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POTTED ORNAMENTAL *Chamaedorea seifrizii, Chamaedorea cataractarum* AND *Rhapis excels* PALM SPECIES: HOSTS FOR THE OPPORTUNISTIC FUNGAL PATHOGEN

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While detecting the fungi associated with indoor and outdoor cultivated potted ornamental palm species the samples comprising of roots and soil were found to be infested with *Acremonium sclerotigenum* (other than the fungal plant pathogens), which is reported fungal opportunistic human pathogen. For further investigation regarding the role of potted indoor and outdoor ornamental plant species in harboring and disseminating human fungal pathogens a total 190 soils and roots samples of three ornamental potted palms species including Bamboo palm (*Chamaedorea seifrizii*), Cat palm (*Chamaedorea cataractarum)* and Lady palm (*Rhapis excels*) were investigated. *A. sclerotigenum* was identified based on morphocultural and molecular characterization. The Sequence (GenBank accession# MF716849) showed 100% homology to the TYPE material of *A. sclerotigenum* (CBS 124.42), which then validated by resolving its phylogenetic hierarchy through genomic and bioinformatics analyses. The culture was deposited to FMB-CC-UAF with culture collection number FMB 0019. Moreover, phylogenetic tree also supported this investigated isolate as *A. sclerotigenum.* The Frequency percentage of *A. Sclerotigenum* association with the decaying root samples of above mentioned three palm species was found 12.27, 11.47, 10.53% from the indoor potted palms; 08, 7.7, 7.4% from the outdoor potted palms respectively, while 7.87, 7.53, 6.00% from the soils of indoor potted palms and 4.5, 4.2, 3.2% from the soils of outdoor potted palms respectively. The results of this study, in relation with the already reported cases in harboring and dispersing the fungal opportunistic pathogens causing mycosis indicated that this type of plantation without any proper antifungal treatment may constitute a serious mycotic hazards to human beings and animals. To our knowledge this is the first report from Pakistan showing that *C. seifrizii*, *C. cataractarum* and *R. excels* are three new host of *A. Sclerotigenum.*

Keywords: Acremonium infections, human pathogen, immunocompromised individuals, Palmae, host plants.

INTRODUCTION

Acremonium is a polyphyletic genus having nearly 150 species (Summerbell *et al*., 2011) and is one of the established fungal opportunistic pathogens plays an important role in humans and animals infectious diseases. For the last few years the importance of opportunistic fungal infections in immunocompromised patients has been increased manifolds and resulted investigations regarding harboring and spread of fungal environmental contamination and mycosis (Miceli and Lee, 2011). Acremonium can be commonly found in the soil, decayed plant parts and may cause disease in animals as well as in plants (Gams, 1973; Simpson et al., 1993). Mycetoma, Onychomycosis and Keratitis are the most common examples of clinical Acremonium infections presentations in humans (McCormack *et al*., 1987, Guarro *et al*., 1997; Grunwald *et al*., 1998).

Palms are members of Palmae family which includes six sub families, 200 genera with 2700 species (Dransfield *et al*., 2008). The member of this family have gained popularity for their high aesthetic values, other than the provision of edible products. It is an established fact that ornamental palms cultivation is increasing day by day across the world and the play vital role in atmospheric purification, improving environmental health controlling air pollution, providing food, contributing in aesthetic values and many other benefits (El-Juhany, 2010). The members of Palms are grown for both indoor and outdoor landscape. Among major cultivation and growth constraints of palms the fungal pathogens are most destructive causing heavy losses both in terms of economical and aesthetic value (Elliott *et al*., 2004; Flood, 2006; Farr and Rossman, 2014). A team comprising of plant pathologists while working on detection of fungi associated with indoor and outdoor palm plantations came across with one of the unique opportunistic fungi i.e. *A. sclerotigenum* along with the range of plant pathogenic and saprophytic fungi. *A. sclerotigenum* was found to be associated with the potted soils and rotted roots of three ornamental palm species including Bamboo palm (*C. seifrizii*), Cat palm (*C. cataractarum)* and Lady palm (*R. excels*). In this study sampling (soil and roots) was done from three palm species grown in pots both indoor and outdoor. The objective of this study was to determine the

role of potting soils and decayed roots of ornamental plants in harboring and the release of opportunistic fungal propagule into ambient air.

MATERIALS AND METHODS

***Sampling*:** The ornamental palms *C. seifrizii*, *C. cataractarum and R. excels* cultivated indoor building landscape of 15 different locations as well as outdoor ornamental commercial nurseries of 10 different locations were selected for sampling. A total of 190 soils and roots samples from the potted plants of three above mentioned palm species were collected by the research team of plant pathologists of FMB Lab., Department of Plant Pathology UAF, Pakistan working on the Higher Education Commissions funded project "Etiology and integrated management of perennial declining ornamental plants in Pakistan".

***Isolation and identification*:** The roots samples were rinsed under tap water, cut into smaller pieces (3-4 mm), superficially sterilized in 2% sodium hypochlorite solution for 30 seconds, washed in sterile water and excessive moisture was removed. Samples were processed on Potato Dextrose Agar (PDA) medium for the isolation of fungal isolate(s). Soil samples were prepared by serial dilution technique. Suspensions were plated out onto Agar medium. Plates were incubated at 25-28°C. After 22-24 hours of incubation; fungal colonies appeared on inoculated plates were picked from the marginal tips of growing hyphae, sub cultured and identified microscopically. Physiological study of the isolates was done observing macroscopic characteristics including colony colour and growth pattern on culture media. Microscopic observations including type of mycelium and spore(s) produced, spore shape and colour were recorded at 10X, 20X and 40X.

***Molecular characterization*:** For molecular characterization, DNA of investigated fungal isolate was extracted from fresh mycelia (Prabha *et al*., 2013) and quantified by NanoDrop™ 8000 Spectrophotometer (Thermo Fisher). PCR analysis was performed on 96 well Veriti thermo cycler (Applied Biosystem) with working DNA dilution, 50ng/µl using ITS based primer pairs. PCR amplified product was purified with FavorPrep PCR Clean-up Kit and sequenced by Eurofins Genomics services USA. Generated sequence was trimmed using BioEdit software. Trimmed and high quality sequence was analysed with BLAST (Basic Local Alignment Search Tool) for finding its homology with available sequences at NCBI database. Generated sequence was submitted to NCBI database. Phylogenetic tree was constructed. The best evolutionary model was selected based on Bayesian Information Criterion (BIC) using MEGA 7.0 software and model with lowest BIC value was selected to construct a maximum likelihood phylogenetic tree. Sequences were aligned with MSA (Multiple sequence Alignment) program, Clustal W. A phylogenetic tree of the 20 aligned sequences was constructed on best evolutionary model with heuristic search in bootstrap mode and 1000 replications by applying the Neighbor-Joining scheme to a matrix of pairwise distances valued through Maximum Composite Likelihood (MCL) method using the MEGA7 (Molecular Evolutionary Genetics Analysis version 7.0) software.

RESULTS

***Morphology*:** The measurements of relevant morphological characters (mentioned earlier) were made from 30 randomly selected conidia from suspension of fungal isolate prepared in sterile distilled water using compound microscope Meji Techno, Japan model HD1600T fitted with Olympus (DP25) digital camera. Whitish, cottony colony was observed on PDA at 25°C with 13–42 µm long Phialides. Single celled hyaline conidia were produced with 3.5–5.6 × 0.6–1.9 µm size. Conidia were cylindrical in shape having smooth walls. Morphologically the isolate was identified *A. sclerotigenum*.

***Molecular characterization*:** Molecular characterization of studied fungal isolate was based on the direct sequencing of ITS region. The generated sequence was subjected to BLASTn tool to find its homology with sequences of the type materials available in NCBI database. The sequence similarity using BLASTn showed significant similarity (100%) with strain of *Acremonium sclerotigenum* (CBS 124.42). Then evolutionary history was deduced by Maximum Likelihood method based on Kimura 2-parameter model with Gamma distribution (K2+G) to resolve its phylogenetic location. The tree with the highest log likelihood is shown in (Fig. 1).

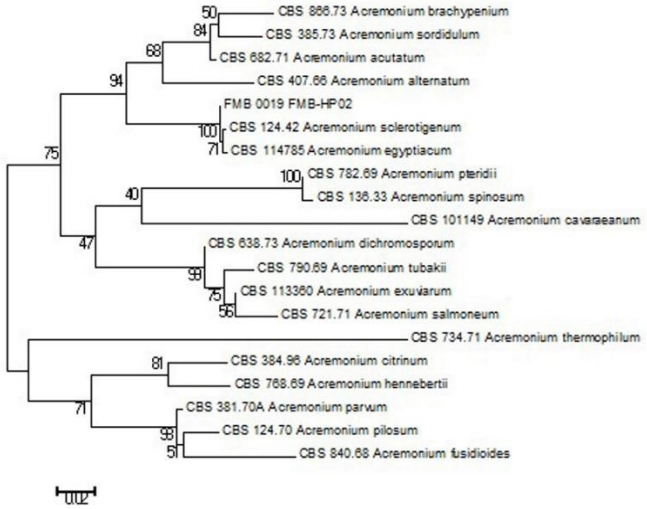

Figure 1. A phylogenetic tree: This phylogram was constructed using Maximum likelihood approach based on best evolutionary model K2+G (Kimura 2-parameter model with Gamma distribution)

A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3459). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 371 positions in the final dataset. In phylogenetic tree, investigated fungal isolate (FMB HP-02) has confirmed and validated its evolutionary hierarchy by placing with strain of *Acremonium sclerotigenum*, CBS 124.42 with 100% bootstrap value. Then its nucleotide sequence was submitted to GenBank with accession# MF716849. However, Culture was deposited to FMB-CC-UAF with culture collection number FMB 0019. Based on morphology, genomics and phylogenetic characterization, the fungal isolate found to be associated with *C. seifrizii, C. cataractarum and R. excels,* was identified as *Acremonium sclerotigenum.* Hence, this study reported these ornamental palm species as new hosts of *A. sclerotigenum*. As the inference of this study, which is to the best of our knowledge, *C. seifrizii, C. cataractarum and R. excels* are three new hosts record of *A. sclerotigenum* from Pakistan

***Frequency of A. sclerotigenum isolated from roots and soil samples*:** Frequency percentage of *A. sclerotigenum* from root samples from indoor and outdoor potted (*C. seifrizii*, *C. cataractarum* and *R. excels*) was recorded. Maximum percent frequency was 13 from indoor *C. seifrizii* at growing location five and minimum percentage frequency was 3 from growing location one. Maximum percentage frequency was 15 from indoor *C. cataractarum* at growing location seven and minimum percentage frequency was 3 from growing location two.

Maximum percentage was 12 from indoor *R. excels* at growing location four and minimum percentage frequency was 3 from growing location two. Maximum percentage frequency was 20 from outdoor *C. seifrizii* at growing location eight and minimum percentage frequency was 4 from growing location seven. Maximum percentage frequency was 20 from outdoor *C. cataractarum* at growing location four and minimum percentage frequency was 3 from growing location six. Maximum percentage was 20 from outdoor *R. excels* at growing location twelve and minimum percentage frequency was 3 from growing location five. (Fig. 2).

Frequency percentage of *A. sclerotigenum* for soil samples taken from plants grown indoor and outdoor potted *C. seifrizii, C. cataractarum* and *R. excels* was also recorded. Maximum percentage frequency was 8 from indoor *C. seifrizii* at growing location five and minimum percentage frequency was 2 from growing location one and nine. Maximum percentage frequency was 8 from indoor *C. cataractarum* at growing location four and seven and minimum percentage frequency was 2 from growing location ten. Maximum percentage was 6 from indoor *R. excels* growing location seven and minimum percentage frequency was 2 from growing location two, six and ten. Maximum percentage frequency was 16 from outdoor *C. seifrizii* at growing location eight and minimum percentage frequency was 2 from growing location seven. Maximum percentage frequency was 11 from outdoor *C. cataractarum* growing location eight and minimum percentage frequency was 2 from growing location six. Maximum percentage was 14 from outdoor *R. excels* growing at location four and minimum percentage frequency was 2 from growing location five. (Fig. 3).

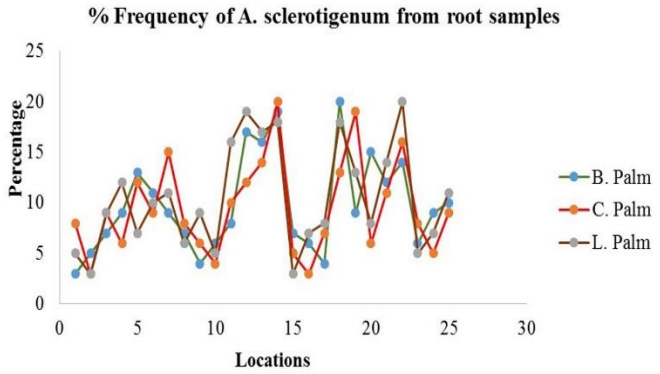

Figure 2. Percent frequency of *A. sclerotigenum* recorded from root samples of potted Bamboo palm (*Chamaedorea seifrizii*), Cat palm (*Chamaedorea cataractarum),* Lady Palm (*Rhapis excels*), grown indoor landscape buildings and outdoor commercial ornamental palm nurseries. On horizontal axis 1-10 locations of outdoor commercial ornamental palm nurseries and 11-25 locations of indoor landscape.

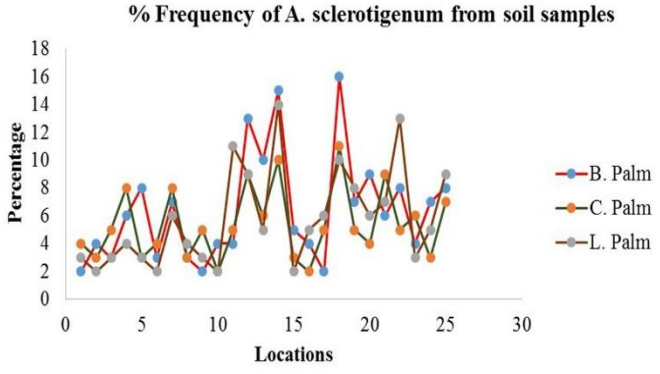

Figure 3. Percent frequency of *A. sclerotigenum* recorded from soil samples of potted Bamboo palm (*Chamaedorea seifrizii*), Cat palm (*Chamaedorea cataractarum),* Lady palm (*Rhapis excels*), grown indoor landscape buildings and outdoor commercial ornamental palm nurseries. On horizontal axis 1-10 locations of outdoor commercial ornamental palm nurseries and 11-25 locations of indoor landscape.

Average Frequency percentage of *A. sclerotigenum* from soil and root samples grown indoor and outdoor potted *C. seifrizii*, *C. cataractarum* and *R. excels* was recorded. Maximum 8% of *A. sclerotigenum* was recorded from root samples of outdoor *C. cataractarum* with 7.7% and 7.4% from *R. excels* and *C. seifrizii* respectively. Maximum 12.27% of *A. sclerotigenum* was recorded from root samples of indoor *R. excels* with 11.47% and 10.53% from *C. seifrizii* and *C. cataractarum* respectively. Maximum 4.5% of *A. sclerotigenum* was recorded from soil samples of outdoor *C. cataractarum* with 4.2% and 3.2% from *C. seifrizii* and *R. excels*, respectively. Maximum 7.87% of *A. sclerotigenum* was recorded from soil samples of indoor *C. seifrizii* with 7.53% and 6% from *R. excels* and *C. cataractarum*, respectively. Above all data showed that maximum frequency of *A. sclerotigenum* from indoor *R. excels* with 12.27% and minimum frequency of *A. sclerotigenum* from outdoor *R. excels* with 3.2% (Fig. 4).

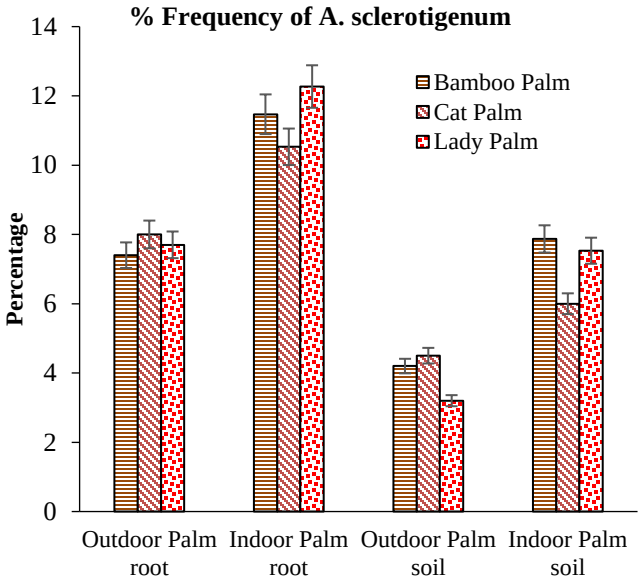

Figure 4. Average percent frequency of *A. sclerotigenum* recorded from root and soil samples of potted Bamboo palm (*Chamaedorea seifrizii*), Cat palm (*Chamaedorea cataractarum)* and Lady palm (*Rhapis excels*) grown for indoor and outdoor landscape.

DISCUSSION

The fungi present in the soils of potted plant (grown as indoor or outdoor ornamental plants) and associated with their roots are a serious matter of discussion. However, in developing countries like Pakistan no routine investigations are carried out by the scientists, therefore very little or no information is available regarding the role of indoor and outdoor ornamental plantation serving as host to harbor and multiplication of opportunistic fungi. It is an established fact that fungi are natural decomposer and that soil supposed as an important biotope for the occurrence and multiplication fungi. Various soil-related dermatophytes, molds and yeasts causing human infections are known Baumgardner, 2012. Several studies have been conducted so far, by various scientists addressing this issue like Summerbell *et al*. (1989) and Hedayati *et al*. (2004) conducted research on nosocomial infections.

In this present study association of *A. sclerotigenum* a unique human opportunistic fungus was detected from soil and root samples grown indoor and outdoor potted Bamboo palm (*C. seifrizii*), Cat palm (*C. cataractarum*) and Lady palm (*R. excels*) was recorded. Similarly, Summerbell *et al*. (1989) analyzed potted soils and reported 16 potentially pathogenic fungi. Hedayati *et al*. (2004) assumed that potted soils in hospitals can play a significant role of dispersal of opportunistic fungi and can be a big risk to immunosuppressed individuals. Gams (1973) and Simpson *et al*. (1993) described the *Acremonium* as cosmopolitan environmental fungus which can be commonly soil borne and associated with plant debris. Some species of this genus also reported as plant, insect pathogens.

***Conclusion*:** Our study has confirmed the association of *A. sclerotigenum* with soils and roots of potted plants with varying frequency. These potted ornamental plants can be source of dissemination of fungal propagule and may cause serious infections in immunosuppressed individuals. Staib *et al*. (1978) showed air dispersal of *Aspergillus fumigatus* propagule from the soil of potted plants.

It is concluded from this study to avoid the risk of dispersal of opportunistic fungi from the soils of potted plants, we must treat the soils and plants with some fungicides at regular interval. Further investigations to determine the opportunistic fungal spore concentrations and kind of fungal species in the potted ornamental indoor and outdoor plants soils and their parts, type of cultivated plants, substrates used for their cultivation would be very useful to keep the environment clean and healthy.

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REFERENCES

Dransfield, J., N.W. Uhl, C. B. Asmussen, W.J. Baker, M. Harley and C. Lewis. 2008. Genera Palmarum: The Evolution and Classification of Palms, 1st Ed. Royal Botanic Gardens, UK.

Baumgardner, D.J. 2012. Soil-related bacterial and fungal infections. J. Am. Board Fam. Med. 25:734-744.

El-Juhany, L.I. 2010. Degradation of date palm trees and date production in Arab countries: Causes and potential rehabilitation. Aust. J. Basic Appl. Sci. 4:3998-4010.

Elliott, M.L., T.K. Broschat, J.Y. Uchida and G.W. Simone. 2004. Compendium of Ornamental Palm Diseases and Disorders, 1st Ed. American Phytopathological Society, USA.

Farr, D.F. and A.Y. Rossman. 2014. Fungal Databases. U.S. National Fungus Collection. Available online at https://nt.ars-grin.gov/fungaldatabases/

Flood, J. 2006. A review of Fusarium wilt of oil palm caused by *Fusarium oxysporum* f. sp. elaeidis. Phytopath. 96:660-662.

Gams, W. 1973. Cephalosporium-artige Schimmelpilze (Hyphomycetes). Gustav Fischer Verlag, Stutgart, Germany. Mycologia 65:253-257.

Grunwald, M.H., M. Cagnano, M. Mosovich and S. Halevy. 1998. Cutaneous infection due to Acremonium. J. Eur. Acad. Dermatol. Venereol. 10:58-61.

Guarro, J., W. Gams, I. Pujol and J. Gene. 1997. Acremonium species: new emerging fungal opportunists *in vitro* antifungal susceptibilities and review. Clin. Infect. Dis. 25:1222-1229.

Hedayati, M.T., A. Mohseni-Bandpi and S. Moradi. 2004. A survey on the pathogenic fungi in soil samples of potted plants from Sari hospitals, Iran. J. Hosp. Infect. 58:59-62.

McCormack, J.C., P.B. McIntyre, M.H. Tilse and D.H. Ellis. 1987. Mycetoma associated with *Acremonium falciforme* infection. Med. J. Aust. 147:187-189.

Miceli, M.H. and S.A. Lee. 2011. Emerging moulds: Epidemiological trends and antifungal resistance. Mycoses 54:666-678.

Prabha, T.R., K. Revathi, M.S. Vinod, S.P. Shanthakumar and P. Bernard. 2013. A simple method for total genomic DNA extraction from water moulds. Curr. Sci. 104:345-347.

Simpson, K.W., K.N. Khan, M. Podell, S.E. Johnson and D.A. Wilkie. 1993. Systemic mycosis caused by Acremonium sp. in a dog. J. Am. Vet. Med. Assoc. 203:1296-1299.

Simpson, K.W., K.N. Khan, M. Podell, S.E. Johnson and D.A. Wilkie. 1993. Systemic mycosis caused by Acremonium sp. in a dog. J. Am. Vet. Med. Assoc. 203:1296-1299.

Staib, F., B. Tompak, D. Thiel and A. Blisse. 1978. *Aspergillus fumigatus* and *Aspergillus niger* in two potted ornamental plants, cactus (*Epiphyllum truncatum*) and clivia (*Clivia miniata*): Biological and epidemiological aspects. Mycopathologia 66:27-30.

Summerbell, R.C., C. Gueidan, H.J. Schroers, G.S. De Hoog, M. Starink, Y.A. Rosete, J. Guarro and J.A. Scott. 2011. Acremonium phylogenetic overview and revision of Gliomastix, Sarocladium and Trichothecium. Stud. Mycol. 68:139-162.

Summerbell, R.C., S. Krajden and J. Kane. 1989. Potted plants in hospitals as reservoirs of pathogenic fungi. Mycopathologia 106:13-22.

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