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Diversity and Density of Endophytic Fungi Isolated from *Calotropis procera* Leaves and their Genotypic Characterization

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Abstract: Endophytic fungi represent a precious source for drug discovery. The present study was designed to explore and identify members of these fungi inhabiting leaves of which is widely spreading in Kingdom Saudi Arabia. Plant leaves from upper, *Calotropis procera* plant middle and lower parts of *C. procera* branches were taken, surface sterilized and cut into small segments (5x5 mm) then cultured on potato dextrose agar and incubated at 25°C for 7-14 days. The mycological analysis of 200 leaf samples revealed the isolation of 150 strains classified into 12 species belonging to 9 genera of endophytic fungi. Taxonomically, the isolated fungi were grouped into two major Divisions; Ascomycota (10 species and 7 genera) and Basidiomycota (two species and two genera). The majority of fungal strains were obtained from leaves at the lower level of branches. The uppermost leaves of *C. procera* were completely free of endophytic fungi. Images illustrating macro and microscopic characteristics of the isolated fungi are included in this study. The identification of species-level was confirmed by sequencing the ITS region of the rRNA gene. *Curvularia* (three species) and *Penicillium* (two species) shared with 37 and 29 CFU matching 24.6% and 19.3% of total fungi respectively. *Cladosporium* was only represented by *C. sphaerospermum* and contributed with 15.3% of the total fungal count. The remaining endophytic fungal genera which comprised *Cochliobolus*, *Periconia*, *Pseudozyma*, *Quambalaria* and *Trichoderma* occurred in low densities ranging from 4.6% - 9.3% of total fungi. Sequencing data of molecularly identified fungal strains were submitted to GenBank and given their respective accession numbers.

Key words: *Calotropis procera* · Endophytic Fungi · Phenotypic Characteristics and Genotypic Identification

INTRODUCTION

Endophytes inhabit the internal plant tissue in a symbiotic association and can spend most of their life cycle within host plants. Approximately one million endophytic species are present in the plant kingdom [1]. The Kingdom of Saudi Arabia possesses a wide range of flora, consisting of manymedicinal herbs, shrubs and trees [2]. A wide variety of endophytes colonize the plant tissues which remain unaffected and functional [3]. In the past two decades, endophytes have been extensively investigated and found producing a wide range of biomolecules, biocatalysts and biological enzyme applied in medicine, agriculture and industry. In addition, many studies have demonstrated that endophytes are helpful in improving plant growth, relieving abiotic stress and protecting from pathogens. The wide presence of an endophyte in plant tissue creates an effective barrier preventing an attack of the pathogens to the host plant. It has been observed that metabolites produced by

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endophytes inhibit the growth of pathogens [3, 4]. Endophytes constitute a major part of the unexplored fungal diversity. Natural products derived particularly from medicinal plants have been exploited for human use for thousands of years to make human life easy. Endophytes are fertile ground for drug discovery. Establishment of fungal repositories from various ecological niches is an important step towards tapping their potential values for novel drug discovery. Fungal Secondary metabolites are defined as low molecular weight compounds not required for growth, but they are produced as an adaptation for specific functions in nature [5]. Many endophytes have the potential to synthesize various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous diseases. Exciting possibilities exist for exploiting endophytic fungi for the production of a plethora of known and novel biologically active secondary metabolites [6].

The Kingdom of Saudi Arabia possesses a varied range of plant, consisting of a large number of medicinal herbs, shrubs and trees [7]. *Calotropis procera* is amedicinal toxic shrub spread widely in different areas of Saudi Arabia. It grows commonly in agricultural areas, around farms and especially in the Tehama plain, it's sandy warm parts [8]. Previous reports mentioned that this plant species has antimicrobial, anticancer, anti-inflammatory, antidiabetic and antioxidant that are used traditionally to treat several diseases [9]. Some researchers found that the medicinal properties of *C. procera* may refer to the presence of the endophytic fungi and their biological activities inside plant tissues [10].

The objective of this work was to investigate the diversity and density of fungal endophyte species in *C. procera* leaves using morphological and molecular techniques.

MATERIALS AND METHODS

Calotropisprocera Leaf Sampling: *Calotropis procera* spread everywhere in Jeddah, Saudi Arabia. This plant was confidently identified by Dr. Faten Filimban, (Assistant Professor of Plant Taxonomy, Faculty of Science and King Abdulaziz University). Leaf samples were selected from a healthy and mature shrub that grows to a height of 3-4 m from October to September 2017 at temperature average of 30°C. Each branch of plant was categorized into different three leaf positions as: lower, middle and upper as shown in Fig. 1. Overall, 200 leaves were sampled and washed with sterile distilled water to remove any associated soil particles. Directly, the leaves were kept in sterile nylon bags with labeling each part individually and transferred to Microbiology Laboratory.

Isolation of Endophytic Fungi: Endophytic fungi were isolated from *C. procera* leaves using a modified method described by Evueh *et al*. [11]. Initially, the surface of fresh leaves were disinfected by immersion in the 70% ethanol for 1 min followed by sodium hypochlorite (5% solution of available chlorine) for 4 mins and then dipping into75% ethanol for 45 secs. Leaf tissues surface were washed thrice with the sterile distilled water to remove all chemicals and air-dried on filter paper (150 mm) for 5 mins under sterilization conditions. Then, 4 drops from these last rinse waters were transferred on to the Petri dishes containing potato dextrose agar (PDA) medium to confirm disinfectant method. Disinfected leaf tissues were cut into small segments of 5×5 mm size and 4 pieces were placed in Petri dishes containing 20 ml of autoclaved potato dextrose agar (PDA) Mediudm supplemented with lactic acid to prevent the growth of endophytic bacteria. The plates were incubated at 28°C for 7-14 days and observed repeatedly for the growth of

Fig. 1: Leaves of *C. procera* categorized into threedifferent leaf positions

endophytic fungi. An inoculum of every new colony was continuously transferred on fresh PDA medium to obtain pure culture.

Diversity and Density Analysis: All funal isolates were phenotypically identified from pure cultures grown on PDA medium. Colony characteristics including growth rate, colony color and texture, reverse pigmentation were considered as important diagnostic criteria for identification. Microscopic appearance of fungal hyphae and spores of the growing fungi was carefully observed in wet mounts prepared on microscopic slides and stained with lactophenol cotton blue (LPCB). All phenotypic structures were approved by comparing them with the characters given by Domsch *et al*. [12] and Watanabe [13].

In density studies, the number of endophytic fungal isolates were calculated as colony forming unit (CFU) of each fungal species as described by Costa *et al*. [14]. In addition, one plug (7mm) of each fungus was transferred into 100 ml loosely capped bottle containing 30 ml of autoclaved potato dextrose broth medium (PDB) and incubated ina shaking incubator at 28°C, 150 rpm to determine biomass dry weight (DW) of all fungal colonies after 7 days as described by Yonni *et al*. [15], Seguin *et al*. [16]. The fungal biomass from the liquid medium was aseptically filtered (pore size, 1.5 µm) through a glass microfiber filter (Ahlstrom Munksjö, Helsinki, Finland). The fungal biomass was washed twice with 10 ml of warm distilled water (60°C). Mycelial mass were kept in an oven at 80°C for 8 hours and cooled at room temperature (25°C) in a desiccator and weighed [7].

Genotypic Identification of Isolated Fungi

DNA Extraction and PCR: The genotypic method described by Al-Samarrai and Schmid [17] was modified and used in fungal identification by polymerase chain reaction (PCR). The fungal isolates were individually inoculated in 100 ml of Erlenmeyer flasks containing 20 ml potatoes dextrose broth (PDB) then incubated at 28°C for 5 days followed by filtration of mycelia using sterilized filter papers. The fungal mycelium was ground to a fine powder by sterilized pestle and mortar using liquid nitrogen. Fine powders were transferred to 1.5 ml Eppendorf tube and stored at -20°C. Thirty mg of frozen ground mycelium were resuspended and lysed in 500 µl of lysis buffer (pH7•8) and incubated in a water bath at 37°C for 60 mins. The lysis buffer was a mixture of 40 mmol/l Tris-acetate, 20 mmol/l sodium acetate, 1mmol/l EDTA and 1% SDS. The DNA was extracted by Gene JET Genomic DNA extraction kit (Thermo Scientific, USA). The PCR technique was performed to amplify internal transcribed spacer (ITS) region of ribosomal DNA (r DNA) using two primers; ITS1 (CTTGGTCATTTAGGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) in a thermal cycler (Esco health care, Swift max pro, Malaysia). The reaction mixture (50 µl) included 3 µl of template DNA, 5 µl each of the primers, 25 µl of green PCR mix (Promega, Go Taq ® Green Master Mix, USA) and 50 µl of nuclease-free water. The cycling conditions were as follows: an initial denaturation of 2 min at 94°C, followed by 39 cycles at 94°C for 20 s, at 40-60°C for 10 s, at 72°C for 20 s and a final extension cycle at 72°C for 5 min. The negative control was prepared with the reaction mixtures in the absence of DNA extract [7].

DNA Visualization: The PCR products were tested onto 1.5% agarose gel and running for 45 min at 130 volts Electrophoretic gel (Horizontal gel electrophoresis, Cleaver Scientific, UK). The gel was stained using ethidium bromide and visualized under UV light (gel doc imager, Biorad, USA). The DNA marker (100 bp DNA Ladder, Invitrogen, USA) was used to quantify and identify PCR products. Then the samples were sent to Macrogen Company, South Korea for purification and sequencing.

DNA Sequencing: PCR product from each fungal strain was sequenced in the sense and antisense strands using the Big Dye terminator cycle sequencing kit (Applied Biosystems, U.K). Sequence identities were characterized using BLAST Genbank general database from National Center for Biotechnology Information (NCBI) and were used to construct neighbor-joining tree using Jukes-Cantor model as described by Najjar *et al*. [18]. The sequencing data were submitted to GenBank and the obtained accession number was recorded for each fungal strain.

RESULTS

The mycological analysis of 200 leaves from *C. procera* revealed the isolation of 12 endophytic fungal species belonging to 9 genera which were identified as *Curvularia*, *Cladosporium*, *Cochliobolus*, *Penicillium*, *Periconia*, *Pseudozyma*, *Quambalaria*, *Trichoderma* and *Wickerhamomyces* (Table 1). A total of 150 CFU of endophytic fungi were distributed at lower (87 CFU) and middle (63 CFU) parts but no fungi were detected in upper leaves. The greatest density of fungal endophytic strains was that of *Cladosporium sphaerospermum*

Table 1: Diversity and counts (CFU) and dry weight (DW) of endophytic fungi isolated from *C. procera* leaves at three different positions of the plant branches

	Isolate					
No.	Leaf positions Fungal species	Lower	Middle	Upper	Total count (CFU)	DW(mg)
DA1	*Curvulariahawaiiensis*Manamgoda, Cai and Hyde	9	7	0	16	140
DA2	*Curvularialunata*(Wakker) Boedijn	8	6	0	14	90
DA3	*Curvulariapapendorfii*Aa	4	3	0	7	90
DA4	*Cladosporiumsphaerospermum*Penz.	13	10	0	23	90
DA5	*Cochliobolus*sp.	9	5	0	14	30
DA6	*Penicilliumchrysogenum*Thom	10	8	0	18	100
DA7	*Penicilliumglabrum*(Wehmer) Westling	6	5	0	11	120
DA8	*Periconiamacrospinosa*Lefebvre &Alb.G.Johnson	5	2	0	7	110
DA9	*Pseudozymaaphidis*(Henninger&Windisch) Boekhout	4	5	0	9	60
DA10	*Quambalariacynescens*(de Hoog& de Vries) de Beer, Begerow& Bauer	9	5	0	14	130
DA11	*Trichodermaharzianum*Rifai	5	4	0	9	70
DA12	*Wickerhamomycesanomalus*(E.C. Hansen) Kurtzman, Robnett&Basehoar-Powers	5	3	0	8	120
	Total	-	-	-	150	-

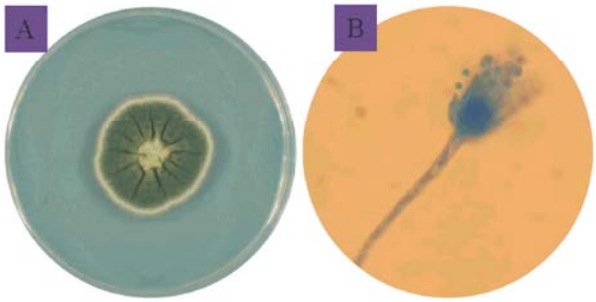
Table 2: Classification of endophytic fungal strains isolated from *C.procera* based on NCBI website.

Genera and species	Family	Order	Class	Phylum
Curvulariahawaiiensis	Pleosporaceae	Pleosporales	Dothideomycetes	*Ascomycota*
Curvularialunata	Pleosporaceae	Pleosporales	Dothideomycetes	*Ascomycota*
Curvulariapapendorfii	Pleosporaceae	Pleosporales	Dothideomycetes	*Ascomycota*
Cladosporiumsphaerospermum	Cladosporiaceae	Capnodiales	Dothideomycetes	*Ascomycota*
Cochliobolus sp.	Pleosporaceae	Pleosporales	Dothideomycetes	*Ascomycota*
Penicilliumchrysogenum	Trichocomaceae	Eurotiales	Eurotiomycetes	*Ascomycota*
Penicilliumglabrum	Trichocomaceae	Eurotiales	Euascomycetes	*Ascomycota*
Pseudozymaaphidis	Ustilaginaceae	Ustilaginales	Ustilaginomycetes	*Basidiomycota*
Periconiamacrospinosa	Pleosporaceae	Pleosporales	Dothideomycetes	*Ascomycota*
Quambalariacyanescens	Quambalariaceae	Microstromatales	Exobasidiomycetes	*Basidiomycota*
Trichodermaharzianum	Hypocreaceae	Hypocreales	Sordariomycetes	*Ascomycota*
Wickerhamomycesanomalus	Wickerhamomycetaceae	Saccharomycetales	Saccharomycetes	*Ascomycota*

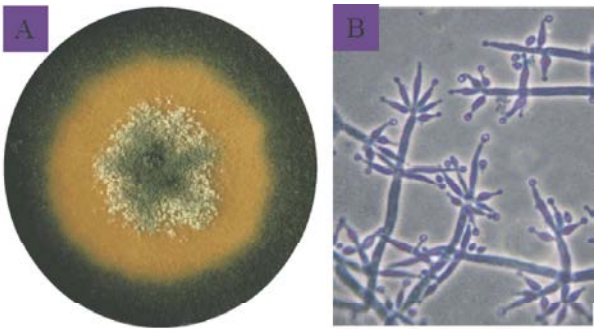
DA4 (23 CFU) and the rarest were indicted by *Curvularia papendorfii* DA3 and *Periconia macrospinosa* DA8 (7 CFU for each). Marked variations were observed in fungal biomass where the dry weight fluctuated between 90 to 140 mg after 7 days of incubation at 28°C.

As follow: The highest weight was obtained from *Curvularia hawaiiensis* (140 mg) followed by *Quambalaria cyanescens* (130), *Penicillium glabrum*, *Wickerhamomyces anomalus* (120 each), *Periconia macrospinosa* (110) and *Penicillium chrysogenum* (100). Each of *Cladosporium sphaerosermum*, *Curvularia lunata* and *Curvularia papendorfii* produced 90 mg of dry mycelium. The remaining fungal species yielded lower amounts of biomass (30 -70 mg) with the least weight detected in broth culture of *Cochliobolus* sp. as shown in Table (1). The majority of endophytic fungal species isolated in the current study belong to Division Ascomycota (10 species out of 12). On the other hand only two species namely *Pseudozyma aphidis* and *Quambalaria cyanescens*are attributed to Division Basidiomycota. The different families, Orders and Classes into which these fungi were classified based on NCBI website are shown in (Table 2). Macro- and microscopic images with brief descriptions are also provided in Fig. 2-5.

The PCR amplification of DNA extracted from fungal isolates was done with one universal fungal primer pair. The intense bands on agarose gel appeared as PCR products in expected size in the range from 523 to 761 bp for the 12 isolates. The PCR profiles of pair ITS1/4 regions amplified from different isolates are shown in Fig. 6. *Curvularia hawaiiensis* recorded a band size of 539 bp, *C. lunata*recorded 546 bp and *C. papendorfii* recorded 596 bp.*Cladosporium sphaerospermum* showed 523 bp of the molecular size of the ITS region. The *Cochliobolus* sp. recorded 565 bp, *P. chrysogenum* showed a band size of 552 bp. *P. glabrum*showed 540 bp of the molecular size, while that of Pseudozyma aphidis was 761 bp. *Periconia macrospinosa* showed a band size of 544 bp. Molecular size of the ITS region of Q. cyanescens 623 bp. while that of *T. harzianum* was 589 bp. Wickerhamomyces anomalus showed a band size near to 583 bp.

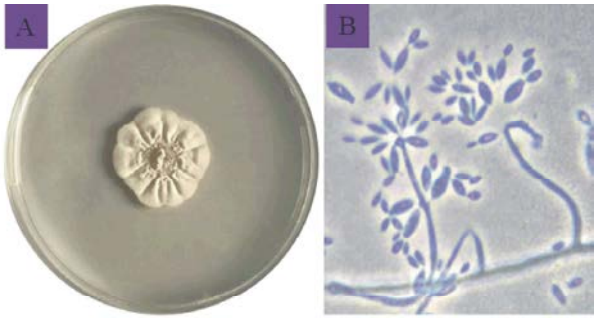


***Penicillium glabrum*:** Blue green colony on CYA after 7 days incubation at 28°C (A) and microscopic appearance Showing unbranched conidiophore, phialides and conidial chains (B)

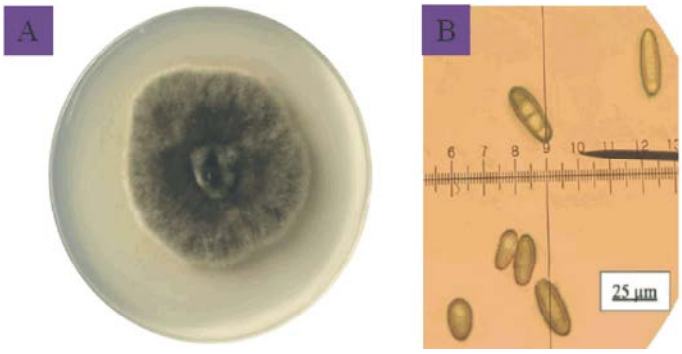


***Trichoderma harzianum*:** Greenish effuse colony on CYA after 7 days incubation at 28°C (A) and microscopic appearance Showing branched hyphae terminating in phialid and conidia (B)

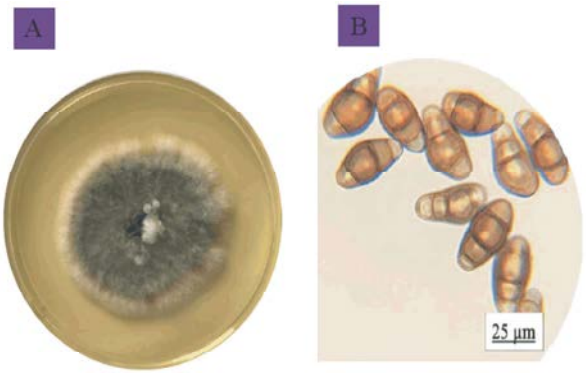
Fig. 2: Colonies and microscopic appearance of *Penicillium* and *Trichoderma* species



***Quambalaria cyaneescens*:** Bluish colony on CYA after 7 days incubation at 28C (A) and microscopic appearance Showing branched hyphae terminating in conidial clusters (B)

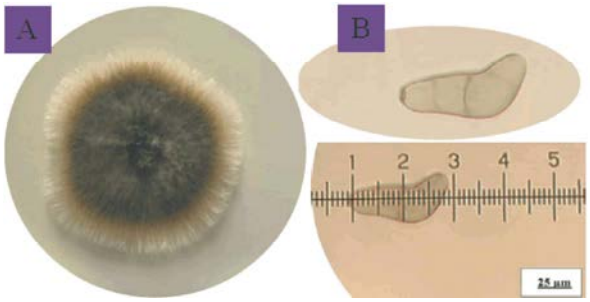


***Curvularia hawaiiensis*:** Dark effuse colony on CYA after 7 days incubation at 28°C (A) and microscopic appearance Showing conidiophores and dark septate conidia (B)

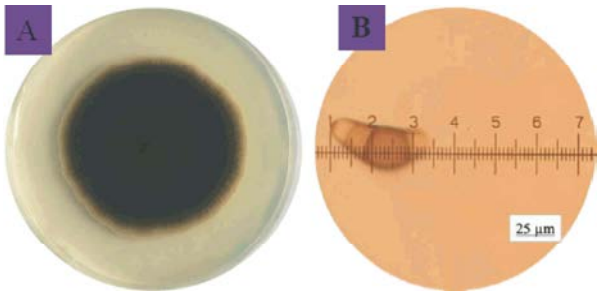


***Curvularia lunata*:** Dark effuse colony on CYA after 7 days incubation at 28°C (A) and microscopic appearance Showing dark brown, curved 3 septate conidia (B)

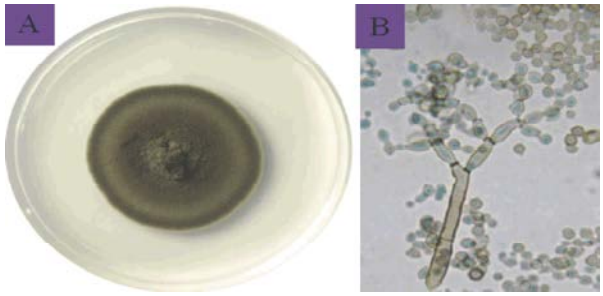
Fig. 3: Colonies and microscopic appearance of *Quambalaria* and *Curvularia* species



Curvularia papendorfii: Dark effuse colony on CYA after 7 days incubation at 28C (A) and microscopic appearance showing conidiophores and dark, curved 3 septate conidia (B).

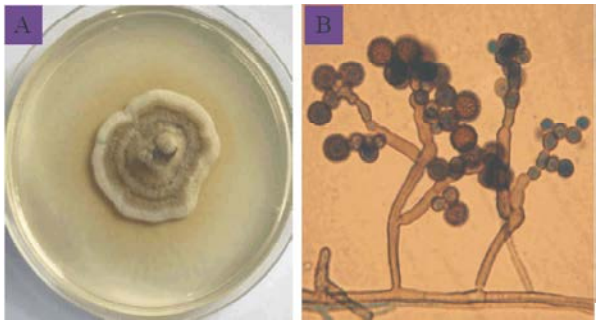


Cochliobolus sp. Dark effuse colony on CYA after 7 days incubation at 28C (A) and microscopic appearance Showing conidiophores and dark, curved 3 septate conidia (B).

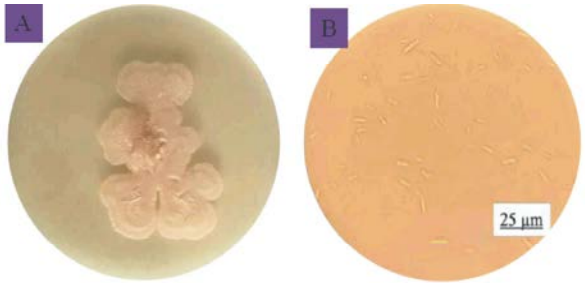


Cladosporium sphaerospermum: Dark cored compact colony on CYA after 7 days incubation at 28ºC (A) and microscopic appearance Showing conidiophores and dark, curved 3 septate conidia (B).

Fig. 4: Colonies and microscopic appearance of *Curvularia, Cochliobolus* and *Cladosporium* species



***Periconia macrospinosa*:** Dark colored colony on CYA after 7 days incubation at 28ºC (A). Pigmented conidiophores bearing dark, spherical and spiny conidia (B)



***Pseudozyma aphidis*:** (= Moesziomyces aphidis): Hyaline soft colony on CYA after 7 days incubation at 28ºC (A). elongated spindle-shaped blastoconidia (B).

Fig. 5: Colonies and microscopic appearance of *Periconia* and *Pseudozyma* species

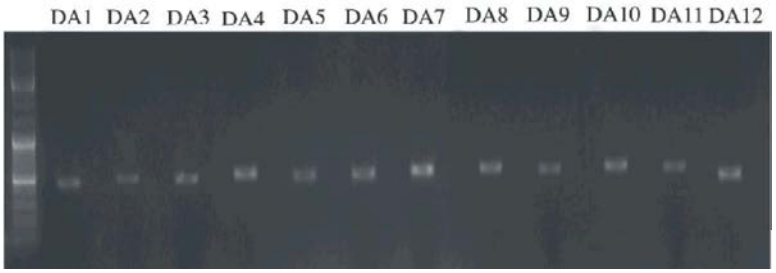


Fig. 6: Gel electrophoresis of PCR product from 12 fungal strains; DA1:*C. hawaiiensis,* DA2:*C. lunata,* DA3:*C. papendorfii,* DA4:*C. sphaerospermum,* DA5: *Cochliobolus*sp., DA6: *P. chrysogenum,* DA7: *P. glabrum,* DA8: *P. macrospinosa,* DA9: *P.aphidis,* DA10: *Q. cyanescens,* DA11: *T. harzianum,* DA12: *W. anomalus*. All data shown were obtained with ITS1/4 primer. The range of DNA molecular size was from 532 to 761 bp compared to M (100 bp marker on the left side of image).

Sequencing data of fungal strains were aligned with the available sequences of closely related strains accessed from the GenBank. Sequences were also submitted to GenBank and given accession numbers as shown in Table (3). Molecular identification of strains to species level was based on 97-100% similarity with sequences of the known species already published in NCBI databases. Sequences of type strains (type material) are preferred for comparison. Based on these criteria it was possible to confidently identify 11 out of 12 strains to species level. Only identification of a strain of *Cochliobolus* was not confirmed. A dendrogram showing phylogenetic analysis of the sequenced fungal strains was shown in Fig. 7. Phylogenetic trees based on ITS sequences of rDNA of the fungal strains isolated in the present study aligned with closely related sequences accessed from the GenBank are illustrated in Fig. 8, 9 and 10.

Table 3: Molecular identification of fungal strains recovered in the present study, their GenBank accession No. and % identity with closely related strains

Fungal strains isolated in the current study			Closely related strains accessed from GenBank				
Fungal species	Strain No.	Accession No.	Fungal species	Strain No.	Accession No.	Coverage (%)	Identity (%)
Curvulariahawaiiensis	DA1	MN398951	*Curvulariahawaiiensis*	B2	KF725777	97	99.74
Curvularialunata	DA2	MN399679	*Curvularialunata*	B2836	MK204512	100	99.34
Curvulariapapendorfii	DA3	MN399677	*Curvulariapapendorfii*	1161	KU128521	99	99.11
Cladosporiumsphaerospermum	DA4	MN399676	*Cladosporiumsphaerospermum*	B6	MK356737	100	99.10
Penicilliumchrysogenum	DA5	MN399379	*Penicilliumchrysogenum*	07SK001	KF938385	99	99.75
*Cochliobolus*species	DA6	MN396743	*Curvularialunata*	-------	MG642982	95	100
Penicilliumglabrum	DA7	MN396740	*Penicilliumglabrum*	136	KU847873	99	99.45
Pseudozymaaphidis	DA8	MN396741	*Pseudozymaaphidis*	DSM101929	KX067825	86	99.10
Periconiamacrospinosa	DA9	MN399378	*Periconiamacrospinosa*	SACCR 110759	JX427048	95	100
Quambalariacyanescens	DA10	MN396738	*Quambalariacyanescens*	CBS 358.73	MH860700	98	99.45
Trichodermaharzianum	DA11	MN396739	*Trichodermaharzianum*	XZ N66-1	MF108874	81	99.67
Wickerhamomycesanomalus	DA12	MN399337	*Wickerhamomycesanomalus*	MY4	KY587123	98	99.18

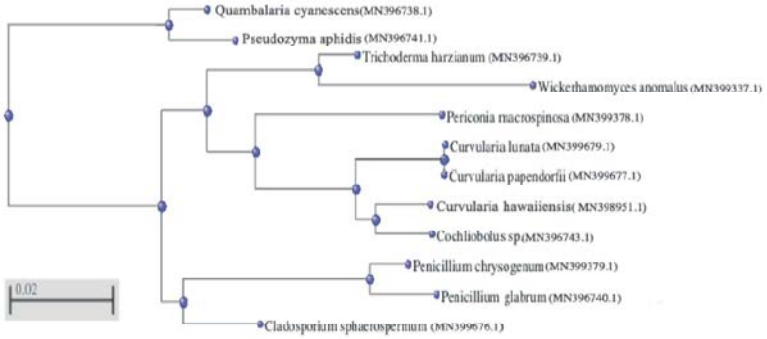


Fig. 7: Dendrogram showing phylogenetic analysis based on the ITS region and NCBI GenBank database for 12 fungal species.

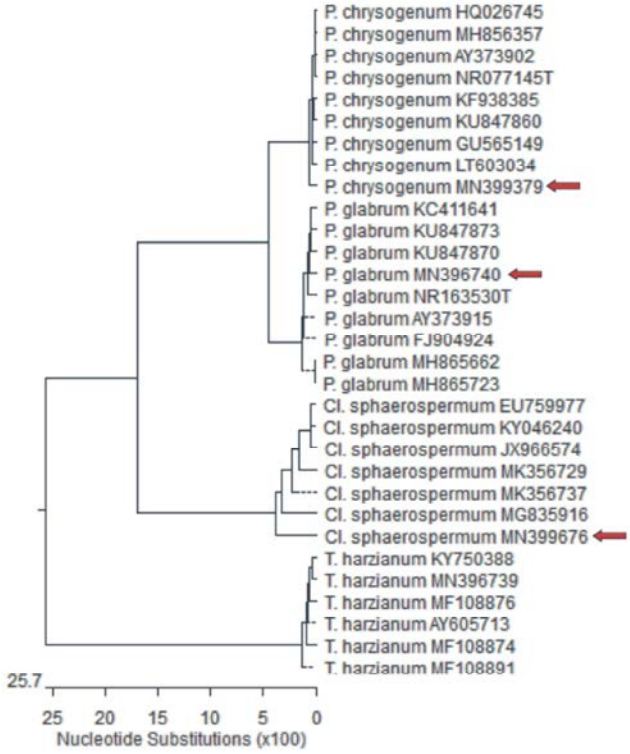


Fig. 8: Phylogenetic tree based on ITS sequences of rDNA of the fungal strains isolated in the present study (arrowed)aligned with closely related sequences accessed from the Gen Bank (*P.* = *Penicillium*, *Cl.* = *Cladosporium* and *T.* = *Trichoderma*)

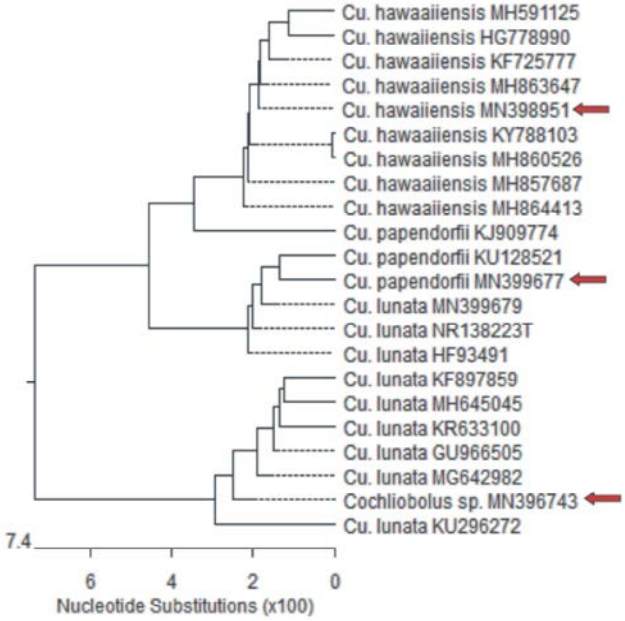


Fig. 9: Phylogenetic tree based on ITS sequences of rDNA of the fungal strains isolated in the present study (arrowed) aligned with closely related sequences accessed from the Gen Bank (*Cu*. = *Curvularia*).

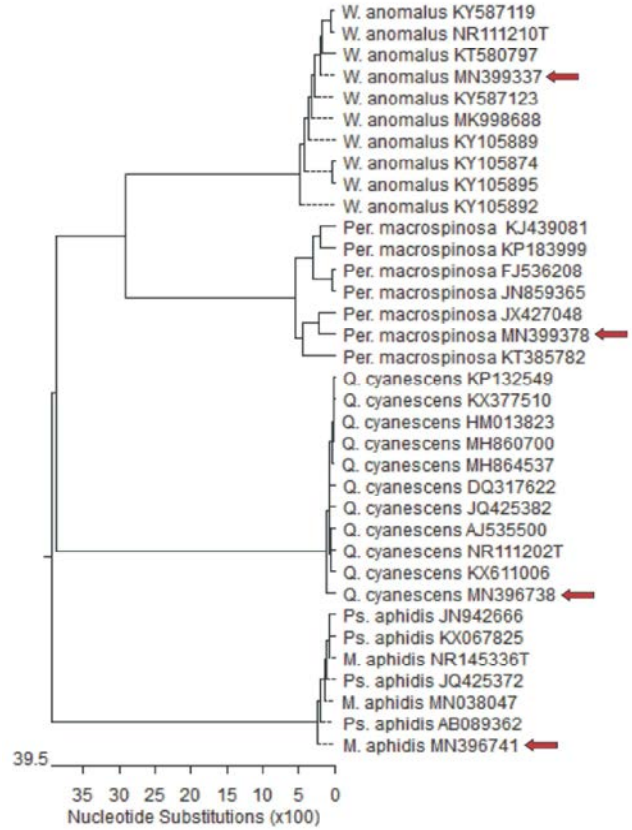


Fig. 10: Phylogenetic tree based on ITS sequences of rDNA of 4 fungal strains isolated in the present study (arrowed) aligned with closely related sequences accessed from the Gen Bank (*W*. = *Wickerhamomyces*, *Per*. =*Periconia, Q*. = *Quambalaria* and *Ps*. = *Pseudozyma* with its synonym *M*. =*Moesziomycesaphidis*).

DISCUSSION

Endophytic microorganisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivores. Endophytic fungi represent a diverse and unexplored group of microorganisms that make symbiotic associations with higher life forms and can produce beneficial substances for the host. The numbers and types of these differ in their hosts depending on geographical position and plant species [2, 10].

Calotropis procera is known as a toxic medicinal plant and the different tissues are reported to have anti-inflammatory, antioxidant and anticancer activities [19]. The medicinal properties of the plant could be attributed to their endophytic fungi. Therefore, the present work was initiated to find out the diversity and density of these endophyticfungi in the dry desert area of Jeddah city, Saudi Arabia. In the present study 9 genera and 12 fungal species were identified the 200 samples of *C. procera* leaves. Most of the isolated fungi have been reported as endophytes from various plant including *C. procera, Cynodon dactylon, Dactyloctenium aegyptium Cannabis sativa* [20, 21].

Additional reports on endophytic fungi were given by Mandyam and Jumpponen [22], Padhi and Tayung [23], Abdel-Hafez *et al*. [24]. In Egypt, Mohamed *et al*. [25] surveyed theendophytic fungi of leaves and latex sample from *C. procera.* Their fungal list included Penicillium chrysogenum and *Trichoderma harzianum.* The authors demonstrated the capability of *P. chrysogenum* to degrade the latex of *C. procera*.

Comparing the density of endophytic fungi recovered in this study(150 CFU) with those reported by previous investigators showed that these counts are relatively lower than those given by Gherbawy and Gashgari [2] who obtained 161 CFU from 150 leaf samples of *C. procera*. In another study by Gherbawy and Elhariry [26] on endophytic fungi from100 samples of twigs from *Juniperus* trees the fungal density was 144 CFU. It is difficult to compare the population size of fungal isolates in different studies due to the variation in experimental design, season of study and type of tested plants.

In the present study, *C. sphaerospermum* had the greatest density than any other endophytic fungus. Generally, it has been published that this fungus exhibited good anti-fungal activities [27] and can be used as ecofriendly alternative in green biological route for extracellular biosynthesis of silver nanoparticles [24] andas effective organism in bioremediation and biodegradation of polycyclic aromatic hydrocarbons in contaminated soil [28]. On the other hand, this species is involved in spoilage of fruits and vegetables and was infrequently reported to affect human health [29]. *Cladosporium sphaerospermum* has been reported from some medical cases such as allergy, brain, eye and skin infections but no myotoxicity diagnosis [30].

The densities of *Curvularia papendorfii* and *Periconia macrospinosa* were generally low in leaves of *C. procera*. *C. papendorfii* (also reported as *Bipolaris papendorfii*) has received little attention, compared with other virulent species that are pathogenic to food crops and humans. It is a rare opportunistic pathogen that has been associated with few cases of keratitis. Like other plant pathogenic *Bipolaris* species, it causes corn leaf spot, but the underlying pathogenic mechanism is totally unclear. More interestingly, a recent report described two bio-active compounds, hamigerone and radicicol, from *C. papendorfii* that exhibited anti-proliferative activity in various cancer cells [31]. *Periconia macrospinosa* is known to produce chlorine containing compounds that have antibiotic like properties and thus helps native grasses in tall grass prairie against biotic stress of herbivores [22].

Periconia macrospinosa was described as endophytic fungus found in association with prairie native grasses of eastern Kansas in the United States [22]. It was considered as one of the main species present in *Anacardium othonianum* plant [32]. To the best of our knowledge, *P.macrospinosa* has not been previously isolated from *C. procera.*

In this research, the fungal biomass was significantly different among the different fungal strains. Such information would be helpful for adjustment of biotechnological processes [33].

Curvularia hawaiiensis recorded a value of band size 539 bp, similar to length other study 543 bp and the *C. lunata* record relative number with 546 bp, the length of the sequences similar to other study which was 555 bp [34].

Curvularia papendorfii record relative number with 596 bp closest to 500 bp [31]. *Cladosporiumsphaerospermum* 523 bp of the molecular size of the ITS region, recorded relative number with 500 bp [35]. *Cochliobolus* sp. recorded 565 bp which means lower molecular size compared to a size of 600-bp

recorded in the study of Berbee *et al*. [36]. The nucleotide sequence of the ITS region of *P. chrysogenum* showed 552 bp which is also lower than that (600 bp) obtained by Avilés-Robles *et al*. [37]. On the other hand, the molecular size of the ITS region of the rDNA of *P. glabrum* showed 540 bp recording a larger molecular size than 460 bp as given by Abastabar *et al*. [38]. Amplicons of *P. aphidis*(761bp) were almost consistent with the report of Liu *et al*. [39] who obtained 784 bp for this fungus. The molecular size of the ITS region of *Q. cyanescens* 623 bp is relatively close to 617 bp as reported by Kuan*et al*. [40]. The band size of *T. harzianum* (589 bp) was almost similar to that found in other studies (600 bp) as recorded by Chakraborty *et al*. [41]. The *W. anomalus* showed a band size of 583 bp that is much higher than that (356 bp) reported by Ricci *et al*. [42]. In case of *P. macrospinosa* the size of 544 bp for ITS region has not been demonstrated in previous studies.

As mentioned by Liu *et al.* [39], *Pseudozyma aphidis* is an epiphytic fungus that widely distributes on the plant surface. Due to technical limitation, the taxonomy of *P. aphidis* was controversial for a long time. It was initially described as a new species in genus *Sterigmatomyces and* named as *Sterigmatomyces aphidis*. Later, it was redefined as anamorphic basidiomycetous yeast which belonged to the order Ustilaginales and reclassified into the genus *Pseudozyma based* on morphological, physiological, biochemical and molecular data. It was named as *P. aphidis* according to the discovery history which was firstly isolated from aphid secretions. Recently, a report has shown that a biologically active strain of *P. aphidis* could reduce the severity of powdery mildew caused by *Podosphaera xanthii* on cucumber plants [43]. This strain of *P. aphidis* may inhibit conidial germination of the pathogen by bioactive compounds and also act as an ectoparasite on *P. xanthii*. It is worthy to mention that Hasnain *et al*. [44] recorded some unidentified species of *Bipolaris*, *Cladosporium*, *Curvularia* and *Periconia* as outdoor aeroallergens in Riyadh, Saudi Arabia.

CONCLUSION

The isolation of endophytic fungi from *C. procera* distributedin the desert of Saudi Arabia can be helpful to explore native beneficial microorganisms. This acquaintance may improve our understanding of their dispersal and interaction inside plant tissues as well as their practicable in a range of medicinal biotechnological processes.

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