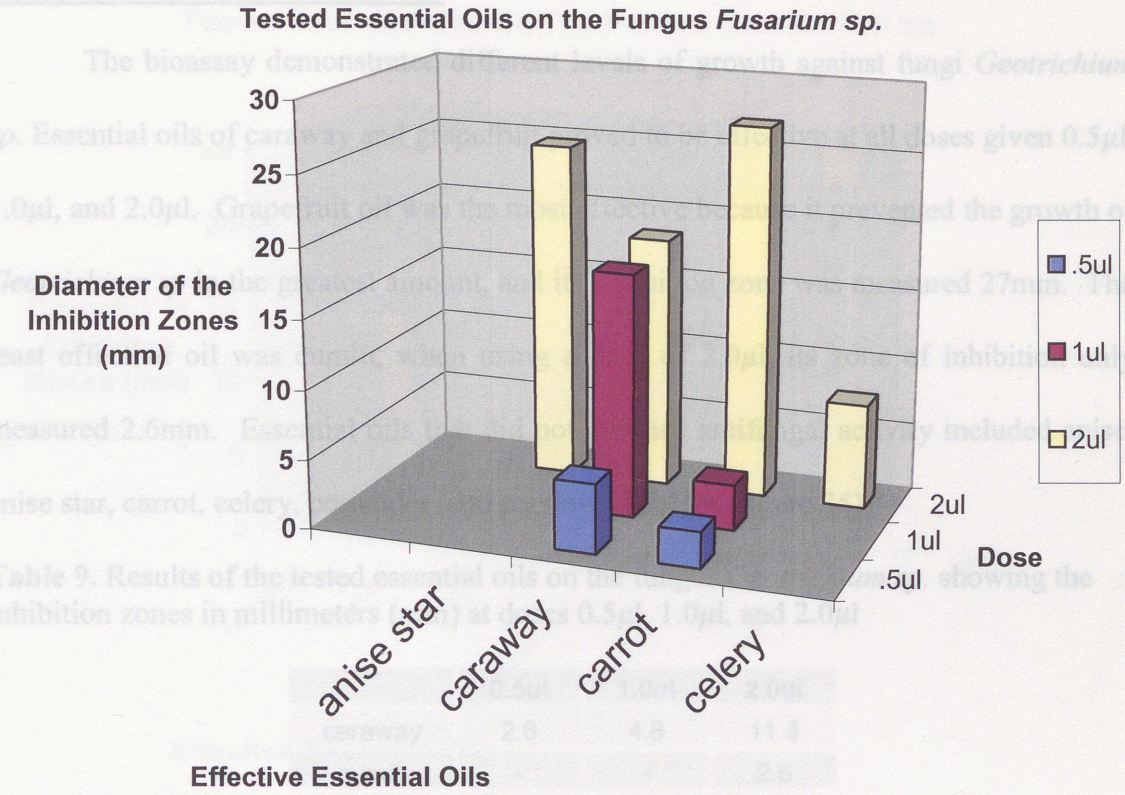
The bioassay demonstrated different levels of growth against fungus *Fusarium sp.* Essential oils of caraway and carrot proved to be effective at all doses used 0.5μl, 1.0μl, and 2.0μl. Carrot oil was the most effective because it prevented the growth of Fusarium and its inhibition zone was measured as 27mm. The least effective oil was carrot, when using a dose of 2.0μl, its zone of inhibition measured 2.6mm. Essential oils that did not produce antifungal activity included anise, cumin, dill, fennel sweet, and mustard. (Table 8, Figure 34)

Table 8. Results of the tested essential oils on the fungus *Fusarium sp.* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	-	-	24.6
caraway	5	17.6	18.3
carrot	2.6	3.3	27
celery	-	-	7.6
coriander	-	-	24.3
cumin	-	-	-
dill	-	-	-
fennel sweet	-	-	-
mustard	-	-	-
parsley	-	-	5.6
grapefruit	-	-	7.3

(-) Essential oils that did not inhibit the growth of the tested organism

Figure 34.


Tested Essential Oils on the Fungus *Fusarium sp.*
Diameter of the Inhibition Zones (mm)
30
25
20
15
10
5
0
.5ul
1ul
2ul
2ul
1ul
.5ul
Dose
anise star
caraway
carrot
celery
Effective Essential Oils

The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Geotrichium sp:*

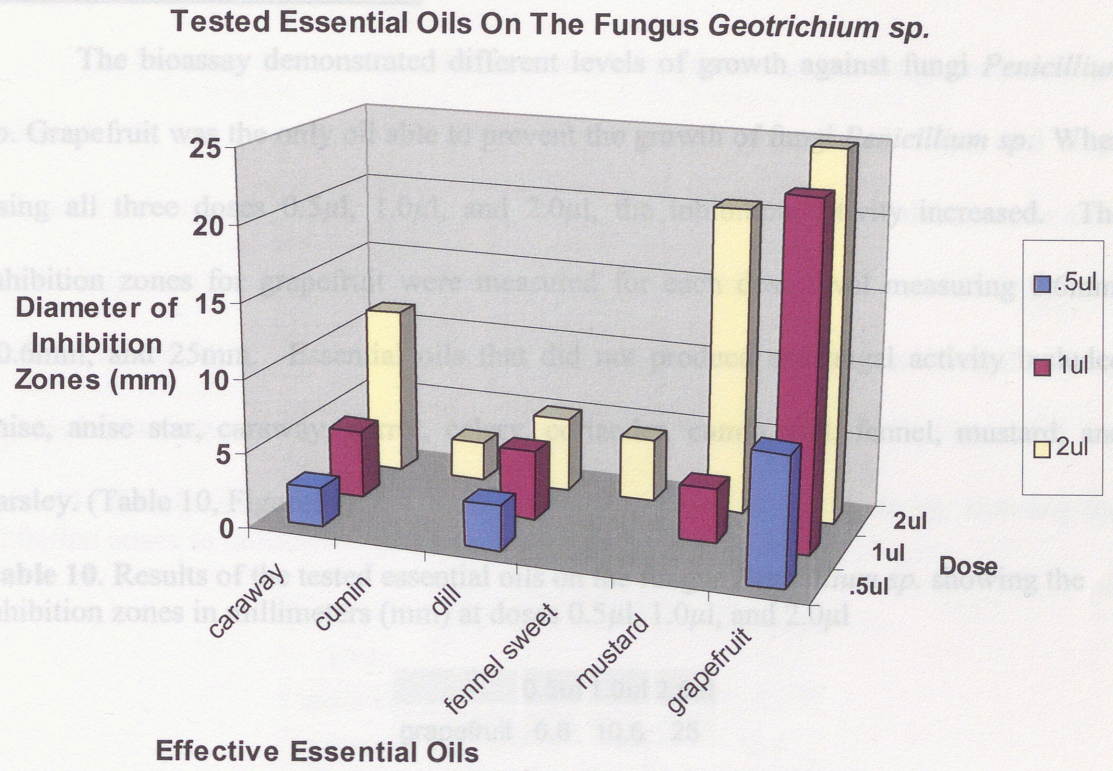
The bioassay demonstrated different levels of growth against fungi *Geotrichium sp*. Essential oils of caraway and grapefruit proved to be effective at all doses given 0.5μl, 1.0μl, and 2.0μl. Grapefruit oil was the most effective because it prevented the growth of *Geotrichium sp* in the greatest amount, and its inhibition zone was measured 27mm. The least effective oil was cumin, when using a dose of 2.0μl, its zone of inhibition only measured 2.6mm. Essential oils that did not produce antifungal activity included anise, anise star, carrot, celery, coriander, and parsley. (Table 9, Figure 35)

Table 9. Results of the tested essential oils on the fungus *Geotrichium sp.* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
caraway	2.6	4.6	11.3
cumin	-	-	2.6
dill	3	4.6	5
fennel sweet	-	-	4.3
mustard		3.6	20.6
grapefruit	8.6	23	25

(-) Essential oils that did not inhibit the growth of the tested organism

Figure 35.


Tested Essential Oils On The Fungus *Geotrichium sp.*
Diameter of Inhibition Zones (mm)
25
20
15
10
5
0
.5ul
1ul
2ul
Dose
caraway
cumin
dill
fennel sweet
mustard
grapefruit
Effective Essential Oils

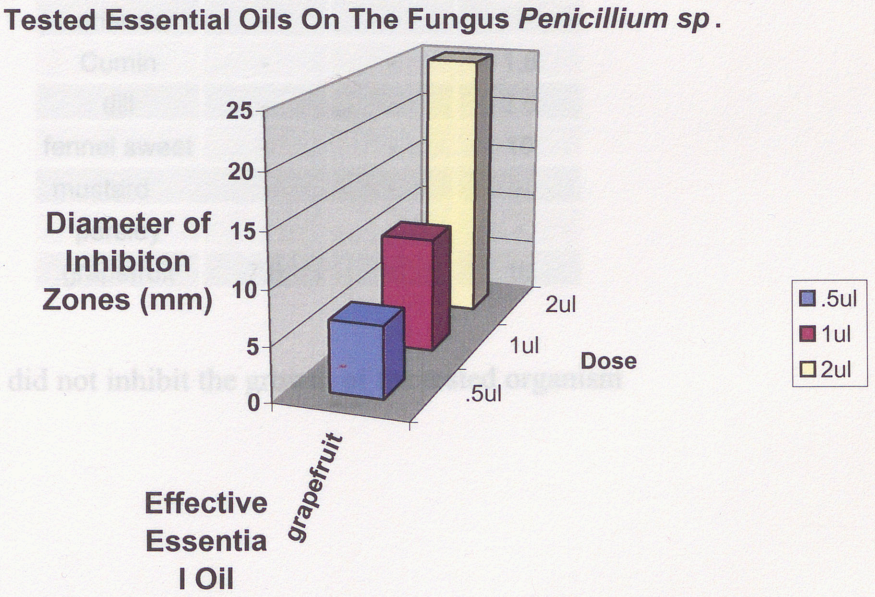
The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Penicillium sp.*

The bioassay demonstrated different levels of growth against fungi *Penicillium sp*. Grapefruit was the only oil able to prevent the growth of fungi *Penicillium sp*. When using all three doses 0.5μl, 1.0μl, and 2.0μl, the inhibition activity increased. The inhibition zones for grapefruit were measured for each dose level measuring 6.6mm, 10.6mm, and 25mm. Essential oils that did not produce antifungal activity included anise, anise star, caraway, carrot, celery, coriander, cumin, dill, fennel, mustard, and parsley. (Table 10, Figure36)

Table 10. Results of the tested essential oils on the fungus *Penicillium sp.* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
grapefruit	6.6	10.6	25

Figure 36.



The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Periconia sp.*

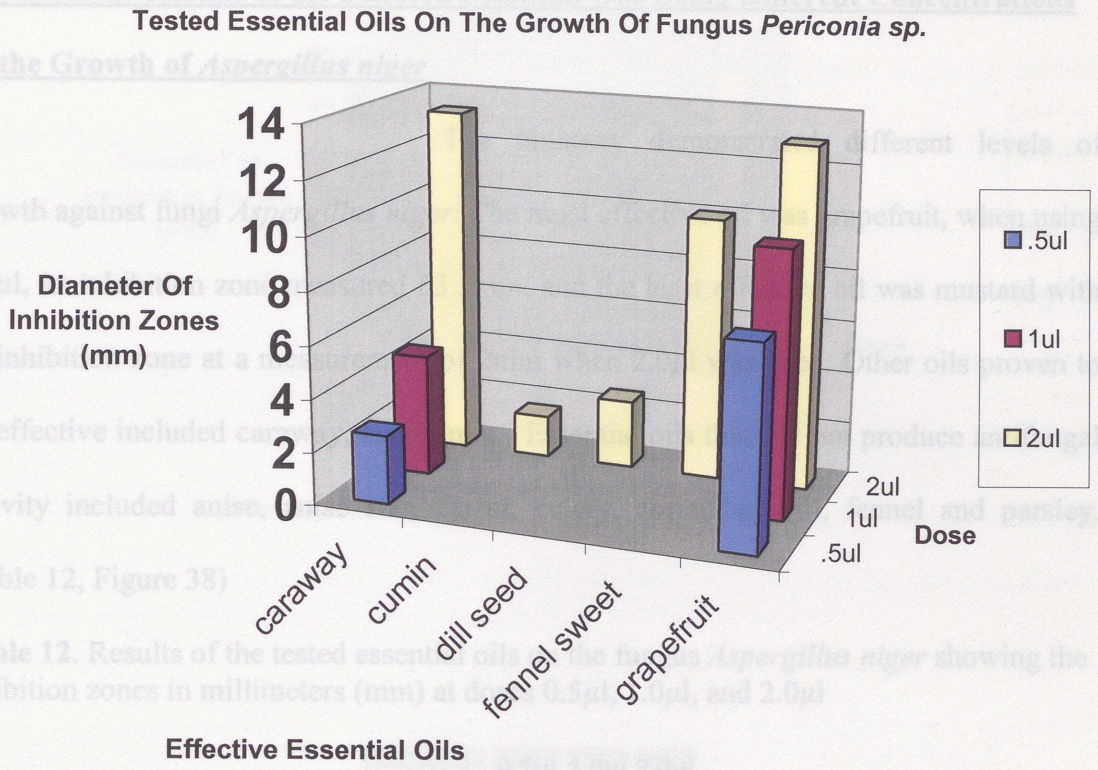
The bioassay demonstrated different levels of growth against fungi *Periconia sp.*. Essential oils that were the most effective at all doses given 0.5μl, 1.0μl, and 2.0μl included caraway and grapefruit. Caraway had the greatest amount of inhibition, its zone of inhibition was measured 13.3mm when using 2.0μl. Other oils proven to be effective included cumin, dill, and fennel. Essential oils that did not produce antifungal activity included anise, anise star, carrot, celery, coriander, and parsley. (Table 11, Figure 37)

Table11- Results of the tested essential oils on the fungus *Periconia sp.* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	-	-	-
caraway	2.6	4.6	13.3
carrot	-	-	-
celery	-	-	-
coriander	-	-	-
Cumin	-	-	1.6
dill	-	-	2.6
fennel sweet	-	-	10
mustard		-	-
parsley	-	-	-
grapefruit	7.6	10	10

(-) Essential oils that did not inhibit the growth of the tested organism

Figure 37.


Tested Essential Oils On The Growth Of Fungus *Periconia sp.*
Diameter Of Inhibition Zones (mm)
14
12
10
8
6
4
2
0
caraway
cumin
dill seed
fennel sweet
grapefruit
Effective Essential Oils
.5ul
1ul
2ul
Dose

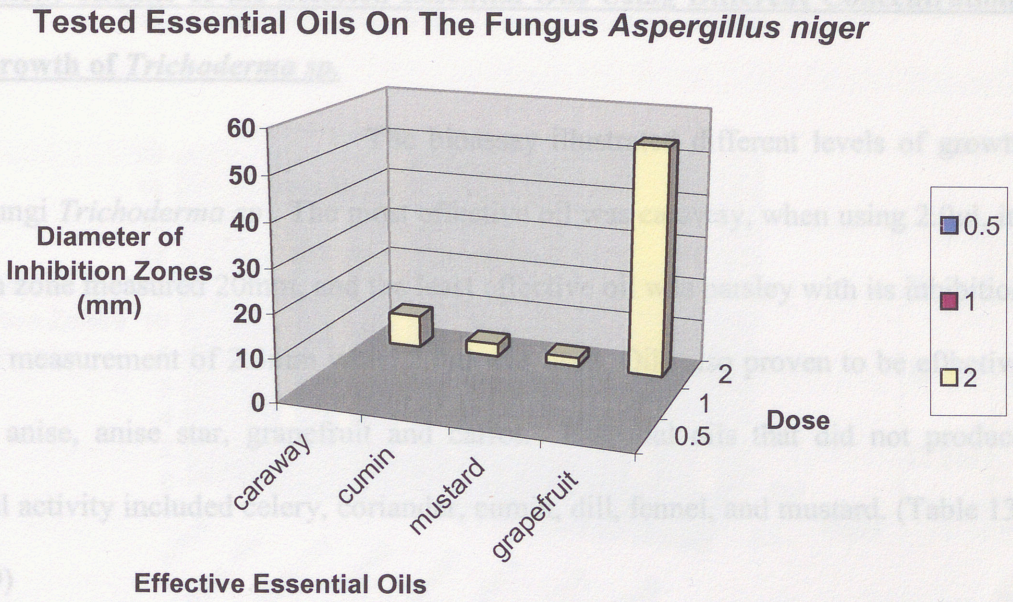
The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Aspergillus niger*

The bioassay demonstrated different levels of growth against fungi *Aspergillus niger*. The most effective oil was grapefruit, when using 2.0μl, its inhibition zone measured 53.3mm, and the least effective oil was mustard with its inhibition zone at a measurement of 2mm when 2.0μl was used. Other oils proven to be effective included caraway, and cumin. Essential oils that did not produce antifungal activity included anise, anise star, carrot, celery, coriander, dill, fennel and parsley. (Table 12, Figure 38)

Table 12. Results of the tested essential oils on the fungus *Aspergillus niger* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
caraway	-	-	7.6
cumin	-	-	3
mustard	-	-	2
grapefruit	-	-	53.3

Figure 38.


Tested Essential Oils On The Fungus Aspergillus niger
Diameter of Inhibition Zones (mm)
60
50
40
30
20
10
0
caraway
cumin
mustard
grapefruit
Effective Essential Oils
2
1
0.5
Dose
0.5
1
2

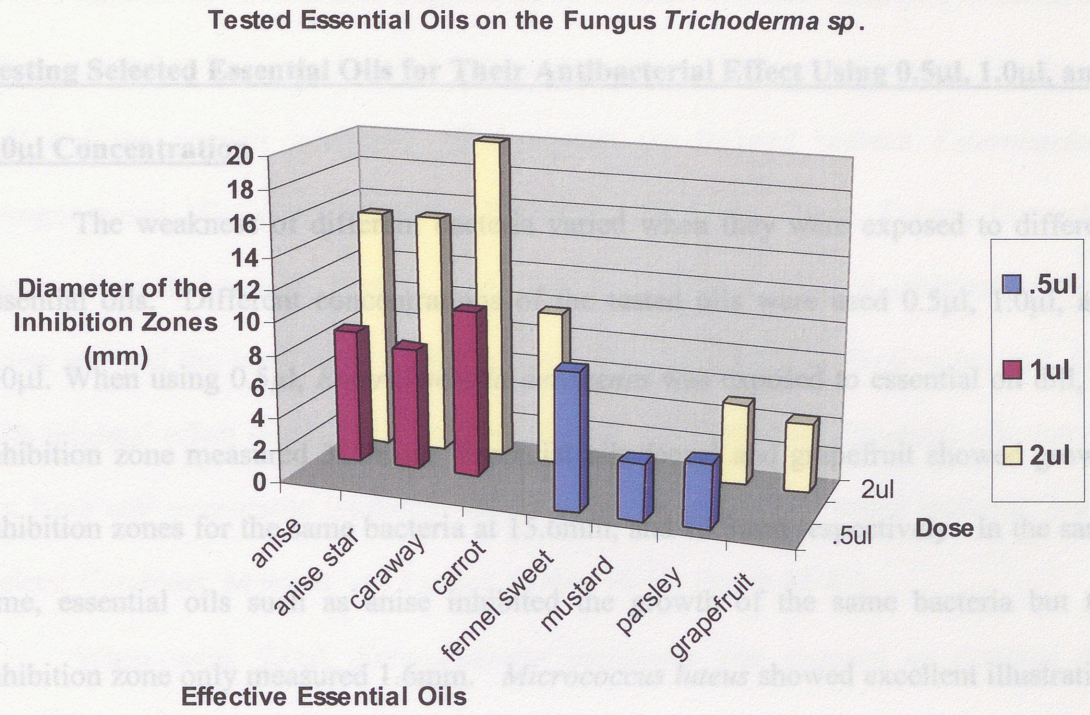
The Bioassay Results of the Selected Essential Oils Using Different Concentrations on the Growth of *Trichoderma sp.*

The bioassay illustrated different levels of growth against fungi *Trichoderma sp* . The most effective oil was caraway, when using 2.0μl, its inhibition zone measured 20mm, and the least effective oil was parsley with its inhibition zone at a measurement of 2.3mm when 2.0μl was used. Oils also proven to be effective included anise, anise star, grapefruit and carrot. Essential oils that did not produce antifungal activity included celery, coriander, cumin, dill, fennel, and mustard. (Table 13, Figure 39)

Table 13. Results of the tested essential oils on the fungi *Trichoderma sp*. showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise	-	8.3	15
anise star	-	7.6	15
caraway	-	10.3	20
carrot	-	-	9.6
celery	-	-	-
coriander	-	-	-
cumin	-	-	-
dill	-	-	-
fennel sweet	8.5	-	-
mustard	3.5	-	-
parsley	4	-	5
grapefruit	-	-	4.3

Figure 39.


Tested Essential Oils on the Fungus *Trichoderma sp*.
Diameter of the Inhibition Zones (mm)
20
18
16
14
12
10
8
6
4
2
0
anise
anise star
caraway
carrot
fennel sweet
mustard
parsley
grapefruit
Effective Essential Oils
2ul
.5ul
Dose
.5ul
1ul
2ul

Testing Selected Essential Oils for Their Antibacterial Effect Using 0.5µl, 1.0µl, and 2.0µl Concentration

The weakness of different bacteria varied when they were exposed to different essential oils. Different concentrations of the tested oils were used 0.5µl, 1.0µl, and 2.0µl. When using 0.5µl, *Enterobacteria aerogenes* was exposed to essential oil dill, its inhibition zone measured 31.3mm. Essential oils fennel and grapefruit showed growth inhibition zones for the same bacteria at 15.6mm, and 15.3mm respectively. In the same time, essential oils such as anise inhibited the growth of the same bacteria but the inhibition zone only measured 1.6mm. *Micrococcus luteus* showed excellent illustration of growth inhibition when treated with caraway and cumin oil, its inhibition zone measured 31mm and 41.3mm respectively. The greatest inhibitory effect was seen with the essential oil Cumin on the isolated bacteria *Micrococcus luteu.* Caraway oil inhibited the isolated bacteria *Bacillus subtilis, Enterobacteria aerogenes*, *Klebsiella pneumoniae, Micrococcus luteus and Staphylococcus aureus.* Cumin and Caraway oils had the greatest inhibitory effect on the bacteria *Micrococcus luteus*. On the isolated bacteria *Staphylococcus aureus* essential oils Caraway, Coriander, and Cumin showed minimal inhibitory effects. Oils that did not have any measurable inhibitory effect under the conditions tested were Mustard, and Parsley. (Table 14-15, Figure 40-41)

When using 1.0µl, *Enterobacteria aerogenes* was exposed to essential oil caraway and dill, its inhibition zones measured 33.3mm and 34.6 respectively. Also, essential oils cumin and fennel showed growth inhibition zones for the same bacteria at 21mm, and

34.6mm respectively. Bacteria *Micrococcus luteus* showed excellent illustration of growth inhibition, when treated with cumin oil its inhibition zone measured 41.3mm, and when exposed to caraway, its inhibition zone measured 38.3mm. Caraway, Cumin and Dill had the greatest inhibitory effect against the isolated bacteria *Enterobacteria aerogenes,and Micrococcus luteus.* Essential oils Anise, Carrot, Celery, Coriander, Cumin, Fennel, Mustard, Parsley, and Grapefruit showed minimal inhibitory effects. Anise star had the least effect on the growth of isolated bacteria *Escherichia coli.* Oils with minimal effect included carrot, Dill, Fennel, and Grapefruit. Essential oils that did not have any measurable inhibitory effect under the conditions tested included Anise, Celery, Corainder, Mustard, and Parsley. (Table 16-17, Figure 42-43)

When using 2.0µl, *Enterobacteria aerogenes* was exposed to essential oils caraway, cumin and grapefruit, its inhibition zones measured 46.3mm and 39mm, and 36.6 respectively. Bacteria *Micrococcus luteus* revealed distinct results of growth inhibition, when treated with caraway oil its inhibition zone measured 44.6mm, and when exposed to cumin, its inhibition zone measured 44.3mm. *Serratia marcescens* displayed excellent results when exposed to caraway oil, its inhibition zone measured 34.6mm. *Staphylococcus aureus* demonstrated the lowest inhibition of growth when it was exposed to fennel oil, its inhibition zone measured 2mm. Essential oils Anise star, Caraway, Carrot, Cumin, and Dill prevented the growth of bacteria *Bacillus cereus.* Oils Anise star, Caraway, Carrot, Celery, Coriander, Cumin, Dill, Fennel, Parsley, and Grapefruit were able to stop the growth of *Bacillus subtilis.* All essential oils tested prevented the growth of *Enterobacteria aerogenes.* Oils Anise star, Caraway, Carrot, Cumin, Parsley, and Grapefruit limited the growth of the isolated bacteria *Escherichia*

coli. Oils Caraway, Carrot, Cumin, Dill, Fennel, Mustard, Parsley, and Grapefruit were able to inhibit the growth of *Staphyloccus aureus*. Oils Cumin, Dill, Fennel, and Grapefruit had minimal inhibitory effect on the growth of *Serratia marcescens*. For the growth of *Klebsiella pneumoniae*, essential oils Anise star, Caraway, Carrot, Dill, Parsley, and Grapefruit displayed minimal growth of the isolated bacteria. All tested oils showed inhibitory effect under the conditions tested. (Table 18-19, Figure 44-45)

Results of the Bioassay Testing Selected Essential Oils for Their Antibacterial Activity

Table 14. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Bacillus cereus	***Bacillus subtilis***	***Enterobacteria aerogenes***	***Klebsiella pneumoniae***
Anise	-	-	1.6	-
Anise star	-	3	3	-
Caraway	-	7.3	19	5.6
Carrot	5.6	3.3	-	-
Celery	-	6	-	-
Coriander	-	4.3	5.3	-
Cumin	-	-	13.3	-
Dill	-	-	31.3	12.3
Fennel	-	-	15.6	-
Mustard	-	-	-	-
Parsley	-	-	-	-
Grapefruit	5	-	15.3	-

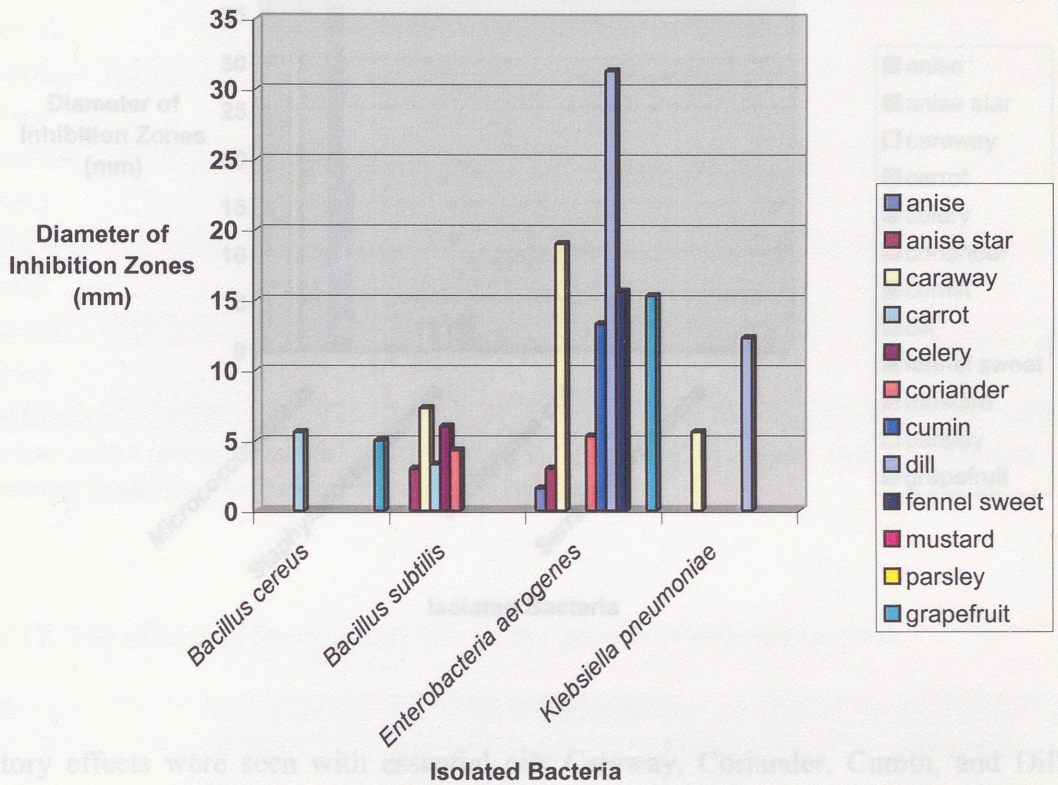
Inhibition zones measured in millimeters (mm); Volume of oil used 0.5μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Table 15. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Micrococcus luteus	***Staphylococcus aureus***	***Escherichia coli***	***Serratia marcescens***
Anise	-	-	-	-
Anise star	-	-	-	-
Caraway	31	3	-	-
Carrot	-	-	-	-
Celery	-	-	-	-
Coriander	-	2.6	-	-
Cumin	41.3	3.3	-	-
Dill	17.3	-	-	-
Fennel	-	-	-	-
Mustard	-	-	-	-
Parsley	-	-	-	-
Grapefruit	-	-	-	-

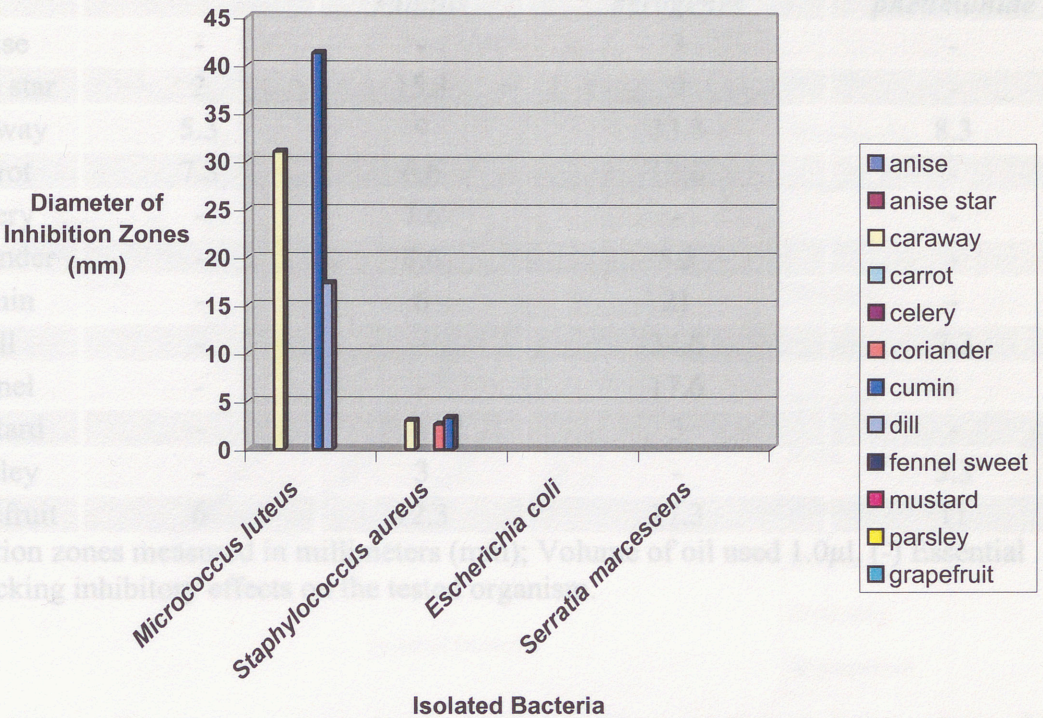
Inhibition zones measured in millimeters (mm); Volume of oil used 0.5μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Figure 40. The Effect of Selected Essential Oils On The Growth of Isolated Bacteria.



An inhibitory effect was seen with Anise, Anise star, Caraway, Carrot, Celery, Coriander, Cumin, Dill, Fennel, Grapefruit oils. Essential oils that showed minimum inhibitory effects included Carrot, Anise star, Celery, Coriander, Cumin, Dill, Fennel, and Grapefruit. The essential oils that showed no inhibitory effects included Mustard and Parsley. (Volume of the oil used was 0.5µl)

Figure 41. The Effect of Selected Essential Oils on the Growth of Isolated Bacteria.



Inhibitory effects were seen with essential oils Caraway, Coriander, Cumin, and Dill. Oils that did not have any measurable inhibitory effect under the conditions tested were Anise, Anise star, Carrot, Celery, Fennel, Mustard, Parsley, and Grapefruit. (Volume of oil used was 0.5µl.)

Table 16. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Bacillus cereus	***Bacillus subtilis***	***Enterobacteria aerogenes***	***Klebsiella pneumoniae***
Anise	-	-	3	-
Anise star	2	15.3	9	-
Caraway	5.3	9	33.3	8.3
Carrot	7.3	6.6	12.6	-
Celery	-	7.6	-	-
Coriander	-	8.6	5.3	-
Cumin	-	6	21	-
Dill	-	-	34.6	7.3
Fennel	-	-	17.6	-
Mustard	-	-	3	-
Parsley	-	3	-	5.3
Grapefruit	6	12.3	17.3	11

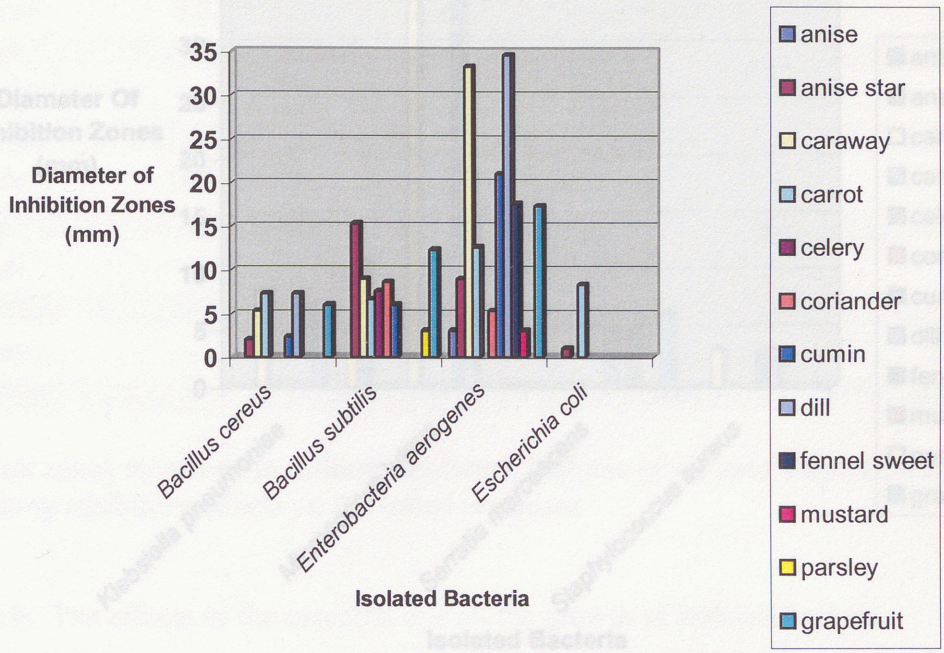
Inhibition zones measured in millimeters (mm); Volume of oil used 1.0μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Table 17. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Micrococcus luteus	***Staphylococcus aureus***	***Escherichia coli***	***Serratia marcescens***
Anise	-	-	-	-
Anise star	-	-	1	-
Caraway	38.3	3.3	-	-
Carrot	-	-	8.3	-
Celery	-	-	-	-
Coriander	-	-	-	-
Cumin	41.3	3.3	-	5
Dill	19	3.3	-	-
Fennel	-	-	-	5
Mustard	-	-	-	-
Parsley	-	-	-	-
Grapefruit	-	-	-	6.6

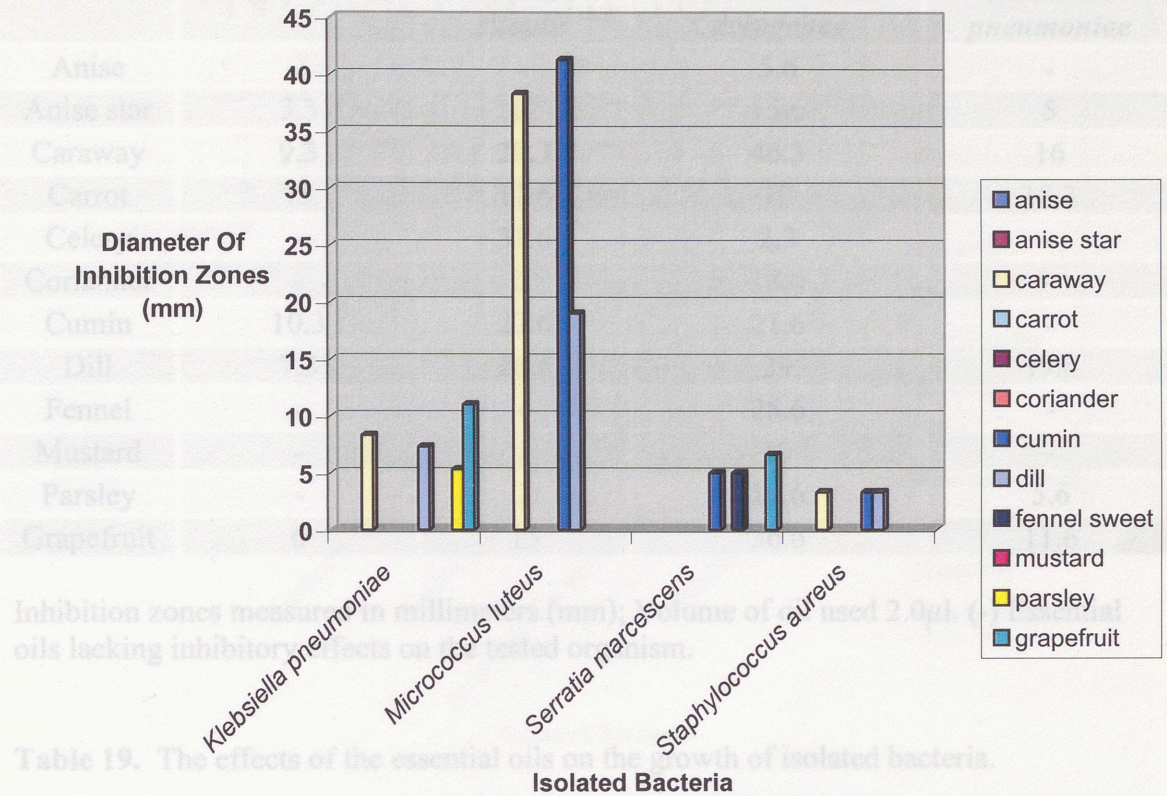
Inhibition zones measured in millimeters (mm); Volume of oil used 1.0μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Figure 42. The Effect of Selected Essential Oils On The Growth of Isolated Bacteria.



An inhibitory effect was seen with Anise, Anise star, Caraway, Carrot, Celery, Coriander, Cumin, Dill, Fennel sweet, Mustard, Parsley and Grapefruit. Essential oils Anise, Carrot, Celery, Coriander, Cumin, Fennel, Mustard, Parsley, and Grapefruit showed minimal inhibitory effects. Anise star showed the least inhibitory effect against the isolated bacteria Bacillus cereus. (Volume of oil used was 1.0µl.)

Figure 43. The Effect of Selected Essential Oils On The Growth of Isolated Bacteria.



An inhibitory effect was seen with Anise Star, Caraway, Carrot, Cumin, Dill, Fennel, and Grapefruit oils. Essential oils that did not have any measurable inhibitory effect under the conditions tested included Anise, Celery, Coriander, Mustard, and Parsley. (Volume of oil used was 1.0µl)

Table 18. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Bacillus cereus	***Bacillus subtilis***	***Enterobacteria aerogenes***	***Klebsiella pneumoniae***
Anise	-	-	5.6	-
Anise star	3.3	17	15.6	5
Caraway	9.3	29.3	46.3	16
Carrot	15	11.6	10	18.3
Celery	-	31.6	2.3	-
Coriander	-	20	18.6	-
Cumin	10.3	22.6	21.6	-
Dill	7.3	26.6	39	19.6
Fennel	-	4	25.6	-
Mustard	-	-	10	-
Parsley	-	3	10.6	5.6
Grapefruit	6	15	36.6	11.6

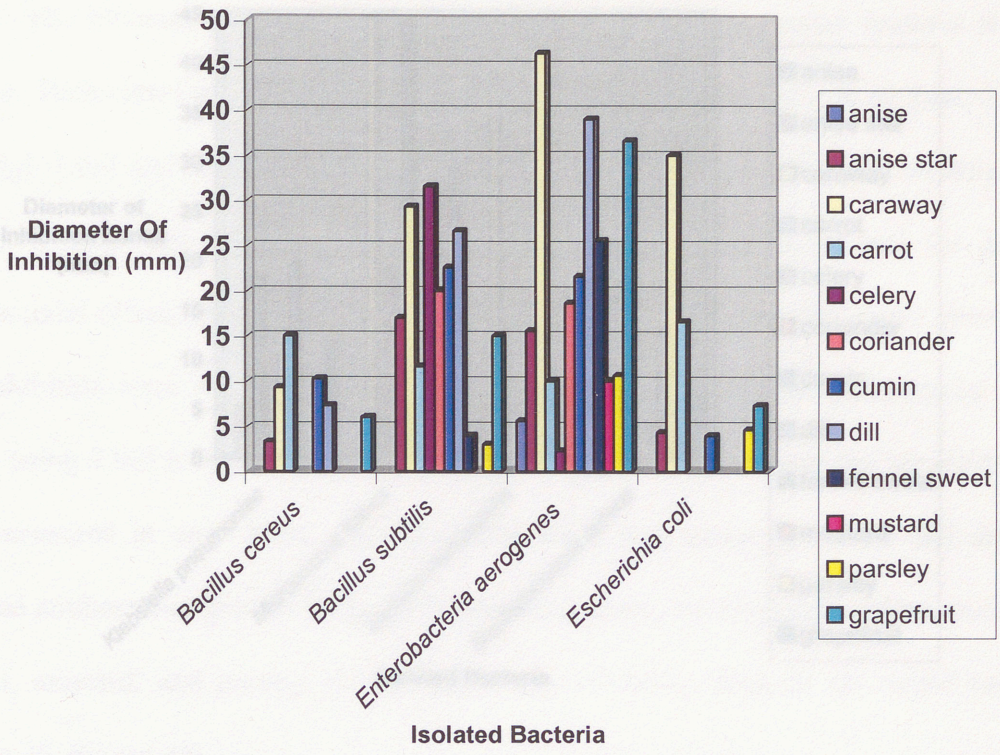
Inhibition zones measured in millimeters (mm); Volume of oil used 2.0μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Table 19. The effects of the essential oils on the growth of isolated bacteria.

Oils	Bacteria			
	Micrococcus luteus	***Staphylococcus aureus***	***Escherichia coli***	***Serratia marcescens***
Anise	-	-	-	-
Anise star	2.6	-	4.3	-
Caraway	44.6	3.3	35	34.6
Carrot	-	17.3	16.6	-
Celery	19	-	-	-
Coriander	-	-	-	-
Cumin	44.3	3.3	4	11.6
Dill	20	13.3	-	13.3
Fennel	-	3.6	-	9
Mustard	-	2	-	-
Parsley	-	2.6	4.6	-
Grapefruit	29.6	6.6	7.3	9.6

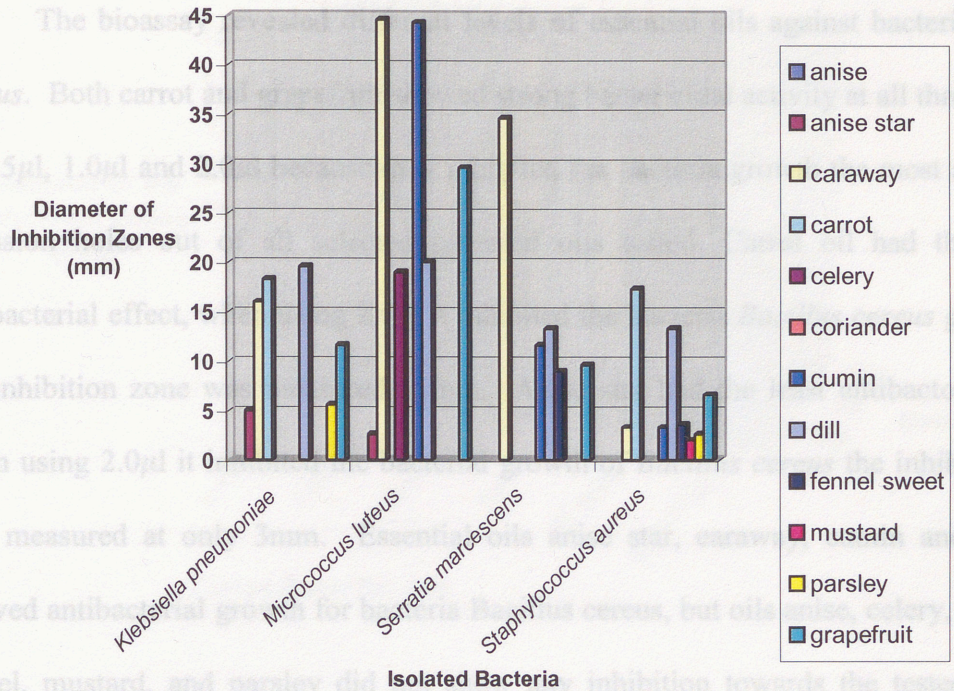
Inhibition zones measured in millimeters (mm); Volume of oil used 2.0μl. (-) Essential oils lacking inhibitory effects on the tested organism.

Figure 44. The Effect of Selected Essential Oils On The Growth of Isolated Bacteria.



All tested oils proved to be effective. Caraway oil showed the greatest inhibition against the isolated growth of *Enterobacteria aerogenes*, and *Escherichia coli*. (Volume of oil used was 2.0µl)

Figure 45. The Effect of Selected Essential Oils On The Growth of Isolated Bacteria.



Caraway and Cumin oils had the greatest inhibitory effect against the growth of isolated bacteria *Micrococcus luteus*. Essential oils Anise and Coriander did not show any inhibitory effect under the conditions tested. (Volume of oil used was 2.0µl)

The Bioassay Results of the Selected Bacteria *Bacillus cereus* using Different Concentrations

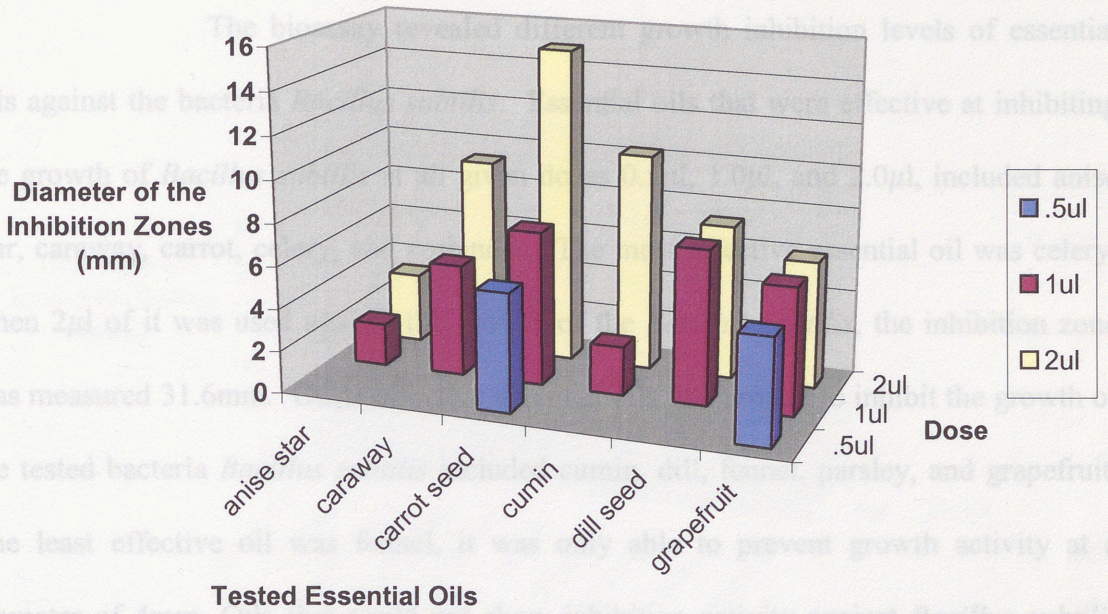
The bioassay revealed different levels of essential oils against bacteria *Bacillus cereus*. Both carrot and grapefruit showed strong bactericidal activity at all three dosages of 0.5µl, 1.0µl and 2.0µl because they inhibited the bacteria growth the most around the diffusion holes out of all selected essential oils tested. Carrot oil had the greatest antibacterial effect, when using 2.0µl it inhibited the bacteria *Bacillus cereus* growth and the inhibition zone was measured 15mm. Anise star had the least antibacterial effect, when using 2.0µl it inhibited the bacterial growth of *Bacillus cereus* the inhibition zone was measured at only 3mm. Essential oils anise star, caraway, cumin and dill also showed antibacterial growth for bacteria Bacillus cereus, but oils anise, celery, coriander, fennel, mustard, and parsley did not show any inhibition towards the tested bacteria. (Table 20, Figure 46)

Table 20. - Results of the tested essential oils on the bacteria *Bacillus cereus* showing the inhibition zones in millimeters (mm) at doses 0.5µl, 1.0µl, and 2.0µl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	-	2	3.3
caraway	-	5.3	9.3
carrot	5.6	7.3	15
celery	-	-	-
coriander	-	-	-
cumin	-	2.3	10.3
dill	-	7.3	7.3
fennel sweet	-	-	-
mustard	-	-	-
parsley	-	-	-
grapefruit	5	6	6

Figure 46.

Tested Essential Oils On The Growth Of The Bacteria *Bacillus cereus*



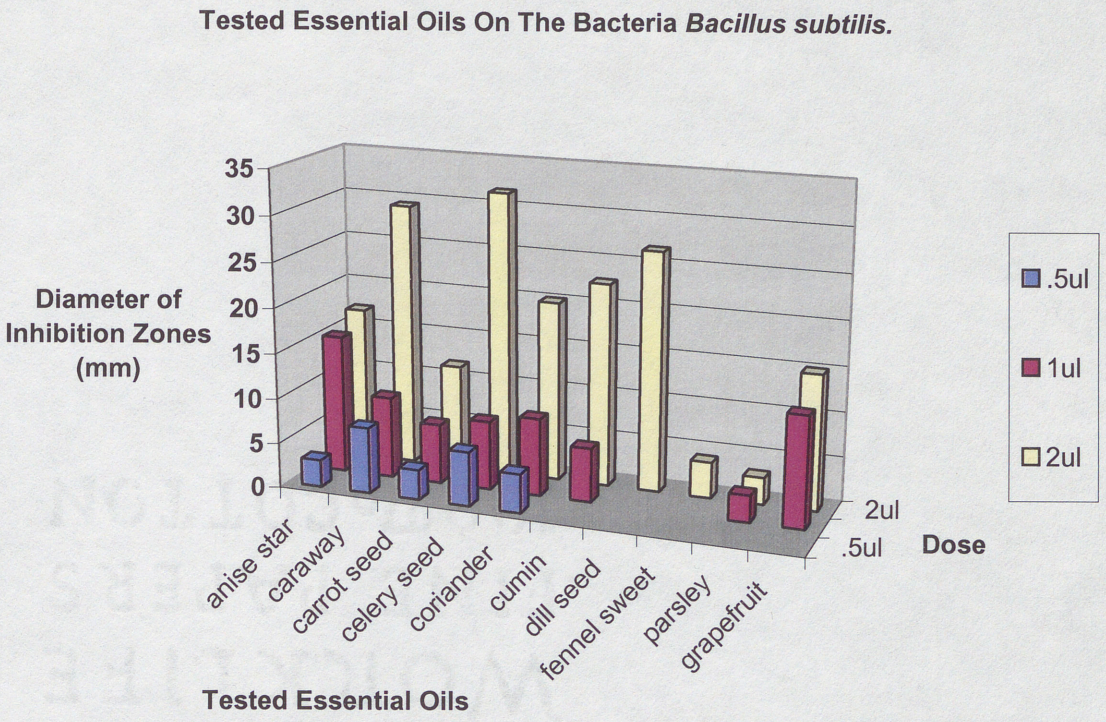
The Bioassay Results of the Selected Bacteria *Bacillus subtilis* using Different Concentrations

The bioassay revealed different growth inhibition levels of essential oils against the bacteria *Bacillus subtilis*. Essential oils that were effective at inhibiting the growth of *Bacillus subtilis* at all given doses 0.5µl, 1.0µl, and 2.0µl, included anise star, caraway, carrot, celery, and coriander. The most effective essential oil was celery, when 2µl of it was used against the growth of the *Bacillus subtilis*, the inhibition zone was measured 31.6mm. Other effective essential oils that proved to inhibit the growth of the tested bacteria *Bacillus subtilis* included cumin, dill, fennel, parsley, and grapefruit. The least effective oil was fennel, it was only able to prevent growth activity at a diameter of 4mm. Oils that could not show inhibition activity against *Bacillus subtilis* included anise, mustard, and dill. (Table 21, Figure 47)

Table 21. Results of the tested essential oils on the bacteria *Bacillus subtilis* showing the inhibition zones in millimeters (mm) at doses 0.5µl, 1.0µl, and 2.0µl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	3	15.3	17
caraway	7.3	9	29.3
carrot	3.3	6.6	11.6
celery	6	7.6	31.6
coriander	4.3	8.6	20
cumin	-	6	22.6
dill	-	-	26.6
fennel sweet	-	-	4
mustard	-	-	-
parsley	-	3	3
grapefruit	-	12.3	15

Figure 47.


Tested Essential Oils On The Bacteria *Bacillus subtilis.*
Diameter of Inhibition Zones (mm)
35
30
25
20
15
10
5
0
anise star
caraway
carrot seed
celery seed
coriander
cumin
dill seed
fennel sweet
parsley
grapefruit
Tested Essential Oils
2ul
.5ul
Dose
.5ul
1ul
2ul

The Bioassay Results of the Selected Bacteria *Enterobacteria aerogenes* using Different Concentrations

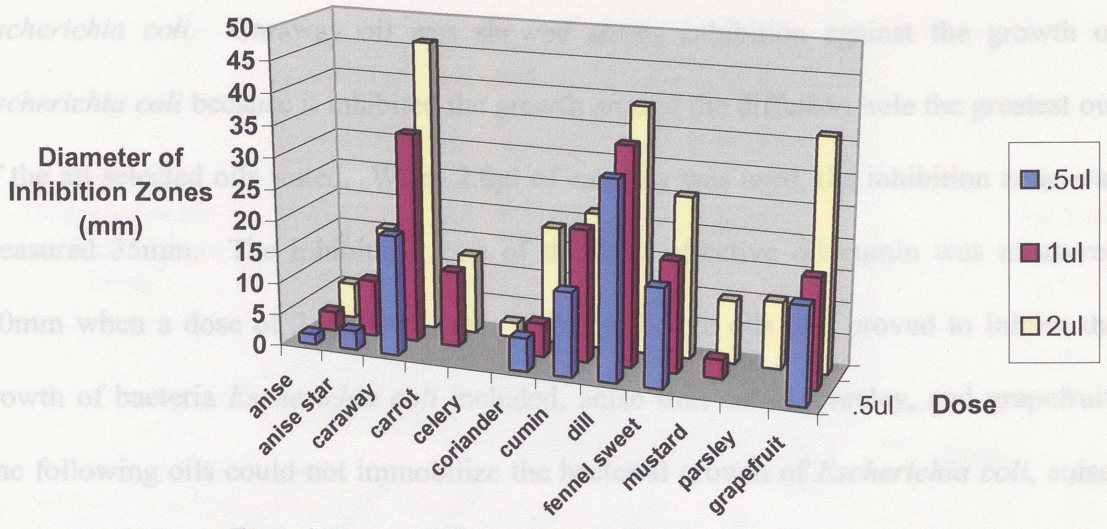
The bioassay demonstrated different inhibition levels using the selected essential oils against bacteria *Enterobacteria aerogenes*. Although all selected essential oils were effective against the growth of *Enterobacteria aerogenes*, caraway was the most effective allowing celery to be the least effective. When 2.0μl of caraway was used, it inhibited the growth of *Enterobacteria aerogenes* and the inhibition zone was measured 46.3mm. Celery was considered to be the least effective oil, when used at 2.0μl, the inhibition zone was measured 2.3mm. Other oils that proved their effectiveness towards the inhibition growth of *Enterobacteria aerogenes* included anise, anise star, caraway, coriander, cumin, dill, fennel, and grapefruit. (Table 22, Figure 48)

Table 22. Results of the tested essential oils on the bacteria *Enterobacteria aerogenes* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise	1.6	3	5.6
anise star	3	9	15.6
caraway	19	33.3	46.3
carrot	-	12	12.6
celery	-	-	2.3
coriander	5.3	5.3	18.6
cumin	13.3	21	21.6
dill	31.3	34.6	39
fennel sweet	15.6	17.6	25.6
mustard	-	3	10
parsley	-	-	10.6
grapefruit	15.3	17.3	36.6

Figure 48.

Tested Essential Oils on the Growth of Bacteria *Entreobacteria aerogenes.*



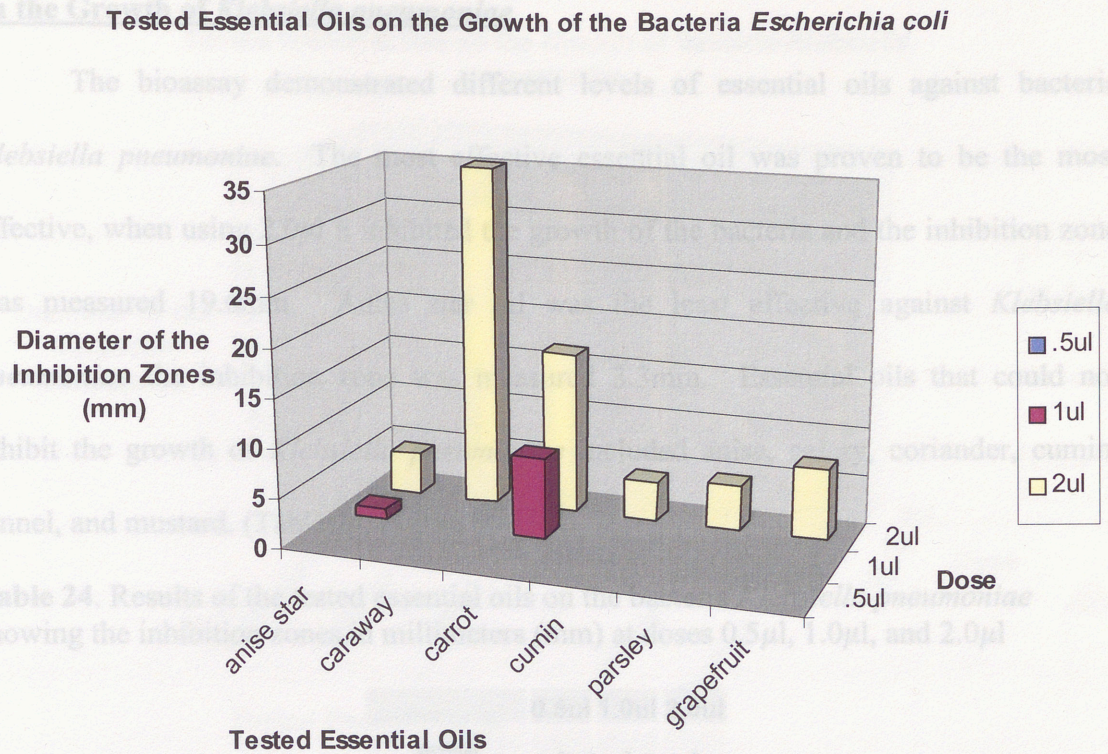
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Escherichia coli*

The bioassay test illustrated different levels of essential oils against bacteria *Escherichia coli.* Caraway oil was showed strong inhibition against the growth of *Escherichia coli* because it inhibited the growth around the diffusion hole the greatest out of the all selected oils tested. When 2.0μl of caraway was used, the inhibition zone was measured 35mm. The inhibition zone of the least effective oil cumin was measured 4.0mm when a dose of 2.0μl was used. Other effective oils that proved to inhibit the growth of bacteria *Escherichia coli* included, anise star, carrot, parsley, and grapefruit. The following oils could not immobilize the bacterial growth of *Escherichia coli*, anise, celery, coriander, dill, fennel, and mustard. (Table 23, Figure 49)

Table 23. Results of the tested essential oils on the bacteria *Escherichia coli* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise star	-	1	4.3
caraway	-	-	35
carrot	-	8.3	16.6
cumin	-	-	4
parsley	-	-	4.6
grapefruit	-	-	7.3

Figure 49.


Tested Essential Oils on the Growth of the Bacteria *Escherichia coli*
Diameter of the Inhibition Zones (mm)
35
30
25
20
15
10
5
0
anise star
caraway
carrot
cumin
parsley
grapefruit
Tested Essential Oils
2ul
1ul
.5ul
Dose
.5ul
1ul
2ul

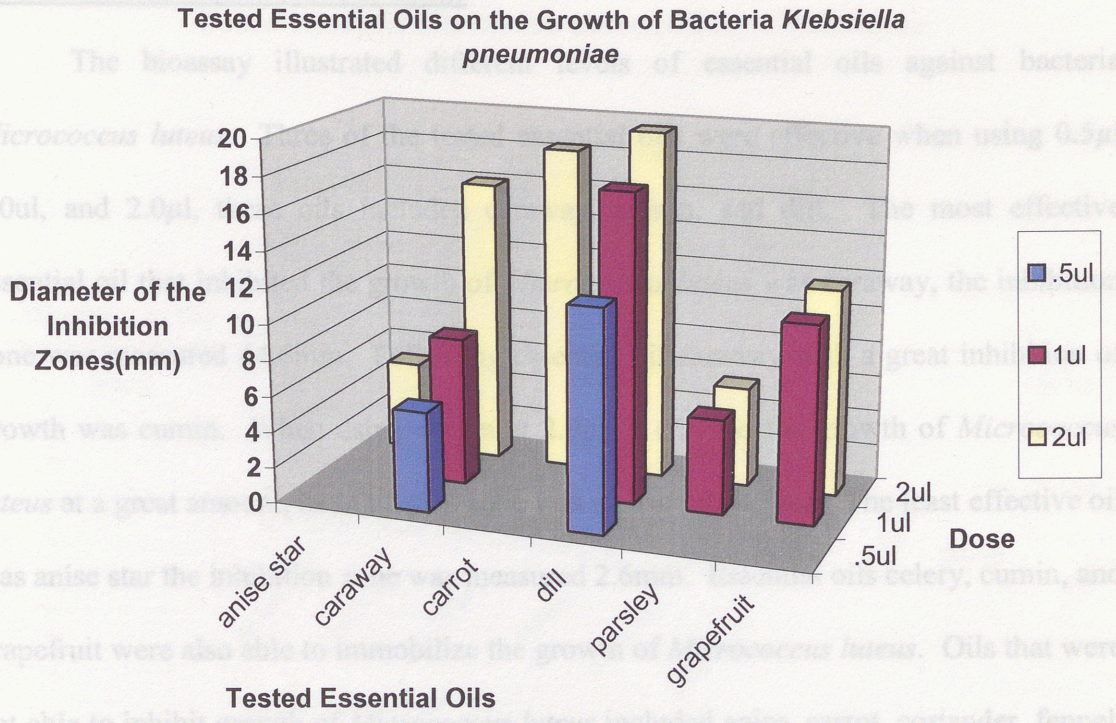
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Klebsiella pneumoniae*

The bioassay demonstrated different levels of essential oils against bacteria *Klebsiella pneumoniae*. The most effective essential oil was proven to be the most effective, when using 2.0μl it inhibited the growth of the bacteria and the inhibition zone was measured 19.6mm. Anise star oil was the least effective against *Klebsiella pneumoniae* the inhibition zone was measured 3.3mm. Essential oils that could not inhibit the growth of *Klebsiella pneumoniae* included anise, celery, coriander, cumin, fennel, and mustard. (Table 24, Figure 50)

Table 24. Results of the tested essential oils on the bacteria *Klebsiella pneumoniae* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	-	-	5
caraway	5.6	8.3	16
carrot	-	-	18.3
celery	-	-	-
coriander	-	-	-
cumin	-	-	-
dill	12.3	17.3	19.6
fennel sweet	-	-	-
mustard	-	-	-
parsley	-	5.3	5.6
grapefruit	-	11	11.6

Figure 50.


Tested Essential Oils on the Growth of Bacteria *Klebsiella pneumoniae*
Diameter of the Inhibition Zones(mm)
20
18
16
14
12
10
8
6
4
2
0
anise star
caraway
carrot
dill
parsley
grapefruit
Tested Essential Oils
2ul
1ul
.5ul
Dose
.5ul
1ul
2ul

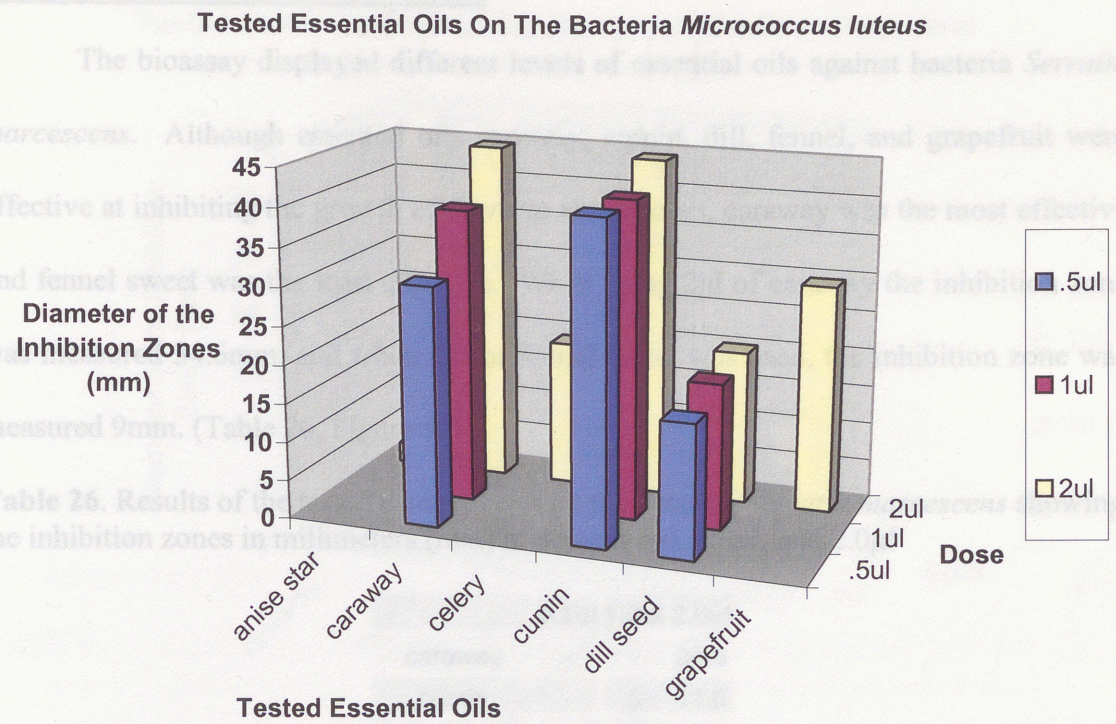
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Micrococcus luteu:*

The bioassay illustrated different levels of essential oils against bacteria *Micrococcus luteus.* Three of the tested essential oils were effective when using 0.5µl 1.0ul, and 2.0µl, these oils included caraway, cumin, and dill. The most effective essential oil that inhibited the growth of *Micrococcus luteus* was caraway, the inhibition zone was measured 44.6mm. Following essential oil caraway with a great inhibition of growth was cumin. When using cumin at 2.0µl, it enabled the growth of *Micrococcus luteus* at a great amount, its inhibition zone was measured 44.3mm. The least effective oil was anise star the inhibition zone was measured 2.6mm. Essential oils celery, cumin, and grapefruit were also able to immobilize the growth of *Micrococcus luteus*. Oils that were not able to inhibit growth of *Micrococcus luteus* included anise, carrot, coriander, fennel, mustard, and parsley. (Table 25, Figure 51)

Table 25. Results of the tested essential oils on the bacteria *Micrococcus luteus* showing the inhibition zones in millimeters (mm) at doses 0.5µl, 1.0µl, and 2.0µl

	0.5ul	1.0ul	2.0ul
anise	-	-	-
anise star	-	-	2.6
caraway	31	38.3	44.6
carrot	-	-	-
celery	-	-	19
coriander	-	-	-
Cumin	41.3	41.3	44.3
dill	17.3	19	20
fennel sweet	-	-	-
mustard -		-	-
parsley	-	-	-
grapefruit	-	-	29.6

Figure 51.


Tested Essential Oils On The Bacteria *Micrococcus luteus*
Diameter of the Inhibition Zones (mm)
45
40
35
30
25
20
15
10
5
0
anise star
caraway
celery
cumin
dill seed
grapefruit
Tested Essential Oils
2ul
1ul
.5ul
Dose
.5ul
1ul
2ul

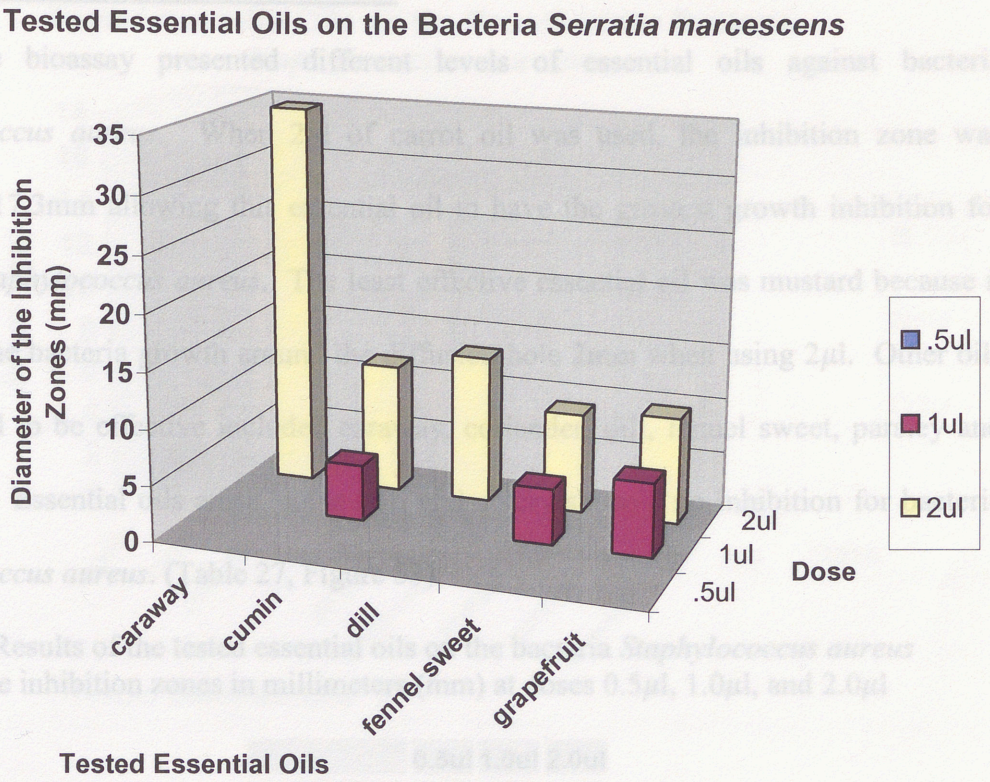
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Serratia marcescens*

The bioassay displayed different levels of essential oils against bacteria *Serratia marcescens*. Although essential oils caraway, cumin, dill, fennel, and grapefruit were effective at inhibiting the growth of *Serratia marcescens*, caraway was the most effective and fennel sweet was the least effective. When using 2μl of caraway the inhibition zone was measured 34.6mm, and when 2μl of fennel sweet was used, the inhibition zone was measured 9mm. (Table 26, Figure 52)

Table 26. Results of the tested essential oils on the bacteria *Serratia marcescens* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
caraway	-	-	34.6
cumin	-	5	11.6
dill	-	-	13.3
fennel sweet	-	5	9
grapefruit	-	6.6	9.6

Figure 52.


Tested Essential Oils on the Bacteria *Serratia marcescens*
Diameter of the Inhibition Zones (mm)
35
30
25
20
15
10
5
0
caraway
cumin
dill
fennel sweet
grapefruit
Tested Essential Oils
2ul
1ul
.5ul
Dose
.5ul
1ul
2ul

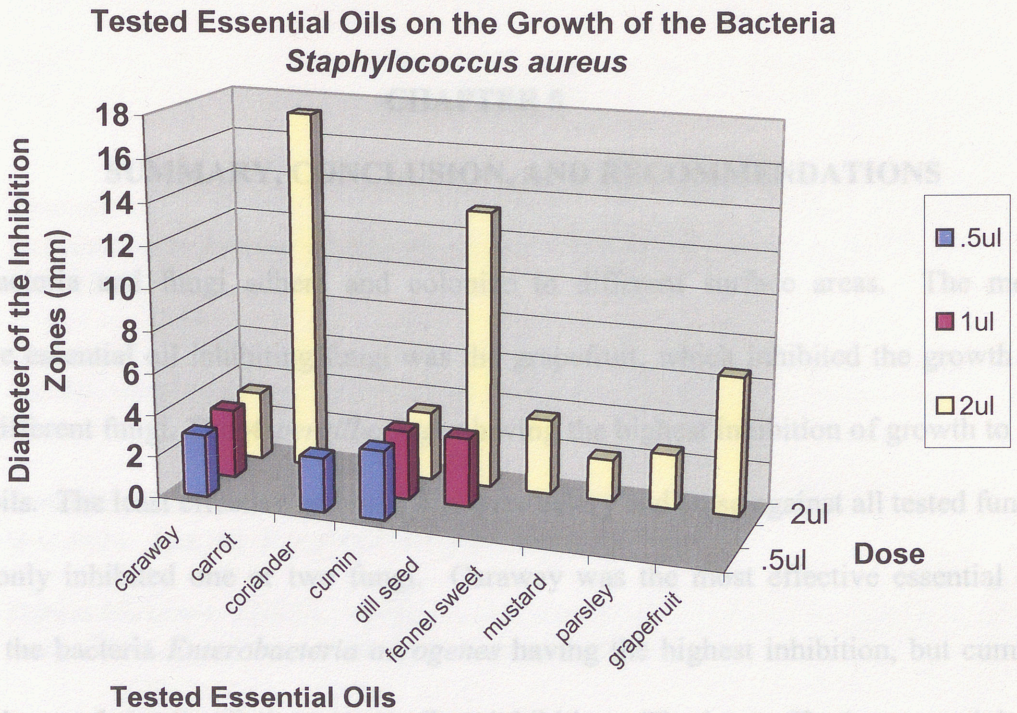
The Bioassay Results of the Selected Essential Oils using Different Concentrations on the Growth of *Staphylococcus aureus*

The bioassay presented different levels of essential oils against bacteria *Staphylococcus aureus*. When 2μl of carrot oil was used, the inhibition zone was measured 17.3mm allowing this essential oil to have the greatest growth inhibition for bacteria *Staphylococcus aureus*. The least effective essential oil was mustard because it inhibited the bacteria growth around the diffusion hole 2mm when using 2μl. Other oils that proved to be effective included caraway, coriander, dill, fennel sweet, parsley and grapefruit. Essential oils anise, anise star, and celery showed no inhibition for bacteria *Staphylococcus aureus*. (Table 27, Figure 53)

Table 27. Results of the tested essential oils on the bacteria *Staphylococcus aureus* showing the inhibition zones in millimeters (mm) at doses 0.5μl, 1.0μl, and 2.0μl

	0.5ul	1.0ul	2.0ul
caraway	3	3.3	3.3
carrot	-	-	17.3
coriander	2.6	-	-
cumin	3.3	3.3	3.3
dill	-	3.3	13.3
fennel sweet	-	-	3.6
mustard	-	-	2
parsley	-	-	2.6
grapefruit	-	-	6.6

Figure 53.


Tested Essential Oils on the Growth of the Bacteria
Staphylococcus aureus
Diameter of the Inhibition Zones (mm)
18
16
14
12
10
8
6
4
2
0
caraway
carrot
coriander
cumin
dill seed
fennel sweet
mustard
parsley
grapefruit
Tested Essential Oils
.5ul
1ul
2ul
2ul
.5ul
Dose

CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Bacteria and fungi adhere and colonize to different surface areas. The most effective essential oil inhibiting fungi was the grapefruit, which inhibited the growth of seven different fungi. The *Aspergillus niger* having the highest inhibition of growth to all tested oils. The least effective essential oils were celery and anise against all tested fungi, which only inhibited one or two fungi. Caraway was the most effective essential oil against the bacteria *Enterobacteria aerogenes* having the highest inhibition, but cumin, dill, and grapefruit oils all showed excellent inhibition. The least effective essential oil against bacteria was anise, and the most widely effective oil for both bacteria and fungi was grapefruit.

This study has shown that in the Bioassay using drill hole cavity method, essential oils have different antimicrobial properties. Overall some essential oils are effective towards the inhibition of some bacteria and fungi growth, but the effectiveness of the essential oils depends on the microorganism and the essential oil tested. These inhibitory effects are interesting in connection with the prevention of biofilm formation, and this study demonstrates that essential oils present an excellent potential for the inhibition of biofilm formation on medical devices, food, cosmetics, monuments, water pipes and other surface areas.

REFERENCES

1. Alarcon G.G. (1995). Microbial communities and alteration processes in monuments at Alcala de Henares, Spain. The Science of the Total Environment. 167, 231-239.
2. Chaidez C. (2004). Comparison of the microbiologic quality of point of use treated water and tap water. International Journal of Environmental Health Research. 14, 253-260.
3. Critchley M.M. (2004). Microbiological influences in blue water copper corrosion. Journal of Applied Microbiology. 97, 590-597.
4. Dautle Melalnie. (2003). Isolation and identification of biofilm microorganisms from silicone gastrostomy devices. Journal of Pediatric Surgery. 38, 216-220.
5. Donalan M.R. (2002). Biofilms: Microbial life on surfaces. Emerg.Infect Dis. 8, 1-23.
6. Elvers K.T. (1998). Bacterial fungal biofilms in flowing water photo-processing tanks. Journal of Applied Microbiology. 84, 607-618.
7. Filius Margaret. (2005). Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization. Antimicrobial Agents and Chemotherapy. 49, 2879-2886.
8. Flores M. (1997). Algae and bacteria on historic monuments at Alcala de Henares, Spain. International Biodeterioration and Biodegration. 40, 241-246.
9. Gryta Marek. (2002). The assessment of microorganism growth in the membrane distillation system. Desalination. 142, 79-88.
10. Gunasekaron G. (2004). Influence of bacteria on film formation inhibiting corrosion. Corrosion Science. 46, 1953-1967.
11. Kalemba D. (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry. 10, 813-829.
12. Klaric M. (2006). Antifungal activity of thyme (thymus vulgarisix) essential oil and thymol against moulds from damp dwellings. 44, 36-42.

13. Liu W. (2002). Investigation of assimilable organic carbon (AOC) and bacterial regrowth in drinking water distribution system. Water Research. 36, 891-898.

14. Miklelsen H. (2006). Interrelationships between colonies, biofilms and planktonic cells of pseudomonas aeruginosa. Journal of Bacteriology. 7, 2411-2416.

15. Niu C. (2004). Colorimetric method for identifying plant essential oil components that affect biofilm formation and strcture. Applied Microbiology. 70, 6951-6956.

16. Senechal Annie. (2005). Quantification of initial adhesion of Enterococcus faecalis to medical grade polymers using a DNA based fluorescence assay. J.Biomater.Sci.Polymer Edn, 16, 115-129.

17. Senpuka H. (2003). Systemic diseases in association with microbial species in oral biofilm from elderly requiring care. Gerontology. 49, 301-309

18. Sheth Neela. (1983). Colonization of bacteria on polyvinyl chloride and Teflon intravascular catheters in hospitalized patients. 18, 1061-1063.

19. Si W. (2006). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. 100, 296-305.

20. Alarcon G.G. (1995). Microbial communities and alteration processes in monuments at Alcala de Henares, Spain. The Science of the Total Environment. 167, 231-239.

21. Chaidez C. (2004). Comparison of the microbiologic quality of point of use treated water and tap water. International Journal of Environmental Health Research. 14, 253-260.

22. Critchley M.M. (2004). Microbiological influences in blue water copper corrosion. Journal of Applied Microbiology. 97, 590-597.

23. Dautle Melalnie. (2003). Isolation and identification of biofilm microorganisms from silicone gastrostomy devices. Journal of Pediatric Surgery. 38, 216-220.

24. Donalan M.R. (2002). Biofilms: Microbial life on surfaces. Emerg.Infect Dis. 8, 1-23.

25. Elvers K.T. (1998). Bacterial fungal biofilms in flowing water photo-processing tanks. Journal of Applied Microbiology. 84, 607-618.

26. Filius Margaret. (2005). Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization. Antimicrobial Agents and Chemotherapy. 49, 2879-2886.

27. Flores M. (1997). Algae and bacteria on historic monuments at Alcala de Henares, Spain. International Biodeterioration and Biodegration. 40, 241-246.

28. Gryta Marek. (2002). The assessment of microorganism growth in the membrane distillation system. Desalination. 142, 79-88.

29. Gunasekaron G. (2004). Influence of bacteria on film formation inhibiting corrosion. Corrosion Science. 46, 1953-1967.

30. Kalemba D. (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry. 10, 813-829.

31. Klaric M. (2006). Antifungal activity of thyme (thymus vulgarisix) essential oil and thymol against moulds from damp dwellings. 44, 36-42.

32. Liu W. (2002). Investigation of assimilable organic carbon (AOC) and bacterial regrowth in drinking water distribution system. Water Research. 36, 891-898.

33. Miklelsen H. (2006). Interrelationships between colonies, biofilms and planktonic cells of pseudomonas aeruginosa. Journal of Bacteriology. 7, 2411-2416.

34. Niu C. (2004). Colorimetric method for identifying plant essential oil components that affect biofilm formation and strcture. Applied Microbiology. 70, 6951-6956.

35. Senechal Annie. (2005). Quantification of initial adhesion of Enterococcus faecalis to medical grade polymers using a DNA based fluorescence assay. J.Biomater.Sci.Polymer Edn, 16, 115-129.

36. Senpuka H. (2003). Systemic diseases in association with microbial species in oral biofilm from elderly requiring care. Gerontology. 49, 301-309

37. Sheth Neela. (1983). Colonization of bacteria on polyvinyl chloride and Teflon intravascular catheters in hospitalized patients. 18, 1061-1063.

38. Si W. (2006). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. 100, 296-305.