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Biodiversity of endophytic fungi associated with some medicinal plants and their responses to essential oils

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ABSTRACT

The present work aimed to study the biodiversity of fungal endophytes inhabiting four medicinal plants namely; *Cymbopogon citratus* L., *Thymus vulgaris* L., *Salvia officinalis* L. and *Mentha viridis* L. collected from Assiut City, Egypt. A total of 15 species related to 8 genera were isolated from leaves, stems and roots of the tested plants. The isolated fungi were screened for their sensitivities to essential oils extracted from thyme, sage, spearmint and lemongrass. The results indicated that the inhibitory concentration was fungal strains dependant. The majority of fungal strains (74.2%) were inhibited by thyme oil at MICs ranging from 3.125% to 25% (V/V). Some isolates of *Aspergillus terreus*, *Botryodiplodia theobromae*, *Penicillium crustosum* and *Talaromyces pinophilus* were only sensitive to higher concentrations of thyme oil (50% - 100%). In case of saga oil, only 42% of fungal strains were inhibited by low oil concentrations (6.25% - 25%). Oils extracted from spearmint and lemongrass exhibited wide spectrum of inhibitory action at concentrations fluctuating from 3.13% to 25%) and were active against 85% and 90% of the tested fungal strains, respectively. ITS sequencing was used to confirm identification of three resistant fungal strains which were diagnosed as *Aspergillus terreus* AUMC 16070 (GenBank accession no. OQ935432), *Lasiodiplodia theobromae* AUMC 16098 (OQ930484) and *Penicillium crustosum* AUMC 16082 (OQ930483).

INTRODUCTION

Endophytic fungi are fascinating species that colonize the internal healthy tissues of plants **[1].** These fungi have attracted researchers due to their capacities to provided

novel sources of anticarcinogenic molecules, antimicrobial substances, as well as biostimulants for essential oil biosynthesis **[2].** Moreover, these fungi often enhance nutrient solubilization in the plant rhizosphere **[3]**, activate growth and systemic resistances of plants **[4].**

As mentioned by some Iranian scientists **[5]**, few studies were conducted to characterize the endophytic fungi of medicinal plants and their capabilities to produce bioactive metabolites. The pharmaceutical properties of *Thymus* sp. (Family: Lamiaceae) can be attributed to its fungal endophytes. The obtained endophytic genera from this plant were *Alternaria, Aspergillus, Cladosporium, Curvularia, Cylindrocarpon, Drecheslra, Fusarium, Phoma, Stemphylium* and *Ulocladium*. In another study the endophytic fungi within roots of Chenopodiaceae species were surveyed [**6**]. The authors collected 192 fungal isolates belonging to the genera *Acremonium, Alternaria, Aspergillus Bipolaris, Chaetomium, Cladosporium, Curvularia, Embellisia, Fusarium Macrophomina, Penicillium, Ulocladium* in addition to twelve types of sterile mycelia.

In Egypt four endophytic fungal species were isolated from leaves of *Thymus vulgaris* and were identified as *Aspergillus terreus* (the most dominant species), *A. japonicas, Penicillium chrysogenum* and Mycelia sterilia **[7]**. The authors found that carvacrol and thymol were present with high percentage in extracts of *A. terreus* and *T. vulgaris*. *A. terreus* extract exhibited the highest antioxidant activity followed by extracts of *T. vulgaris, A. japonicus,* Mycelia sterilia and *P. chrysogenum* with IC_{50} values of 13.2, 14.3, 23.1, 34.2, 132.7 μg/ml, respectively. *In vitro* cytotoxicity assay was also tested against human liver cancer cell line (HEPG-2). Results revealed that *A. terreus* extract had the highest cytotoxicity effect on HEPG-2 followed by extracts of *T. vulgaris, P. chrysogenum*, Mycelia sterilia, and *A. japonicus* with IC_{50} value of 3.53, 4.19, 112.2, 12,2 and 14.8 μg/ml, respectively.

Studies on endophytic fungi associated with sage plants (*Salvia aegyptiaca*) collected from Gebel Elba region revealed the isolation of twenty fungal species belong to 14 genera with the highest colonization frequencies (21.38%, 20.64%, 20.00%, and 14.71% being recorded by *Alternaria alternata, Humicola grisea, Colletotrichum* sp. and *Trichoderma viride.* Other fungal species belonging to *Aspergillus, Chaetomium, Curvularia, Fusarium, Penicillium* and *Pestalotiopsis* were less frequently isolated from

S. aegyptiaca [8]. From leaves of *Ocimum basilicum*, three endophytic fungi were isolated and identified as *Aspergillus flavus*, *A. fumigatus* and *A. nidulans* [9].

Reports from India showed the isolation of 343 endophytic fungal strains from *Mentha arvensis* L., *Ocimum basilicum* L., *Origanum majorana* L., *Rosmarinus officinalis* L. and *Thymus vulgaris* L. **[10]**. The authors were able to isolate several fungal species belonging to *Alternaria*, *Aspergillus*, *Chaetomium*, *Curvularia*, *Fusarium*, *Penicillium, Rhizopus* and *Talaromyces*

To reduce the use of chemicals in medical and nutritional fields scientists pay a great attention to natural products including essential oils (EOs). These natural compounds can play a fundamental and benificial role in human life **[11].** Essential oils are generally considered flavourings or essences among the natural compounds. They have a wide range of biotechnological applications due to their therapeutic qualities and significance in the cosmetics and food industries. They are typically odoriferous and colourless or light yellow. They have a strong biological potential, and among their other properties are antioxidant, antiseptic, antibacterial, antifungal, anti-inflammatory, and repelling ones **[12]**. One of the most promising natural products for fungal suppression could be EOs. In fact, a wide variety of EOs extracted from various plants or herbs showed potent antifungal activities and could attenuate the microbial growth and biofilm development through specific mechanisms **[11].**

Literature studies on endophytes isolated from some plants of Lamiaceae and Poaceae families are still limited, thus this investigation aims to isolation and identification the endophytic fungi associated with these plants. *In vitro* susceptibility of the isolated fungi to some essential oils was also tested.

MATERIALS AND METHODS

1- Collection of medicinal plant samples:-

During the period from July 2018- July 2019, four species of healthy medicinal plants were freshly collected from Assiut City, Egypt. These plants included *Thymus vulgaris* L, *Salvia officinalis* L. and *Mentha viridis* L. (Family Lamiaceae, Order Lamiales) in addition to *Cymbopogon citrates* L. (Family Poaceae, Order Poales). Plant samples were brought to the Mycological laboratory in sterile plastic bags and processed within 8 hours after sampling.

2- Isolation and identification of endophytic fungi:-

(a) Surface sterilization of the plant materials.

Plant materials (leaves, stems and roots) were washed with running tap water prior to rinsing in 70% ethanol for 30 seconds, 0.5% sodium hypochlorite (NaOCl) for 2-3 minutes, and finally in sterile distilled water for 2-3 times. Samples were then dried between sterile Whatman no.1 filter papers **[13]**.

(b) Culturing of endophytic fungi:

After proper drying, the surface sterilized plant materials were cut into small pieces of $1cm^2$ in case of leaves or 1 cm long in case of stems and roots. Five segments of the plant material were placed in 9 cm Petri plates (3plates/sample) containing sterile potato dextrose agar (PDA) medium **[14]** supplemented with chloramphenicol (250 mg/ml) as antibacterial agent. Samples of stems and roots were cut vertically to expose the interior cells to the PDA medium. The previous steps were repeated using water agar (WA) medium. All inoculated plates were incubated at 28ºC for 7-10 days to promote the growth of endophytic fungi. Each endophytic culture was checked for purity and transferred to freshly prepared PDA plates and slants **[15]**.

(c) Morphological identification of fungal isolates:

Fungal cultures were identified on the basis of their macroscopic and microscopic features as described in specialized identification manuals **[16 - 21]**. Pure cultures of the isolated strains were preserved in the culture collection of the Assiut University Mycological Center (AUMC).

(d) Molecular identification of some fungal isolates:

Three fungal strains which showed partial or complete resistant to one or more of the tested essential oils were choosen for molecular identification. Pure cultures were grown on potato dextrose agar (PDA) and incubated at 28°C for 5 days. The genomic DNA was extracted using Patho-gene-spin extraction kit. Pure DNA was shipped to SolGent Company South Korea for polymerase chain reaction (PCR) and sequencing of the internal transcribed spacer region (ITS) of rRNA gene using the primer pair ITS1 (forward) and ITS4 (reverse) **[22**]. The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National

Center of Biotechnology Information (NCBI) website **[23]**. Sequence alignments and phylogeny were performed using MegAlign (DNA Star) software version 5.05.

3- Antifungal Activity of essential oils:

Purified essential oils were purchased from an essential oil factory in the industrial city located in the New Fayoum City, Egypt. The antifungal activity was carried out using the agar well diffusion method **[24]**. For each fungal strain, 6 Sabouraud`s dextrose agar (SDA) plates were prepared and seeded with $1x10^6$ spores/ml. Using sterile 5 mm cork borer one well was made in center of each plate. Descending concentrations (100%, 50%, 25%, 12.5%, 6.25% and 3.125 % v/v) of each of the four types of essential oils were prepared in dimethylsulfoxide (DMSO) solvent. Aliquots of 50µl of each concentration were transferred into the wells using sterile micropipette. The synthetic antifungal agent clotrimazole (10 mg/ml) was used as a positive control. All cultures were incubated at 28^oC for 7 days. The results were recorded as the diameter of inhibition zone around each well (mm).

RESULTS AND DISCUSSION

(a) Endophytic mycobiota isolated from the tested medicinal plants:

A total of 31 endophytic fungal isolates belonging to 15 species and 7 genera were identified from the four medicinal plant types. These species are listed in **Table (1)** with their respective AUMC numbers.

Different samples of *Thymus vulgaris* plant were colonized by 11 fungal species namely; *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Chaetomium globosum, Lasiodiplodia theobromae, Penicillium chrysogenum, P. oxalicum, Talaromyces duclauxii* and *T. pinophilus.* The broadest spectrum of fungi (6 species) was obtained from *Thymus* leaves while stems and roots yielded 3 and 2 fungal species respectively.

From *Salvia officinalis* nine endophytic fungal species were isolated andn identified namely; *A. flavus, A. fumigatus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, F. proliferatum, L. theobromae, P. crustosum* and *T. pinophilum.*Four fungal species were recovered from *Cymbopogon citratus* and these were *A. flavus, A. terreus, L. theobromae* and *P. crustosum.*

Only three endophytic fungal species were recovered from *Mentha viridis* which included *A. fumigatus, P. crustosum* and *T. pinophilus.*

Several studies have shown that medicinal plants are precious sources of endophytic fungi. *Alternaria alternata, Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Penicillium* sp. and *Phoma* sp. were isolated from superficially disinfected leaves, stems and roots of *Thymus vulgaris* plant [**23**]. Also, *Aspergillus japonicas, A. terreus* and *Penicillium chrysogenum* were identfied as endophytic fungal species from *Thymus vulgaris* **[25]**. Some indian researchers **[26]** isolated 325 endophytic fungi belonging to hyphomycetes, coelomycetes, ascomycetes in addition to sterile mycelium from 800 segments of *Cymbopogon citratus*. The most frequently isolated endophytes included *Cladosporium cladosporioides* (22.46%), *Drechslera* sp. (6.76%), *Colletotrichum gloeosporioides* (6.76%) and *Phyllosticta* sp (5.53%). Leaf samples contained more endophytes than rhizome samples. Three medicinal plants of Lamiaceae (*Ocimum sanctum, O. bacilicum* and *Leucas aspera*) were screened for endophytic fungal diversity [**28**]. The authors were able to obtain 103 fungal endophytic isolates belonging to14 genera. Leaves of all tested plants were generally colonized by a great number of endophytic fungi. The fungal list comprised unidentified species belonging to the genera *Alternaria, Aspergillus* and *Fusarium.* A kind of host specificity was observed where certain species of *Curvularia, Hymenula, Trichoderma* and *Tubercularia* exclusively colonized *O. sanctum* plant. *Alternaria*. and *Spicaria* were only cultured from *L. aspera*. Other endophytic fungi belonging to *Alternaria, Aspergillus, Chaetomium, Fusarium, Penicillium* and *Talaromyces* were obtained from different medicinal plants **[29]**. *A. flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, F. moniliforme, P. chrysogenum* and *P. oxalicum* were isolated from different parts of *Thymus vulgaris, Origanum majorana* and *Rosmarinus officinalis* **[30]**. Recently**,** several genera of fungal endophytes including *Diaporthe, Stemphylium, Botryosphaeria, Talaromyces, Fusarium, Cephalotheca, Cladosporium, Penicillium, Aspergillus,* and *Phoma* were reported from Lamiaceae plants **[31].**

It is worthy to mention that representative species of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Fusarium, Phoma* and *Trichoderma***,** which appeared in the current study, were also reported as endophytes from *Jasminum sambac, Camellia sinensis* and *Ocimum basilicum* **[32].**

Table (1): Endophytic fungi isolated from the tested medicinal plants on potato dextrose agar and water agar media at 28ºC.

Fungal Strains	AUMC NO.	Source of isolation	Media of isolation
Alternaria alternata	16086	Stems of *Thymus vulgaris*	PDA
Aspergillus flavus	16071	Leaves of *Salvia officinalis*	PDA
Aspergillus flavus	16079	Leaves of *Cymbopogon citratus*	WA
Aspergillus flavus	16094	Roots of *Thymus vulgaris*	PDA
Aspergillus fumigatus	16073	Leaves of *Thymus vulgaris*	PDA
Aspergillus fumigatus	16074	Stems of *Salvia officinalis*	PDA
Aspergillus fumigatus	16080	Leaves of *Mentha viridis*	PDA
Aspergillus niger	16076	Leaves of *Thymus vulgaris*	PDA
Aspergillus niger	16096	Stems of *Salvia officinalis*	PDA
Aspergillus terreus	16072	Leaves of *Thymus vulgaris*	PDA
Aspergillus terreus	16070	Roots of *Cymbopogon citratus*	PDA
Chaetomium globosum	16093	Stems of *Thymus vulgaris*	WA
Cladosporium cladosporioides	16081	Stems of *Salvia officinalis*	PDA
Fusarium oxysporum	16100	Roots of *Salvia officinalis*	WA
Fusarium proliferatum	16092	Stems of *Salvia officinalis*	PDA
Lasiodiplodia theobromae	16085	Leaves of *Salvia officinalis*	PDA
Lasiodiplodia theobromae	16088	Roots of *Thymus vulgaris*	PDA
Lasiodiplodia theobromae	16089	Leaves of *Thymus vulgaris*	PDA
Lasiodiplodia theobromae	16091	Roots of *Cymbopogon citratus*	WA
Penicillium chrysogenum	16077	Stems of *Thymus vulgaris*	WA
Penicillium crustosum	16078	Stems of *Cymbopogon citratus*	PDA
Penicillium crustosum	16082	Roots of *Mentha viridis*	PDA
Penicillium crustosum	16084	Roots of *Mentha viridis*	WA
Penicillium crustosum	16087	Roots of *Salvia officinalis*	PDA
Penicillium crustosum	16090	Leaves of *Mentha viridis*	WA
Penicillium crustosum	16098	Roots of *Salvia officinalis*	PDA
Penicillium oxalicum	16083	Leaves of *Thymus vulgaris*	WA
Talaromyces duclauxii	16075	Leaves of *Thymus vulgaris*	PDA
Talaromyces pinophilus	16095	Stems of *Thymus vulgaris*	PDA
Talaromyces pinophilus	16097	Stems of *Salvia officinalis*	PDA
Talaromyces pinophilus	16099	Stems of *Mentha viridis*	WA

(a) Fungal strains identified by molecular techniques

Three fungal strains which showed high resistance to the inhibitory action of one or more of the tested essential oils were sequenced using the ITS region of rRNA gene. Phylogenetic trees of *Aspergillus terreus*, *Penicillium crustosum* and *Lasiodiplodia theobromae* are illustrated in Figures 1, 2 and 3. Molecular results confirmed the morphological identification of these fungal strains.

***Aspergillus terreus* AUMC 16070 (580 letters),**
GenBank accession number: OQ935432

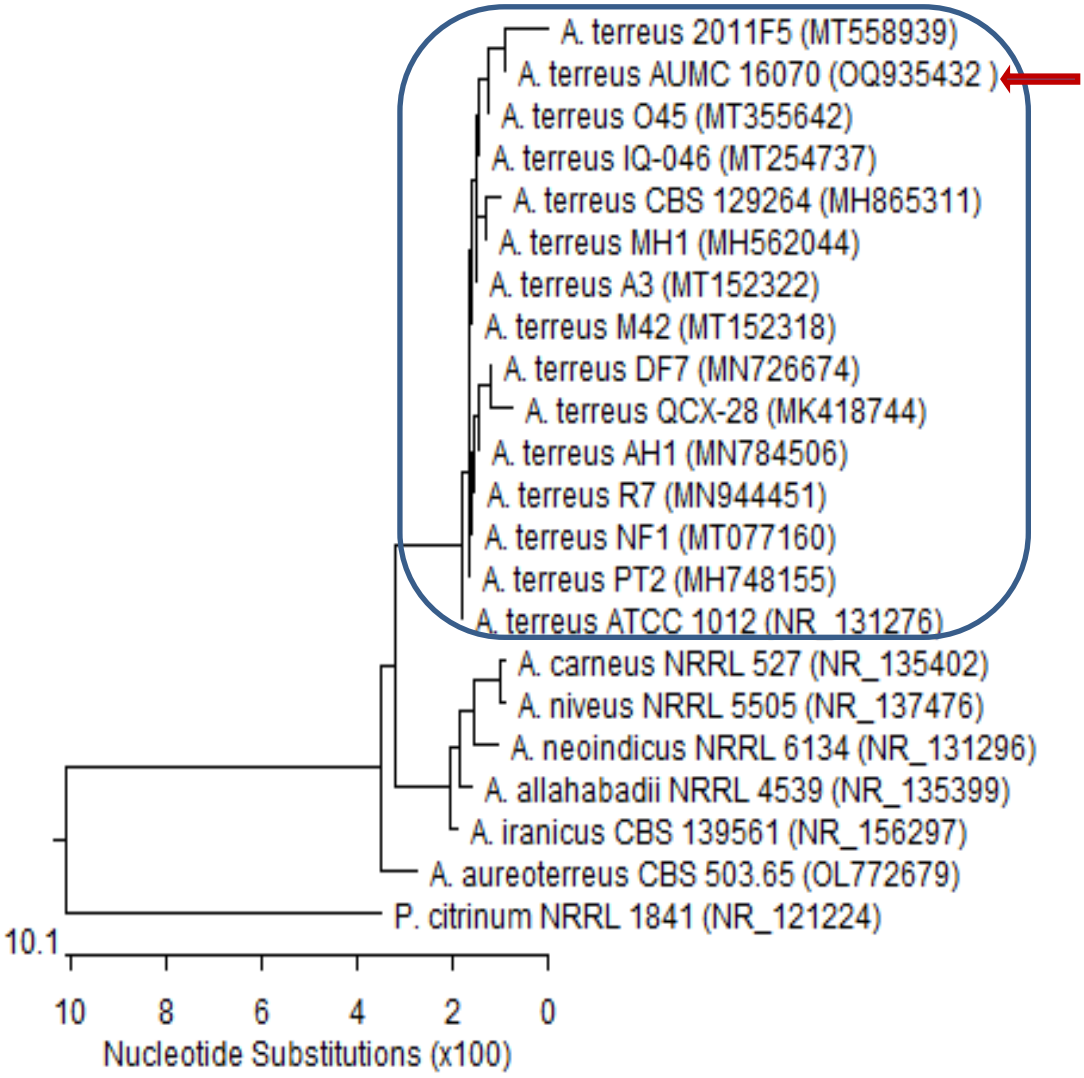

Figure (1): Phylogenetic tree based on ITS sequences of rDNA of the fungal strain (*A. terreus* AUMC16070) with GenBank accession no. OQ 935432 (arrowed) aligned with closely related sequences of the same species accessed from the GenBank. A.= *Aspergillus*, P.= *Penicillium*

Notes: *A. terreus* AUMC 16070 showed 100% identity and 100% coverage with several related species including the type strain of *A. terreus* ATCC 1012 (NR_131276)

***Penicillium crustosum* AUMC16082 (571 letters),**
GenBank accession number: OQ930483

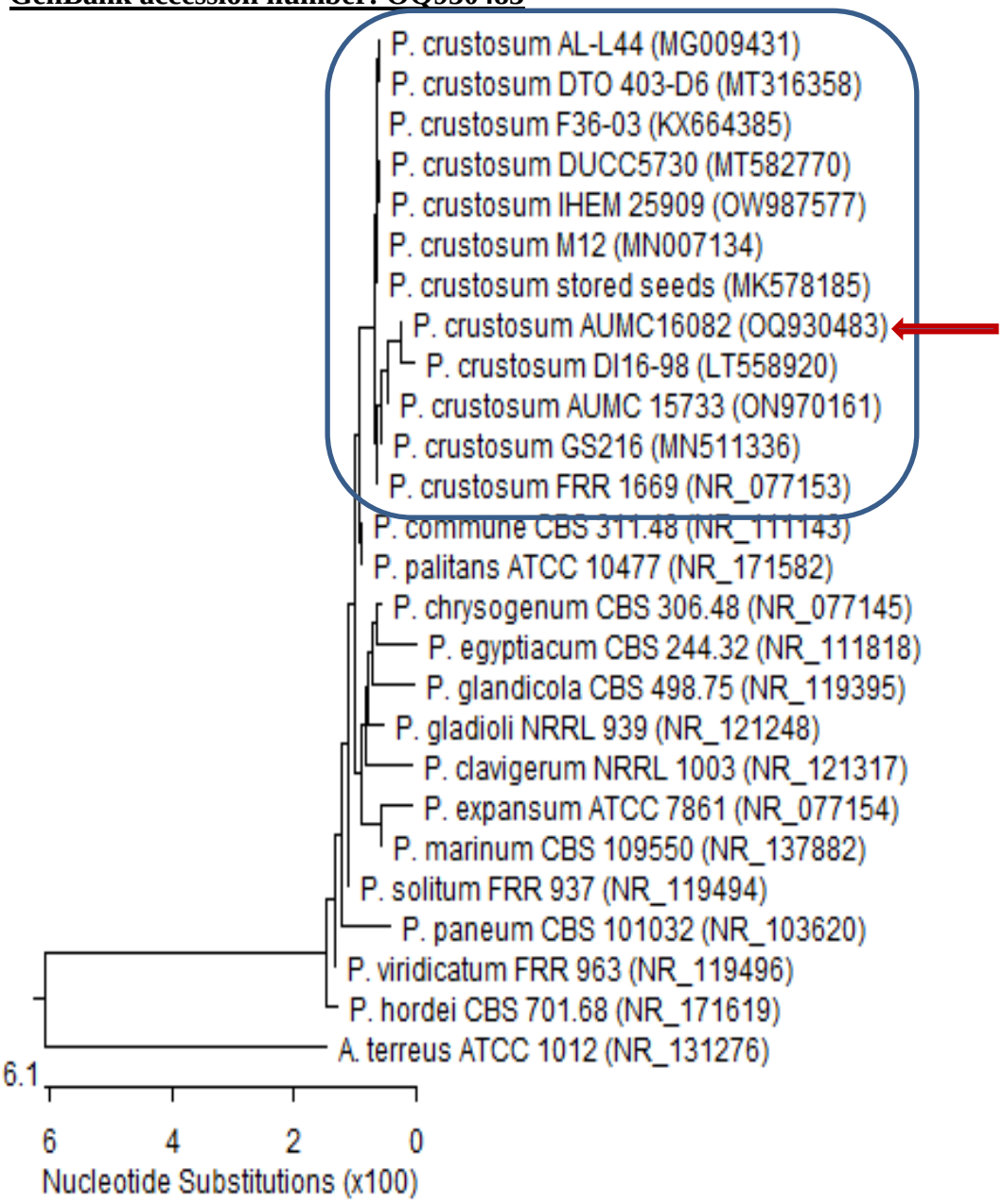

Figure (2): Phylogenetic tree based on ITS sequences of rDNA of the fungal strain (*P. crustosum* AUMC16082) with GenBank accession no. OQ930483 (arrowed) aligned with closely related sequences of the same species accessed from the GenBank. A.= *Aspergillus*, P.= *Penicillium*

Notes: *P. crustosum* AUMC 16082 showed 99.65% - 99.82% identity and 100% coverage with several related species including the type strain *P. crustosum* FRR1669 (NR_077153).

***Lasiodiplodia theobromae* AUMC16089 (527 letters),**
GenBank accession number: OQ930484

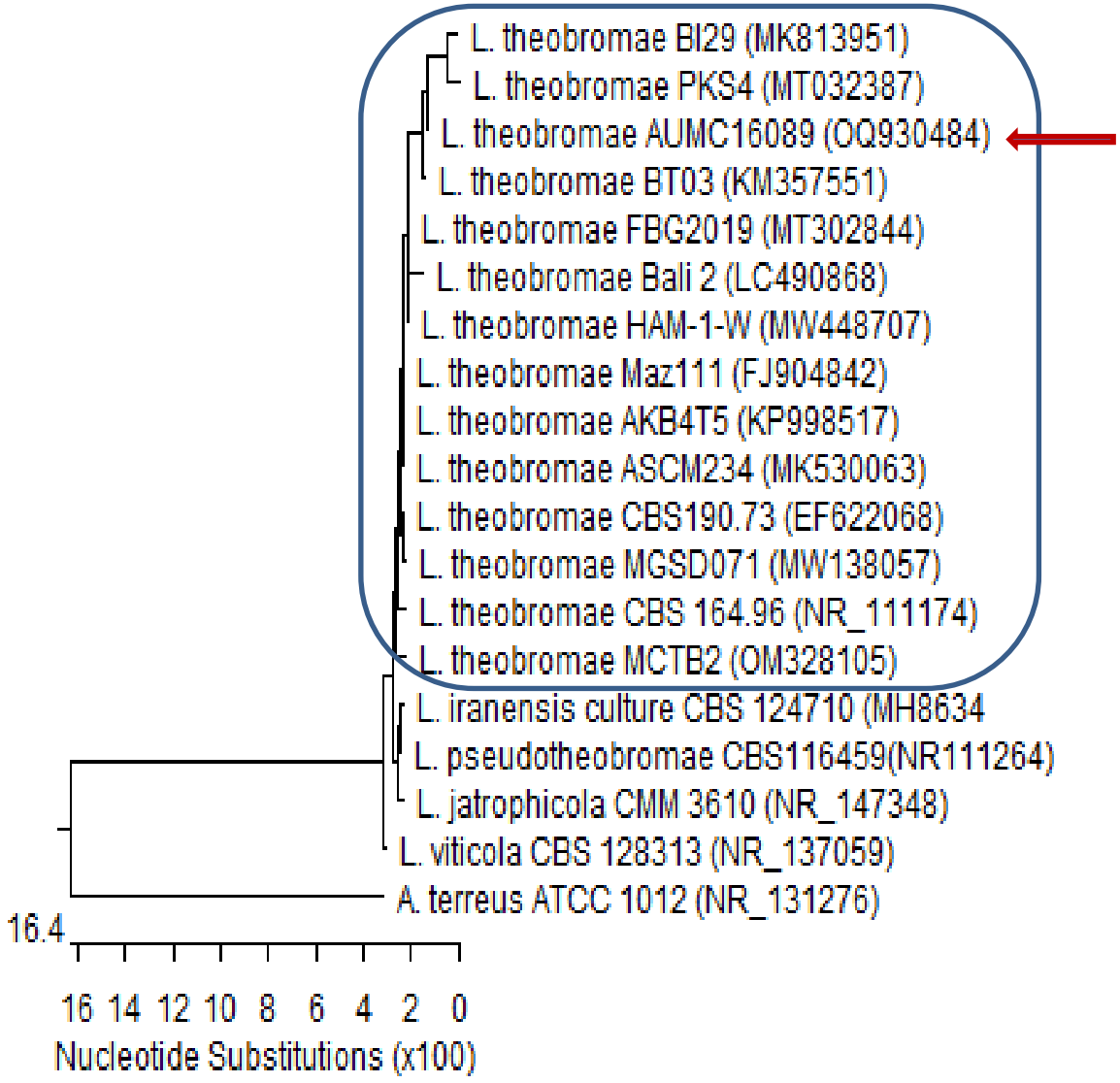

Figure (3): Phylogenetic tree based on ITS sequences of rDNA of the fungal strain (*L. theobromae* AUMC16089) with GenBank accession no. OQ930484 (arrowed) aligned with closely related sequences of the same species accessed from the GenBank. A.= *Aspergillus*, L.= *Lasiodiplodia*

Notes: *L. theobromae* AUMC 16089 showed 99.05% - 99.62% identity and 99% - 100% coverage with several related species including the type strain *P. crustosum* FRR1669 (NR_077153).

(c) Sensitivity of endophytic fungi to essential oils

The current results **(Table 2)** revealed that thyme oil exhibited high activity against several fungal species with MICs ranging from 3.125% in case of *Penicillium crustosum* AUMC16084 and 6.25% in case of *Alternaria alternata* AUMC16086, *Aspergillus flavus* AUMC16071, *A. fumigatus* AUMC16073, *A. fumigatus* AUMC16074, *A. fumigatus* AUMC16080, *A. niger* AUMC16076, *A. niger* AUMC16096, *Chaetomium globosum* AUMC16093, *Penicillium chrysogenum* AUMC16077, *P. crustosum* AUMC16078, *P. crustosum* AUMC16098, *Talaromyces duclauxii* AUMC16075 and *T. pinophilus* AUMC16097. Good activity of thyme oil was also observed at 12.5% against *Aspergillus flavus* AUMC16079, *Lasiodiplodia theobromae* AUMC16085, *Cladosporium cladosporioides* AUMC16081 and *P. crustosum* AUMC16087. Higher concentrations of thyme oil (25% to 100%) showed variable effects against the remaining fungal strains and the most resistant was *L. theobromae* AUMC16089 which was only inhibited by pure thyme oil **(Table 2)**.

Sage oil exhibited high activity at 6.25% against *Alternaria alternata* AUMC 16086, *P. crustosum* AUMC 16098 and *T. pinophilus* AUMC 16097. Good activity of this oil was observed at 12.5% against *L. theobromae* AUMC 16091 and *C. globosum* AUMC 16093. On the other hand, certain strains of *A. flavus* AUMC16071, *A. terreus* AUMC16070, *A. terreus* AUMC16072, *P. crustosum* AUMC16084 and *P. crustosum* AUMC16087 were completely resistant to sage oil even at 100% concentration. Variable inhibitory effects was recorded against the remaining fungal species at concentration ranging from 25% to 100% **(Table 2)**.

Spearmint and lemongrass oils proved to be a widespectrum antifungal compounds showing excellent activity against several fungal species with the lowest MIC (3.125%) being observed with *Alternaria alternata* AUMC16086 and *P. crustosum* AUMC 16084. At cocentrations of 6.25% and 12.5%. These oils exhibited high activity against *Aspergillus, Chaetomium, Fusarium, Lasiodiplodia, Penicillium* and *Talaromyces* as shown in **(Table 2)**.

Clotrimzole (10 mg / ml) was effective against the majority of tested fungi showing inhibition zones ranging from 20–50 mm **(Table 2)**. Response to this drug appeared to be strains dependent where some strains of the same fungal species were

markedly inhibited but others were resistant. It is worthy to mention that three *Lasiodiplodia theobromae* AUMC 16088, *L. theobromae* AUMC 16091 and *Fusarium proliferatum* AUMC 16092 were not affected by Clotrimzole (10 mg / ml) but they were markedly inhibited by thyme oil at (50%), Sage oil (25% and 12.5%), spearmint oil (50%, 25% and 12.5%) and Lemongrass oil (50% and 25%) as indicated in **(Table 2)**.

Table (2) :Inhibition zone (in mm) and MICs (% in parenthes) of essential oils tested against fungi isolated from medicinal plants. Clotrimazole (10 mg/ml) served as control

Fungal Strain	Thyme oil	Sage oil	spearmint oil	Lemongrass oil	Control
Alternaria alternata AUMC 16086	18(6.25%)	17(6.25%)	19(3.125%)	19(3.125%)	27
Aspergillus flavus AUMC 16071	20(6.25%)	0(100%)	18(12.5%)	45(25%)	38
Aspergillus flavus AUMC 16079	19(12.5%)	28(50%)	25(25%)	21(12.5%)	35
Aspergillus flavus AUMC 16094	19(25%)	21(25%)	18(25%)	18(12.5%)	35
Aspergillus fumigatus AUMC 16073	20(6.25%)	18(50%)	18(12.5%)	25(6.25)	38
Aspergillus fumigatus AUMC 16074	20(6.25%)	30(50%)	20(6.25%)	25(6.25%)	43
Aspergillus fumigatus AUMC 16080	21(6.25%)	30(100%)	18(25%)	18(6.25%)	40
Aspergillus niger AUMC 16076	20(6.25%)	20(50%)	17(12.5%)	22(12.5%)	30
Aspergillus niger AUMC 16096	19(6.25%)	20(50%)	20(12.5%)	25(6.25%)	35
Aspergillus terreus AUMC 16070	25(25%)	0(100%)	18(50%)	23(25%)	35
Aspergillus terreus AUMC 16072	28(50%)	0(100%)	23(25%)	32(50%)	40
Chaetomium globosum AUMC 16093	19(6.25%)	18(12.5%)	20(6.25%)	33(25%)	43
Cladosporium cladosporioides AUMC 16081	27(12.5%)	20(100%)	23(25%)	23(6.25%)	40

Fusarium oxysporum AUMC 16100	30(50%)	19(25%)	28(50%)	35(50%)	33
Fusarium proliferatum AUMC 16092	25(50%)	20(25%)	20(12.5%)	18(50%)	0
Lasiodiplodia theobromae AUMC 16085	27(12.5%)	50(100%)	19(12.5%)	32(50%)	33
Lasiodiplodia theobromae AUMC 16088	28(50%)	17(25%)	21(50%)	19(25%)	0
Lasiodiplodia theobromae AUMC 16089	18(100%)	20(25%)	30(25%)	19(25%)	25
Lasiodiplodia theobromae AUMC 16091	38(50%)	19(12.5%)	18(25%)	22(25%)	0
Penicillium chrysogenum AUMC 16077	18(6.25%)	20(50%)	21(12.5%)	27(6.25%)	35
Penicillium crustosum AUMC 16078	21(6.25%)	18(50%)	22(25%)	27(6.25%)	40
Penicillium crustosum AUMC 16082	18(25%)	33(100%)	20(50%)	17(25%)	40
Penicillium crustosum AUMC 16084	18(3.125%)	0(100%)	23(25%)	37(3.125%)	45
Penicillium crustosum AUMC 16087	18(12.5%)	0(100%)	20(25%)	20(12.5%)	50
Penicillium crustosum AUMC 16090	35(50%)	25(100%)	30(25%)	18(25%)	38
Penicillium crustosum AUMC 16098	18(6.25%)	21(6.25%)	21(6.25%)	19(25%)	42
Penicillium oxalicum AUMC *16083*	18(25%)	40(100%)	28(100%)	18(6.25%)	40
Talaromyces duclauxii AUMC *16075*	20(6.25%)	19(25%)	45(50%)	25(6.25%)	45
Talaromyces pinophilus AUMC 16095	35(50%)	20(50%)	23(100%)	33(100%)	38
Talaromyces pinophilus AUMC 16097	37(6.25%)	18(6.25%)	22(12.5%)	32(6.25%)	20
Talaromyces pinophilus AUMC 16099	17(25%)	25(100%)	23(50%)	18(6.25%)	40

Previous studies on EOs of *Oreganum vulgare* and *Thymus vulgaris* referred to their inhibitory action towards several plant pathogens such as *Botrytis cinerea, Fusarium oxysporum, F. solani, Macrophomina phaseolina, Penicillium* sp., *Pythium* sp., *Phytophthora infestans, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotium rolfsii* **[33].** Other reports demonstrated that essential oils of origanum, thyme, clove, lavander, and sage were effective against *Alternaria alternata, Aspergillus fumigatus, Cladosporium cladosporioides, Chaetomium globosum* and *Penicillium chrysogenum* **[34].**

Currently, plant essential oils are gaining more popularity, particularly due to their safety and various pharmacological properties, including bacteriostatic, free radical scavenging, anti-inflammatory and anti-proliferation of cancer cells [**35, 36]**. Moreover, EOs have a high potential as air disinfectants in indoor environments [**37**]. In this respect, Eos extracted from Origano was reported to be effective against the allergenic fungi *Alternaria alternata*, *Botrytis cinerea* and *Cladosporium cladosporioides* at the concentration of 0.1 mg/mL causing decay of fungal mycelium and spores **[38]**. The antimicrobial effects of individual or mixed EOs obtained from lemongrass, oregano, rosemary, peppermint, and eucalyptus were evaluated against *Cladosporium cladosporoides, Aspergillus fumigatus*, and *Penicillium chrysogenum*, which are commonly found on archive papers were studied. A mixture of oregano, lemongrass and peppermint (1:1:1) was more effective against these fungi showing MIC of 0.78% during a vapour test at a distance of 5.5 cm compared with individual Eos **[39]**.

Many EOs have hydrophobic or lipophilic properties which facilitates interaction with the fungal membranes causing alterations of membrane properties including the fluidity where most of sphingolipid and glycosphingolipid metabolism-related genes were downregulated as a result of thymol treatment **[40].** On the other hand, genes involved in cell wall modification, chitin biosynthesis and antioxidant activities, were up-regulated. It was proposed that EO from thymol acts by disrupting fungal cell wall and cell membranes by increasing the production of reactive oxygen species (ROS) on the fungal cell surface as well as by blocking the fungal genes required for cell wall fortification and synthesis of cell membranes **[41].** Thymol was reported to cause strong inhibition of hyphal growth and conidial production of Fusarium graminacearum by induction of lipid

peroxidation and disruption of ergosterol biosynthesis, which are vital components of plasma membrane **[42].** A similar mechanism of action was observed with EOs of carvacrol and thymol acting against wine spoilage yeasts **[43].**

The antimicrobial mechanism of action by the EOs was repoted to depend on the type of the EOs and the microbial strain. Researchers observed that accumulation of the essential oils in the cell cause disruption of membranes and morphological alterations leading to leakage and death of the organism. Concerning the antifungal activity, the action of Eos seems to involve cell wall penetration and direct deterioration of both cytoplasmic and mitochondrial membranes **[44]**. Extensive damage of fungal cell wall and cytoplasmic membrane was detected after application of thymoquinone extracted from black cumin seed **[45]**. Other adverse effects on fungal spore germination, germ tube elongation and growth of fungal mycelia was reported after treatment with EOs **[46]**. Moreover, detachment of the plasma membrane from the cell wall, extensive folding of lomasomes, formation of cellular vacuoles, and malformation of the fibrillary layers of the cell wall were frequently observed on the fungal mycelia or fungal spores **[47].**

CONCLUSION

Medicinal plants harbor different species of endophytic fungi which are treated as a precious source for several bioactive compounds. These fungi can activate growth and systemic resistances of plants and may serve as biostimulants for essential oil biosynthesis. Essential oils from medicinal plants proved to be a potential and promising antifungal agents that find application in various medical and biotechnological fields.

REFERENCES

[1] B. Alam, J. Li, Q. Ge, M. A. Khan, J.Gong, S. Mehmood, Y. Yuan and W. Gong, Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa?, Frontiers in Plant Science, 12, (2021), 1-24.

[2] H. A. El Enshasy, S. Z. Hanapi, R Abd Malek, S. A. Abdelgalil and O. M. Leng, Endophytic fungi: the desired biostimulants for essential oil production., in Advances in Endophytic Fungal Research: Present Status and Future Challenges, ed. B. P. Singh (Cham: Springer International Publishing), (2019), 211–232.

[3] P. Mehta, R. Sharma, C. Putatunda and A. Walia, Endophytic Fungi: Role in phosphate solubilization, Advances in Endophytic Fungal Research: Present Status and Future Challenges, (2019), 183-209.

[4] J. Poveda, *Trichoderma* as biocontrol agent against pests: New uses for a mycoparasite, Biological Control, 159, (2021), 1-8.

[5] S. Masumi, S Mirzaei1, D. Zafari, R. Kalvandi, Isolation, identification and biodiversity of endophytic fungi of Thymus, Progress in Biological Sciences, 5 (1), (2017), 43-50.

[6] R. Aletaha, S. A. A. Safari and D. Zafari, A survey on endophytic fungi within roots of *Chenopodiaceae* species under different environmental conditions, Mycosphere, 9(4), (2018), 618–634.

[7] E. A. M. Ali, A. A. Ahmed, M. A. Sayed and M. R. Hamed, Antioxidant activity and cytotoxicity of extracts of *Thymus vulgaris* L. and their associated endophytic fungi, The Egyptian Society of Experimental Biology, 14(1), (2018), 107 – 116.

[8] Mohamed El-Bondkly, A.A.; El-Gendy, M.M.A.A.; El-Bondkly, E.A.M.; Ahmed, A.M. Biodiversity and biological activity of the fungal microbiota derived from the medicinal plants *Salvia aegyptiaca* L. and *Balanites aegyptiaca* L. 10, (2020), 1-16.

[9] M. H. Sharaf, A. M. Abdelaziz, M. H. Kalaba, A. A. Radwan and A. H. Hashem, Antimicrobial, Antioxidant, Cytotoxic Activities and Phytochemical Analysis of Fungal Endophytes Isolated from *Ocimum Basilicum,* Applied Biochemistry and Biotechnology (2021), 1-20.

[10] R. Jagadish and S. Chowdappa, Diversity and antimicrobial potential of endophytic fungi from aromatic plants of Bhadra Wildlife Sanctuary, Western Ghats, Karnataka, Journal of Applied Biology & Biotechnology, 9(05), (2021), 1-12.

[11] F. Nazzaro, F. Fratianni, R.Coppola and V. De Feo, Essential oils and antifungal activity, Pharmaceuticals, 10(4),(2017) 1-20.

[12] D.A.C.S. Rezende, R.V. Souza, M.L. Magalhães, A.R.S. Caetano, M.S.S. Carvalho, E.C. de Souza, L.G. de Lima Guimarães, D.L. Nelson, L.R. Batista and M. das Graças Cardoso, Characterization of the biological potential of the essential oils from five species of medicinal plants, American Journal of Plant Sciences, 8(2), (2017) 154-170.

[13] H Yao, X Sun, C He, P Maitra, X.C. Li, L.D. Guo, Phyllosphere epiphytic and endophytic fungal community and network structures difer in a tropical mangrove ecosystem. Microbiome 7(1), (2019), 1-15.

[14] J. I. Pitt and A. D. Hocking, Fungi and Food Spoilage, Springer Dordrecht Heidelberg London New York (2009), 524 pages.

[15] M. Ahmed, M. Hussain, M.K. Dhar and S. Kaul, Isolation of microbial endophytes from some ethnomedicinal plants of Jammu and Kashmir, Scholars Research Library, 2(2), (2012), 215-220.

[16] M. Ismail, S. Abdel-Hafez, N.A Hussein and N.A Abdel-Hameed, Contributions to the genus *Fusarium* in Egypt with dichotomous keys for identification of species, Tmkarpiński Publisher Suchy Las, Poland (2015), 179 pages.

[17] J.I. Pitt, The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces* Academic Press, London, **(1979),** 634 pages.

[18] K. Raper and D. Fennell, The Genus *Aspergillus* The Williams and Wilkins Company Baltimore, (1965), 686 pages.

[19] E.G. Simmons, *Alternaria*: An indentification manual, CBS Fungal Biodiversity Center, Utrercht, (2007).775 pages.

[20] J.F. Leslie and B.A. Summerell, The *Fusarium* laboratory manual, Blackwell publishing, USA, (2006) 388.

[21] K.H. Domsch, W. Gams and T.H. Anderson, Compendium of soil fungi 2nd IHW-verlag Eching, (2007), 672 pages

[22] T.J. White, T. Bruns, S. Lee, and J.W. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to Methods and Applications* (ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White),. Academic Press: San Diego, U.S.A. (1990) pp. 315-322.

[23] X. Huang, A. Madan, CAP: A DNA sequence assembly program, Genome Res. 9, (1999), 868–877.

[24] I. S. Rana, A. S. Rana and R. C. Rajak, Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* L. by extraction, purification and analysis of its main component. Eugenol Brazilian Journal of Microbiology, (42), (2011), 1269-1277.

[25] Z. Machowicz-stefaniak, B. zimowska and E. Zalewska, Fungi colonizing various organs of thyme (*Thymus vulgaris*) L. cultivated in the region of Lublin, Acta Agrobotanica, 55, (2002), 185-197.

[26] E. A. M. Ali, A. A. Ahmed, M. A. Sayed and M. R. Hamed, Antioxidant activity and cytotoxicity of extracts of *Thymus vulgaris* L. and their associated endophytic fungi,
Egyptian Journal of Experimental Biology (Bot.), 14(1) (2018), 107 – 116.

[27], S. K. Deshmukh, , M. J. Kolet , S. A Verekar Distribution of endophytic fungi in lemon grass (Cymbopogon citratus (DC.) Stapf.) Journal of Cell and Tissue Research Vol. 10(2) (2010) 2263-2267.

[28] D. Banerjee, S. Manna, S. Mahapatra and B.R. Pati, Fungal endophytes in three medicinal plants of Lamiaceae, Acta Microbiological et Immunological Hungarica, 56(3), (2009), 243-250.

[29] K. L. Rana, D. Kour, T. Kaur, R. Devi, C. Negi, A. N. Yadav, N. Yadav, K. Singh and A. K. Saxena, Endophytic fungi from medicinal plants: Biodiversity and biotechnological applications, In A. Kumar and E. K. Radhakrishnan Microbial Endophytes: Functional Biology and Applications, (2020), 273-305.

[30] A. S. Yassein, Incidence of fungi contaminating some medicinal plants and their antimicrobial and anticancer properties at Qena Governorate (Egypt), Egyptian Journal of Food Science, 49 (1), (2021), 157-171.

[31] J. Mamangkey, A. Hartanto and C. G. P. Rumahorbo, Biodiversity and biopharmaceuticals of endophytic fungi associated with medicinal plants and therapeutic activity: Case studies of Lamiaceae Crops, Acta Microbiologica Bulgarica 39 (2), (2023), 140-146.

[32] H. F. Al-Harthi, A. M. Elgorgan, B. Ahmed, A. H. Bahkali, M. ElSheshtawi, J. P. Shaik, A. M. Al-Falih and A. Syed, Identification, molecular characterization, and plant growth promoting activities of endophytic fungi of *Jasminum sambac*, *Camellia sinensis*, and *Ocimum basilicum*, Journal of King Saud University Science, 35 (3), (2023), 1-10.

[33] R. S. R. El-Mohamedy, M. M. Abdel-Kader, F. Abd-El-Kareem and N. S. El-Mougy, Essential oils, inorganic acids and potassium salts as control measures

against the growth of tomato root rot pathogens in vitro, Journal of Agricultural Technology, 9(6), (2013), 1507-1520.

[34] A. Puskarova, M. Buckova, L. Krakova, D. Pangallo and K. Kozics, The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells, Scientific Reports 7(1), (2017), 1-11.

[35] Y. Bhalla, V. K. Gupta and V. Jaitak, Anticancer activity of essential oils: A review, Journal of the Science of Food and Agriculture, 93(15), (2013), 3643–3653.

[36] A.M. Elgamal, R.F. Ahmed, A.M. Abd-ElGawad, A.E.N.G. El Gendy, A.I. Elshamy and M.I. Nassar, Chemical profiles, anticancer, and anti-aging activities of essential oils of *Pluchea dioscoridis* (L.) and *Erigeron bonariensis* L. plants, 10(4), (2021), 667-683.

[37] H. Whiley, S. Gaskin, T. Schroder and K. Ross, Antifungal properties of essential oils for improvement of indoor air quality: A review. Reviews on *Environmental Health* , 33(1), (2018), 63–76.

[38] E. D. Zalewska, G. Zawiślak, R. Papliński, M. W. Janusz and R. Gruszecki, Antifungal effects of some essential oils on selected allergenic fungi *in vitro,* Acta Scientiarum Polonarum Hortorum Cultus, 21(6), (2022), 115–127.

[39] A. Tomic, O. Šovljanski, V. Nikolic, L. Pezo, M. Acimovic, M. Cvetkovic, J. Stanojev, N. Kuzmanovic and S. Markov, Screening of antifungal activity of essential oils in controlling biocontamination of historical papers in archives, Antibiotics, 12(103), (2023), 1-16.

[40] M. P. Arraiza, A. González-Coloma, M. F. Andres, M. Berrocal-Lobo, J. A. Domínguez-Núñez, A. C. D. Costa Jr, J. Navarro-Rocha and C. Calderón-Guerrero, Antifungal effect of essential oils, in H. A. El-Shemy, Ed., Potential of Essential Oils. In Tech, (2018), 196 pages.

[41] M. Zhang, J. Ge and X. Yu, Transcriptome analysis reveals the mechanism of Fungicidal of thymol against *Fusarium oxysporum* f. sp. *niveum*, Current Microbiology, 75(4), (2018), 410-419.

[42] T. Gao, H. Zhou, W. Zhou, L. Hu, J. Chen and Z. Shi, The Fungicidal activity of thymol against *Fusarium graminearum* via inducing lipid peroxidation and disrupting ergosterol biosynthesis, Molecules, 21(6), (2016), 770-783.

[43] P.S. Chavan and G. T. Santosh, Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeast, Food Control, 6, (2014), 115-120.

[44] M. S. Omar and S. Kordali, Review of essential oils as antifungal agents for plant fungal diseases, Ziraat Fakültesi Dergisi, 14 (2), (2019), 294-301.

[45] G. Iscan, A. Iscan and F. Demirci, Anticandidal effects of thymoquinone : Mode of action determined by transmission electron microscopy (TEM), Natural Product Communications, 11, (2016), 977– 978.

[46] D. Sivakumar and S. Bautista-Baños, A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Protection, 64, (2014), 27–37.

[47] C.L. Da Cruz, V.F. Pinto and A. Patriarca, Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. International Journal of Food Microbiology, 166, (2013), 1-14.