Contaminated Fungi in the Biology Department laboratories and Antagonistic Potentiality of *Myrtrus Communis* Volatile oil Against the Isolated Fungi

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Abstract

Antagonistic activity of *Myrtrus communis* volatile oil was evaluated against fungi isolated and detected in different hoods in Biology Department in order to eliminate and purge the hoods of laboratories from contaminant fungi which are rendered scientific projects. Many fungi samples were detected in four laboratories which were *Aspergillus fumigates, Asp.niger, Asp.flavus, Cryptococcus albidus, Fusarium solani, Asp.terrus, Asp.terriola, Penicillium notatum and Mucor* sp. Only four pure isolates were gained due to difficulties in purification of the isolates included *Fusarium solani, Asp.fumigatus, Asp.niger* and *Asp.Flavus.* Volatile oil of *Myrtrus communis* showed the highest fungicidal effect causing 100 % inhibition against *Asp.fumigates* and the lowest was against *Fusarium solani.* [DOI: 10.22401/JNUS.21.3.15]

Introduction

Enormous medical, industrial and agricultural avails had been achieved from the whole plant [1]. Unripe fruits are bitter, and become sweet in test in ripeness. Tolerance for desiccation "Soil should be allowed to dry in-between watering". These plants are flourished in summer [2]. Myrtle is a vernacular name of *Myrtus communis* means common plant growing in groups. Root showed antibacterial properties [3]. The volatile oil was exhibited fungicidal against *Aspergillus* as reported by[4]. Despite the notorious role of *Aspergillus* as a major contaminant of laboratory experiments or its clinical participant as a pathogens, but it's had been occupied a significant parts in many scientific and industrial fields [5]. *Penicillium* is widespread in a diverse habitats, this fungi presence in soil, on decaying vegetation, tembers, in addition to the aquatic systems. Penicillia represent a very serious issues as contaminant invasive agents in laboratories. There is no certainty that most *Penicillium* species are the recognizable causes of systemic mycosis [6]. *Fusarium* is a genus of filamentous fungi, part of a group called hyphomycetes which are widely distributed in soil and developed symbiotic relationships with plants. Fumonisins and trichothecenes are the major toxins produce by *Fusarium* manifest a serious health hazard since these fungi infect cereals the main crops for the nourishment for human and animals. In humans with normal immune systems, fusarial infections may occur in the nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis)" [7]. This study aimed to eliminate contaminated fungi in the hoods of Biology Department hoods which are obstacles confronting under and post graduate students in addition to researchers in their pursuing the maximum sterilization conditions in addition to antimicrobial evaluating the effect of myrtle volatile oil against these contaminated fungi.

Materials and Methods

Collection of the studied plant

Myrtus communis leaves, were collected midday from the gardens of Baghdad University, Iraq and air dried at room temperature.

Identification of the studied plant

The plant was identified in the Biology department's Herbarium, College of Science University of Baghdad.

Preparation of plants extracts

Volatile oil extraction

A quantity of 250 g. of dried leaves of *Myrtus communis* was ground into small pieces and subjected to hydro distillation using the Clevenger apparatus for 5 hours according to British Pharmacopeia. The aqueous phase poured in separating funnel which contained sodium chloride, subsequently the aqueous phase was separated into two layers, the lower layer was discard while the upper one which contains the oil retrieved and stored in a sealed marked glass vial in a refrigerator until use [8].

Isolation of contaminant fungi

Culture medium (potata dextrose agar) was prepared and poured in petri dishes. Plates of enrichment medium were inoculated with swabs taken from all hoods in the Department of Biology. The inoculums were diffused on the medium surface, the plates were incubated at 28 C for 3-5 days at optimum temperature for fungi growth.

Purification of contaminant fungi

Fungi appeared on Petri dishes after incubation, showed a variation in their types according to distinctions in their shape, texture and color, each of those colonies was subcultured continuously on PDA till pure isolated colonies were gained. The isolated colonies were preserved on sealed plates and kept in a refrigerator until use.

Identification of the isolated fungi

A-Morphological identification

Shapes, colors and textures of isolated colonies of fungi were primarily used to identify their types, and this was accomplished with the help of specific keys of fungi identification and experienced mycologists in Biology Department, College of Science, Baghdad University.

B- Microscopic examination

A small inoculum from a fresh fungal isolate was transferred on a slide and a drop of lacto phenol dye was added and pressed gently with a cover slide, then examined under (10x) and (40x) of a compound microscope [9].

Evaluation of the inhibitory effect of the extracted oil against isolated fungi

The technique which was used in this study to determine the fungicidal activity of the oil, and then applied for each oil and mold as described by [10]:

0.1, 0.2 and 0.5% concentrations of the oil were prepared by mixing 100, 200 and 500 µl of oil with equal amount of dimethyl sulfoxide (DMSO) in addition to potato dextrose agar approaching the final volume 100 ml then left to solidify. Mycelial growth (8 mm) from mold culture of 15 days was deposited in the center of each plate which contained a certain concentration of oil. The plates were incubated for 3-5 days. Two replicates were made. After incubation period, fungicidal effect of the oil was estimated by measuring the diameter of the growth of the isolated fungi. The data were obtained according to [10].

Percentage of fungal growth = [Growth of control – Growth of treatment] /Growth of control × 100

Results

Oil yield of *Myrtus communis*

4 ml of *Myrtrus communis* volatile oil was extracted by a Clevenger apparatus from 250 g. of myrtle dried and ground leaves. The yield of oil was 1.6% , which calculated using above data.

Sampling of fungi

Over one hundred samples from four different laboratory hoods in Biology department were cultured in replicate pattern on plates in order to identify, isolate and purificate the fungi.

Identification of fungi

Samples were incubated for 3-5 days, then identified morphologically and microscopically as shown in Table(1).

Table (1)
Isolated and identified fungi in the all labs. of Biology Department labs.

Lab.	Fungi
Pathology	*Asp.fumigates, Asp.niger, Asp.Flavus, Cryptococcus albidus, Penicillium notatum, Mucor* sp.
Food microbiology	*Asp.niger, Asp.Flavus, Yeast, Penicillium notatum, Asp.fumigates, Fusarium solani, Mucor* sp.*Asp.terrus, Asp.terriola, cryptococcus albidus*
Botany for postgraduate students	*Penicillium notatum, Asp.niger, Asp.Flavus, Asp.fumigates, Cryptococcus albidus*
mycology for postgraduate students	*Asp.fumigates, Asp.flavus, Asp.niger, Mucor* sp., *Penicillium notatum, Cryptococcus albidus*

Purification of fungi

Isolation and purification and identification were carried out. The isolates were: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Fusarium solani.*

Antifungal assessment of *Myrtus* volatile oil

Antifungal activity of the extracted oil at different concentrations was tested against the isolated fungi from the selected laboratories. The maximum antifungal activity of oil was against *Asp. fumigates* caused 100% inhibition at all concentrations 0.1, 0.2 and 0.5% as shown in Table (2) and Fig.(1,2,3 and 4). Results also revealed that *A. flavus* and *Asp.niger* were the lowest. However, *Fusarium solani* exhibited minimum susceptibility to *Myrtus* volatile oil in this study.

Table (2)
The variations in the fungicidal effects of Myrtus oil against four strains of molds measured by growth inhibition (G.I.%).

Oil (%)	*Asp. niger* G.I (%)	*Asp. flavus* G.I (%)	*Asp. fumigatus* G.I (%)	*Fusarium solani* G.I (%)
0.1	60	71	100	29.5
0.2	60	69	100	22.5
0.5	62	65	100	30

Fig.(1): Growth inhibition of Asp. niger due to the effect of Myrtus essential oil.

Fig.(2): Highly inhibition of Asp. Fumigatus due to the effect of Myrtus essential oil.

Fig.(3): Growth inhibition of Asp. flavus due to the effect of Myrtus essential oil.

Fig.(4): Growth inhibition of Asp. flavus due to the effect of Myrtus essential oil.

Discussion

Myrtus inhibited fungal growth inhibition with significant variations at different concentrations (0.1, 0.2 and 0.5%). High fungicidal activity reached 100% mycilial growth inhibition for *Asp. fumigates* also its exhibited antifungal action against *Asp.niger*, *Asp.flavus*, *Fusarium solanius albidus*. [11] experimented *Myrtus* volatile oil at different concentrations (0.1, 0.2 and 0.5%) against numbers of food poisoning fungi included *Asp. niger* and *Asp. flavus* revealed that the oil have fungicidal activites against all tested fungi which is however less than those which recorded in this study. *Thymus vulgaris* and *Eugenia caryophyllata* oils exhibited strongest activity while *Myrtus communis* oil showed the lowest activity against *A. flavus*. [12] reported that mycelial growth inhibition accompained with degeneration of hyphae occurs after treatment with *Thymus vulgaris* volatile oil. According to [13] "the antimicrobial action of essential oils may be due to impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis". Many scientific researches had been conducted to figure out the inhibitory mode of action of essential oils in attempt to spot the hazard effects on cellular membranes, i.e. permeability and proton motive force [14]. Such actions lead to impairment of cells membranes. [15] experimented the impact of

different concentrations of pennyroyal, savory, chamomile, thyme and myrtle volatile oils against certain fungal isolates *A. flavus, A. fumigatus, S.parasitica, A. niger* manifested decreasing in fungal growth. Essential or volatile oils varied in their antimicrobial capacities may due to many reasons; composition of major and minor chemical compounds and their concentrations which vary according to habitat, age of the plant and accuracy of the collection, drying and extraction methodologies. Many targets in cell could be affected by essential oil due to the numerous number of compounds with different actions including: 1) inactivation of many enzymes and disruption of many chemicals [16](2) affecting membrane permeability [17]. (3) Disfiguration of fatty acids, polysaccharides, and phospholipids layers present in the cell wall and cell membrane [18] "The antimicrobial or antifungal mode of action of essential oils including myrtle essential oil may be due to terpenoids, including monoterpenes, sesquiterpenes and their oxygenated derivatives. These compounds are highly lipophilc and are of low molecular weight which disrupt the cell membrane causing the cell death and inhibition of sporulation and germination of food spoilage fungi " [19].

Conclusion

Laboratories of Biology Department inhabited by many contaminated fungi, therefore this study was conducted to identify these contaminated fungi and eliminate four isolates out of these fungi by using the volatile oil of Myrtrus communis which showed different inhibitory effects, the highest fungicidal effect causing 100 % inhibition against Asp.fumigates while the lowest was against Fusarium solani.

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