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Molecular Identification of Pseudallescheria and Scedosporium Species complex from Soil in Sudan

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Research Article

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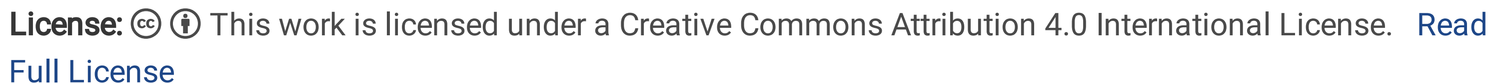

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Abstract

Background: Species of the genus Scedosporium is a complex of ubiquitous filamentous fungal species that distributed varies according to geographic region. Several *Pseudallescheria* and *Scedosporium* species are indicator organisms of pollution in soil and water. In addition, the fungi may cause life-threatening infections in both immunocompromised and immunocompetent patients. This work aimed to investigate the environmental fungal occurrence of Pseudallescheria and Scedosporium species by using morphological characters and molecular sequencing.

Results: Our results demonstrate that the most frequently recovered species of Scedosporium from environmental samples belonged to the *Scedosporium apiospermum* species complex in soil and water collected from 18 Sudanese states. These strains were identified according to their microscopic morphology and further confirmed by BT2 gene (98-100%sequence similarity) *Scedosporium apiospermum* was the microorganism most commonly recovered with 1662 isolates, followed by *Scedosporium. boydii* with 1168 , *Scedosporium aurantiacum* with 192 isolates, *Scedosporium dehoogii* with 22 isolates and *Scedosporium angustum* with 15 isolates .

Conclusion: In conclusion, the data herein presented suggest that the presence of high numbers of *Scedosporium apiospermum* and *Scedosporium boydii* isolates in Sudanese environment may be relevant to their relative prevalence as causative agents of infection. Further surveys targeted at the environments of particular at risk groups patients, are indicated as are studies examining the genetic relatedness of environmental and clinical isolates using molecular-based tools.

Introduction

Species of the genus Scedosporium (family *Microascaceae*,) are responsible for a wide range of opportunistic human infections in both immunocompromised and immunocompetent patients, and have a low susceptibility to most antifungal drugs. It is a complex of ubiquitous filamentous fungal species that distributed varies according to geographic region [1–3].The complex contain five species: *Scedosporium apiospermum*, *Scedosporium boydii* (= *Pseudallescheria boydii*), *Scedosporium aurantiacum*, *Scedosporium dehoogii* [4, 5] and *Scedosporium minutispora* [4]. These fungi are known causing agents of subcutaneous euomycetoma infection [6–12]. Recently, Infections caused by Scedosporium species are increasingly encountered in seriously ill and immunocompromised patients [13–20]. Given the highly morbidity and poor clinical outcomes associated with Scedosporium infections, early preventive and/or therapeutic strategies are importance [2]. The successful implementation of such strategies requires good understanding of the epidemiology and mode of transmission of infection.

Environmental occurrence of the *Scedosporium* complex has been investigated, with authors reporting that *Scedosporium/Pseudallescheria* species found as saprophytes in soil and are especially abundant in human-impacted areas, such as public gardens, agricultural soils,, fluids obtained from wastewater treatment plants [2, 21–24]. Most soil samples that cultured positive for the *Scedosporium apiospermum* species complex exhibited pH values in the range of 6 to 8 and the highest concentration of ammonium

and nitrogen [21]. The authors suggested that the different species have different degrees of virulence [5, 25, 26]. These environmental surveys offer useful information for the characterization of ecological areas where *Scedosporium* species may be present [21] as well as to establish probable sources of infection in outbreaks [25, 27].

Unfortunately, there is no report on the environmental distribution of *Scedosporium* complex in Sudan. Also there is no genetic studies on the species belonging to this genus in Sudan. Therefore, this work aimed to investigate the environmental fungal occurrence *Scedosporium* complex by using morphological characters and molecular sequencing.

Materials And Methods

A total of 900 environmental samples were collected in a period between 2021- 2022 from 18 states of Sudan were included in this study (figure-1). from area representing various types of human activities included: Urban industrialized area , recreational parks, public gardens and crowded playgrounds agricultural areas, fluids obtained from wastewater treatment plants, sandy riverbanks of the White Nile and muddy riverbanks of the Blue Nile , house plant or surface soil samples from houses , soil from animals manure were the selected zones for sampling. From each state soil and water were collected.

Soil samples collection: Approximately 150-200 g was collected from each soil sample at a depth of 0-10 cm after scraping and removing leaves and other plant debris on the soil surface. A total of eight hundred soil samples were collected and were put in clean bags.

Water samples collection: A total of one hundred water samples have been randomly collected in sterile bottles (500ml) from surface river water, drinking water, sewage water , wastewater treatment plants and animal drinking water

All the samples were then transferred to the laboratory, and maintained at 4°C until further use.

Measurements of Physicochemical Parameters

We employed the Sudan Soil Information System (SUSIS) digital soil map of the Sudan from Land and Water Research Centre -Agricultural Research Corporation Sudan Webadmin@susis.sd as a reference on the type of soil where the species occurred. Soil important characteristics, Soil texture,Soil type, salinity and nitrogen concentrations were retrieved from SUSIS database (table -1).

pH measurement. pH was measured on soil samples using a cyberscan pH 510 pH-meter (Eutech instruments). 20 g of dried and sieved (2 mm) soil sample were suspended in 50 ml of distilled water. After stirring for 10 min, the suspension was allowed to rest for 10 min before being stirred again for 10 min. The soil suspension was maintained under agitation during pH measurement. All assays were performed in triplicates and mean values were calculated [21].

Isolation of fungi

Scedosporium species complex were isolated from soil using the plate dilution method [28]. Briefly, from each soil sample a total of 10 g was suspended in 100ml sterile distilled water and shaking for 10 min at 25C° and 120 rpm. The suspensions of soil were serial diluted in sterile distilled water up to 10^{-6} ·approximately 1ml was removed by pipette and streaked.

For primary isolation, potato dextrose agar (PDA; Hi media, India), Sabouraud dextrose agar (SDA; Hi media, India) and oatmeal agar (OA; Hi media, India) supplemented with the antibiotic chloramphenicol (50 mg/L) were used with duplicates. All the dishes were incubated at 25 °C and 37 °C for 7-14 days in dark. All distinct colonies were subjected to additional purification by sub culturing onto PDA and Malt extract agar, (MEA 2%; Merck, Germany), and the plates were incubated for 5–7 days at 25 °C and 37 °C in dark until growth of fungal colonies was observed. The pure cultures were preserved on 20% glycerol stock at 4°C for further studies.

Furthermore strains were isolated from water samples using filtration technique [29]. One hundred milliliters of each water sample was filtered through membrane filters (pore diameter 0.45 µm), followed by incubation of the membrane filters on PDA, SDA and OA supplemented with the antibiotic chloramphenicol (50 mg/L) were used with three replicates. All the dishes were incubated at 25 °C and 37 °C for 7-14 days in dark.

All colonies displaying typical characteristics were presumptively identified as *Scedosporium/Pseudallescheria* spp. according to their microscopic morphology and isolated in pure culture [30].

Morphological characterization

Morphological characteristics of isolates were studied on PDA,OA ,MEA, and SDA. After incubation, the diameters of the colonies on each agar media were measured. Colony color (obverse and reverse sides) and the degree of sporulation were also observed. Appropriate keys were used for the phenotypic identification of the isolated fungi [30] Mounted needle was used for fungal structures , in which small portion of colonies were placed on slides using lactophenol cotton blue as the mounting medium and then examined under Olympus microscope (Olympus Optical Co., Ltd., Tokyo, Japan) connected with digital camera (KOPTIC Korea Optics, Seoul, Korea) .

DNA extraction, amplification and sequencing

Genomic DNA was extracted from a fresh culture grown on MEA plates using the cetyltrimethyl ammonium bromide (CTAB) protocol of Möller *et al*. [31]. The ribosomal DNA (rDNA) internal transcribed spacer (ITS) gene, the first β-tubulin locus (BT2), the second β-tubulin locus (TUB), partial sequences of the actin(ACT1), a portion of the calmodulin gene (CAL) , DNA-dependent RNA polymerase II largest subunit (RPB1) and second largest subunit (RPB2). and elongation factor 1α (TEF1) genes were amplified and sequenced. Primers used for amplification and sequencing are according to Luplertlop *et al.* and Zeng *et al.* [24,26] .The PCR conditions were the same as those described by Luplertlop *et al*. [24] .

Alignment and phylogenetic analyses

Consensus sequences from forward and reverse primers were generated using Seqman assembly programme from Lasergene software package (DNASTAR, Madison, WI, U.S.A.). These sequences were compared to the GenBank sequence database using nucleotide BLAST tool.

The bioinformatics data were analyzed and used to assess inter- and intra-species nucleotide variation of the in all strains analyzed in this study. To assess evolutionary relationships among isolates, all generated BT2 gene sequences as well as 30 sequences of *Scedosporium*. *apiospermum* and related species were downloaded from GenBank. Gene sequences of *Parascedosporium tectoneae* (CBS 120338) were used as outgroup. For identification at the species level 98-100% sequence similarity to isolates of the GenBank was used to identify our isolates. When similarity result was lower than 98%, an additional analysis was performed consisting in sequencing ITS 4 and 5 genes, a portion of the CAL gene, TUB , ACT1, RPB1, RPB2 and TEF1 genes , as previously described [5].

Phylogenetic analyses were based on Bayesian inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) as described previously [24]. Gaps and missing data were deleted .For BI, the best evolutionary model for each locus was determined using MrModeltest v. 2.0 [32]. Confidence values were assessed from 1000 bootstrap replicates of the original data. Bootstrap values below 50% were removed from tree.

Results

Characterization of the Isolate of *Scedosporium* Species Complex Population from Soil and water

Our results showed that the species of Scedosporium were recovered from environmental samples from soil and water collected from 18 Sudanese states. The pH of the soil samples ranged between 6.0 and 8.0, with nitrate, concentrations varying from 0–20 mg/100 g (table-1). As shown in figure-2, we found colonies with different morphologies on each PDA, OA, MEA, and SDA agar plate. From each plate, we selected one representative colony of each morphological type of Scedosporium, which yielded 3,059 single colonies: 2,891 colonies from 800 soil samples and only 168 colonies from 100 water samples.

These strains were identified according to their microscopic morphology [30] and further confirmed by BT2 gene (98-100%sequence similarity) and those sequences for which identification was ≤ 98.% were confirmed by ITS and another region of the β-tubulin gene sequencing [5,33]. *Scedosporium apiospermum* was the microorganism most commonly recovered with 1662 isolates, followed by *Scedosporium. boydii* with 1168, *Scedosporium aurantiacum* with 192 isolates, *Scedosporium dehoogii* with 22 isolates and *Scedosporium angustum* with 15 isolates .

Colonies of *Scedosporium boydii* grow rapidly at 25°C on PDA, as white aerial mycelia and later becomes dark gray or smoky brown. Wooly to cottony from the surface, and it is pale with brownish black colour from the reverse. Microscopy: Hyphae hyaline and septate. Conidia Unicellular, finely smoothened, ovoid with truncate bases formed singly at the ends of the conidiophores [30] (figure3-1).

The cleistothecia of *Scedosporium boydii* are dark brown fruiting bodies (completely closed ascocarps) measuring 100 to 200 µm in diameter.. Asci are subglobose to globose measure 8 to 13 µm by 12 to 18 µm and bear 8 ascospores inside. Ascospores are unicellular, ovoid to ellipsoidal, smooth, and pale yellow brown to copper in color measuring 4 to 5 µm by 7 to 8 µm [30].

Material examined: This fungus was isolated from Urban gardens, agricultural areas, sports parks, recreational parks Urban industrialized area, sandy riverbanks of the White , house plant , surface soil samples from houses , soil from animals manure , surface river water , sewage water , and wastewater treatment plants samples taken from River Nile (250 isolates), El-Obeid (193 isolates), North Darfour (188 isolates), Sennar (135 isolates), El Geteina (125 isolates), Dongla (122 isolates) ,Khartoum (85 isolates), El-Gazira state (70 isolates).

Colonies of *Scedosporium apiospermum* grew at 25°C on PDA as white gray, and later becoming darker gray or brown in colour from the surface .The colour from the reverse, it is dark brown or gray to black .Microscopy: Hyphae hyaline and septate. conidiogenous cells percurrent, lateral or terminal, subhyaline, smooth-walled, usually cylindrical, producing obovoidal or ellipsoidal, 5-14 x 3-5 µm, smooth-walled conidia(figure 3-2) *Scedosporium apiospermum* has large cleistothecia 140-480 µm [30].

Material examined: This fungus was isolated from Urban gardens, agricultural areas, sports parks, recreational parks Urban industrialized area, sandy riverbanks of the White Nile , house plant, surface soil samples from houses , soil from animals manure , surface river water , sewage water , and wastewater treatment plants samples taken from Khartoum (350 isolates), North Darfour (235 isolates), El- Gazira (270 isolates), River Nile (225 isolates). El Geteina (210 isolates), Sennar (175 isolates), El-Obeid (122 isolates) and Dongla state (75 isolates)

Scedosporium aurantiacum colonies at 25°C on PDA, expanding, cottony, with a light yellow diffusible pigment; reverse in orange colour. Microscopy:. Conidia produce directly from undifferentiated hyphae abundant, sessile or on short protrusions, brown, usually obovoidal 6-10 x 3-5 µm, thick-walled [30] (figure 3-3).

Material examined: This fungus was isolated from soil samples from Urban gardens, agricultural areas, sports parks, recreational parks, Urban industrialized area and sandy riverbanks of the White Nile taken from Khartoum (75 isolates),El- Gazira (62 isolates), and El Geteina (55 isolates).

Scedosporium dehoogii colonies at 25°C on PDA growing rapidly, cottony to lanose, initially dirty white, becoming pale grey with age; reverse pale, greenish or whitish. Microscopy.:Conidiogenous cells arising from undifferentiated hyphae, cylindrical to somewhat flask-shaped, 6-50 x 1.0-1.5 µm, producing slimy heads of 1-celled, smooth-walled, subhyaline to brown, ovoidal conidia, 6-12 × 4-5 µm [30] (figure 3-4).

Material examined: This fungus was isolated from soil samples from agricultural areas, house plant , surface soil samples from houses , soil from animals manure taken from AlQadarif state (22 isolates).

For the distribution of *Scedosporium* species complex, between the area studied, we found that The highest density of *Scedosporium* species was found in urban gardens (30%), agricultural areas(21%), industrial parks (16%), sports parks (12%), home gardens (8%) surface soil samples from houses(5%) , soil from animals manure (4%) and wastewater treatment plant activated sludges (4%) . In contrast, the lowest densities of these fungi were encountered in areas in contact with water (riverbanks) sewage water and surface river water, house plant. No *Scedosporium boydii* / *Scedosporium apiospermum* complex colonies were recovered from the evaluated water samples of drinking water and animal drinking water.

For the distribution of *Scedosporium* complex species over the country, we find the following: while most isolates of *Scedosporium apiospermum* (78.5%) were obtained from the north and centre of the country. On the other hand, *Scedosporium boydii* was equally distrusted in the centre, west and north of the country. *Scedosporium aurantiacum* was recovered in 85.7% of the soil samples from the centre of the country and 14.3% from river bank water. The isolation of *Scedosporium dehoogii* was restricted to the samples collected from the southern east. where *Scedosporium angustum* was found in the centre of the country from Khartoum and El- Gazira state and was only isolated from wastewater treatment plant activated sludges.

Phylogenetic Analyses

The phylogenetic relationships of *Scedosporium* species complex were studied using combined sequences of thee-locus alignment (ITS, CAL, and BT2) on the basis of a dataset consisting of 30 sequences of *Scedosporium*. *apiospermum* and related species (figure-4)`. The concatenated alignment consisted of 989 characters (including alignment gaps).The ML analysis was congruent with the BI analysis, both displaying a similar topology. In the combined ITS- BT2 tree, our strains were distributed across four clades, into a well-supported main clade grouping all Scedosporium species (figure-4). 2.814 of the isolates (92%) were placed into the *Scedosporium apiospermum* species complex clade A , and distributed across several terminal clades: A terminal clade containing the type strains of *Scedosporium boydii* ,*Scedosporium apiospermum* and *Scedosporium* angustum and our strains Clade B containing the type strain of *Scedosporium apiospermum* and our strains .Clade C included only two type strain of *Scedosporium dehoogii* and 12 of our strains. Clade D included four type strain of *Scedosporium aurantiacum* and our strains .

Discussion

In order to understand the increasing incidence of *Scedosporium* species complex in human infections, a better knowledge of their habitat is necessary. Previous studies have been performed in Austria and Netherlands [21], Australia [22], France [23] and Thailand [24] highlighting different distribution patterns.

The generic name was proposed by Saccardo (1911) [34], who described a new fungus, which he named *Monosporium apiospermum*, that was isolated from a patient in Italy with mycetoma [34]. Shear in 1922 was described the life cycle of *AUescheria boydii* that isolated from a patient with mycetoma in Texa .[35]. In 1943, Negroni and Fisher [36] isolated the asexual and sexual reproductive structures, from purulent

material from a case of knee arthritis. These two isolates were considered to be different agents of mycetoma until 1944, when Emmons showed that the first was the anamorph (asexual form) of the second [37]. In 2014, in order to remove the dual nomenclature that had been based on the anamorph/teleomorph concept, Pseudallescheria was accepted as a synonym of Scedosporium [1]. Ten species of Scedosporium are currently recognized which are easily distinguishable phylogenetically by comparing the sequences of a fragment of the β-tubulin gene BT2 rather than by phenotype [3, 38].

As *Scedosporium boydii* was found more frequently found in the patients with white grains eumycetoma [6–12] and Sudan is one of more endemic countries with this type of subcutaneous infection. In the last years there was a change in both the route of transmission and the people at risk for mycetoma. There was a progressive increase in the number of cases involving especially children and the elderly. In addition to new species were identified and classified through advanced molecular tools [39–41]. Therefore the present study was conducted in order to clarify distribution of *Scedosporium boydii* / *Scedosporium apiospermum* complex in our country and to characterize their natural habitat and to set the stage for future detailed studies focusing on the investigation of a possible connection between environmental sources and clinical colonization/infection.

A culture-based approach was used to obtain a picture of the fungi living in soils. Despite the inability to culture some microorganisms, this is a fast and inexpensive method that provides information on active populations [42].

The genus comprises species that are highly polymorphic and all them produce the scedosporium-like asexual morph characterized by the production of solitary conidia from annelidic conidiogenous cells; a graphium-like synnematous synanamorph; unicellular sessile conidia; a polycytella-like synanamorph, a sort of “macroconidia” only seen in certain strains of *Scedosporium apiospermum* [43, 44]; and a sexual morph, comprising dark, non-ostiolate globose ascomata with a thin peridial wall of textura epidermoidea, 8-spored, soon evanescent asci, and unicellular, yellowish or reddish brown, broadly fusiform to ellipsoidal ascospores with (usually) a germ pore at each end. .

In agreement with previous results [21, 22] the highest densities of *Scedosporium boydii* / *Scedosporium apiospermum* complex isolates were recovered from human-impacted areas: agricultural areas, playgrounds, industrial areas and wastewater treatment plants fluids. Riverbanks revealed a low fungal density and no *Scedosporium boydii* / *Scedosporium apiospermum* complex colonies were recovered from the evaluated water samples of drinking water and animal drinking water. Interestingly, results from wastewater treatment plants fluids raise the question of the risk on human health using sludge for enrichment of agricultural lands. This environmental investigation also confirmed the influence of pH on *Scedosporium boydii* / *Scedosporium apiospermum* complex occurrence. No *Scedosporium boydii* / *Scedosporium apiospermum* complex isolates were found at acid pH or at a pH value ≥ 8.5.

A positive correlation between ammonium concentrations and *Pseudallescheria* density in soil from industrial areas, parks and playgrounds was demonstrated by Kaltseis *et al*. [21]. These results confirmed earlier observations [45], suggesting that high nitrogen levels in soil are required for the growth of these

species [21]. In addition they found that *Pseudallescheria* spp. were abundant in soils with a pH range of 6.0–7.5 [21]

We found that *Scedosporium apiospermum* was the microorganism most commonly recovered, followed by *Scedosporium. boydii*. *Scedosporium aurantiacum*, *Scedosporium dehoogii* and *Scedosporium angustum*. These proportions differed in Austria, where *Scedosporium apiospermum* was the most common species followed by *Scedosporium dehoogii* [21], and in Australia, where *Scedosporium aurantiacum* accounted for 54.6% of all environmental isolates [22], and in Mexico *Scedosporium dehoogii* was the most frequent species in the environment [46]. These discrepancies in the distribution patterns of *Scedosporium boydii* / *Scedosporium apiospermum* complex between countries may be related to differences in climate or agricultural practices.

Moreover, ecological preferences were observed for each species. As in Austria [21]. *Scedosporium. dehoogii* was more abundant in industrial areas and was associated with agricultural areas. In Australia, *Scedosporium boydii* / *Scedosporium apiospermum* complex were recovered mainly in urban areas [22]. *Scedosporium apiospermum* was associated with playgrounds [22]. In our results we found that The highest density of *Scedosporium* species was found in urban gardens (30%), agricultural areas(21%), industrial parks (16%), sports parks (12%), home gardens (8%) surface soil samples from houses(5%), soil from animals manure (4%) and wastewater treatment plant activated sludges (4%). Interestingly, we isolates *Scedosporium apiospermum* and *Scedosporium boydii* from surface soil samples from houses. Similar result was obtained by Sidot *et al* who recovered *Scedosporium apiospermum* from home environments of CF patients[47].

This suggested the presence of these fungi is strongly associated with organic contamination, such as hydrocarbons from human, animal and industrial waste in the environment as a consequence of human and animal activities [20, 21]. This association is most likely due to a number of factors including the ability of *Scedosporium boydii* to survive at very low oxygen partial pressure and to its ability to tolerate 5% NaCl [48]. They are able to utilize natural gas and/or aromatic compounds as carbon sources [20, 49, 50].

In conclusion, *Pseudallescheria/Scedosporium* species were ubiquitous in soil and water in Sudan. There was a close association between density of fungi recovered and degree of human activity. However, the clinical implications of the environmental presence of *Pseudallescheria/ Scedosporium* are not yet known. The data herein presented suggest that the presence of high numbers of *Scedosporium apiospermum* and *Scedosporium boydii* isolates in Sudanese environment may be relevant to their relative prevalence as causative agents of infection. Further surveys targeted at the environments of particular at risk groups patients, are indicated as are studies examining the genetic relatedness of environmental and clinical isolates using molecular-based tools. To further differentiate closely related Scedosporium species, the partial β-tubulin gene is needed. Therefore, precise species identification is essential to determine virulence and antifungal susceptibility between these fungal species.

Abbreviations

The ribosomal DNA (rDNA) internal transcribed spacer gene ; ITS, the first β-tubulin locus; BT2, the second β-tubulin locus; TUB, partial sequences of the actin ;ACT1 , a portion of the calmodulin gene;CAL , DNA-dependent RNA polymerase II largest subunit; RPB1, second largest subunit; RPB2 and elongation factor 1α genes ; TEF1.

Declarations

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Authors' contributions: NAM was provided conceptual framework for the project, participated in data collection and analysis , participated in the molecular performance and writing the manuscript

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Availability of data and materials:

The datasets used and/or analyzed during the current study are included in this published article and its supplementary information files are given in Additional file 1 :Source information of *Scedosporium* species Complex isolated from soil and water using sequences and primers used in this study are also given in Additional file 2. .

Ethics approval and consent to participate: Not applicable.

Consent to publish: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Tables

Table 1 Characterization of sampling sites and *Pseudallescheria* and *Scedosporium* Species complex collections. Climatic conditions and soil properties at the sampling sites.

No	Site Name	Ecoregion[a]	Soil type[b] (Common description)	Average Annual temperature (°C)	Average Annual rain (mm)	Soil pH	Nitrogen (mg/100 g)
1.	Khartoum	Sahelian Acacia savanna	Yermosols (Flat gravel silt loam with clay)	29.9	168	7.0-7.5	0.01-0.20
2.	El-Obeid	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay	27.5	236	6.5-7.5	0.00-0.10
3.	Dongla	Desert	Volcanic(alluvium sand)	28.9	9.28	7.5-8.0	0.01-0.20
4.	Kassala	East Sudanian savanna	Fluvisols (Riverside silt)	29.6	251	8.0-8.5	$000 - 0$ 01
5.	Blue Nile	East Sudanian savanna	Vertisols(Black clay	28.9	698	8.0-8.5	0.01-0.20
6.	North Darfour	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay)	26.3	213	6.0-6.5	>0.20
7.	SouthDarfour	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay)	29.2	398	7.0-7.5	0.05-0.20
8.	South Kordfan	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay	27.9	736	6.5-7.5	0.00-0.10
9.	El- Gazira	Sahelian Acacia savanna	Vertisols(Black clay	30.4	427	7.5-8.0	0.01-0.20

10.	El Geteina	Sahelian Acacia savanna	Yermosols (Flat gravel silt loam with clay)	28.3	427	7.5-8.0	0.01-0.20
11.	River Nile	Desert and semi desert	Volcanic(alluvium sand)	21.0		7.0-7`.5	$005 - 0$ 20
12.	Red Sea	Desert and semi desert	Volcanic(alluvium sand)	28.4	165	8.0-8.5	$000 - 0$ 01
13.	AlQadarif	East Sudanian savanna	Vertisols(Black clay)	29.3	601	7.5-8.0	0.01-0.20
14.	Sennar	Sahelian Acacia savanna	Vertisols(Black clay	30.1	305	7.5-8.0	0.01-0.20
15.	West Darfur	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay)	25.6	553	7.0-7.5	0.04-1.00
16.	Central Darfur	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay)	24.2	615	7.0-7.5	0.05-0.20
17.	East Darfur	Sahelian Acacia savanna	Arenosols (Stabilized sand dunes with silt or clay loam)	28.4	377	6.0-6.5	0.01-0.05
18.	West Kordofan	East Sudanian savanna	Arenosols (Stabilized sand dunes with silt or clay)	28.0	485	6.5-7.5	0.00-0.10

Ecoregion[a] and Soil typing [b] based on Sudan Soil Information System (SUSIS) digital soil map of the Sudan from Land and Water Research Centre -Agricultural Research Corporation Sudan Webadmin@susis.sd

Figures

Figure 1

Sampling location in Sudan States: 1:Khartoum;2: El-Obeid ;3: Dongla ;4: Kassala ;5: Blue Nile ;6: North Darfour ;7 SouthDarfour;8: South Kordfan;9: El- Gazira;10: El Geteina;11: River Nile;12: Red Sea;13: AlQadarif;14: Sennar;15: West Darfur;16: Central Darfur;17: East Darfur;18: West Kordofan. From Comersis.com.

Figure 2

Colonies of Scedosporium on PDA plate after inoculation of 1ml of soil suspension from each sample and incubation at for 7 days at 25 °C.

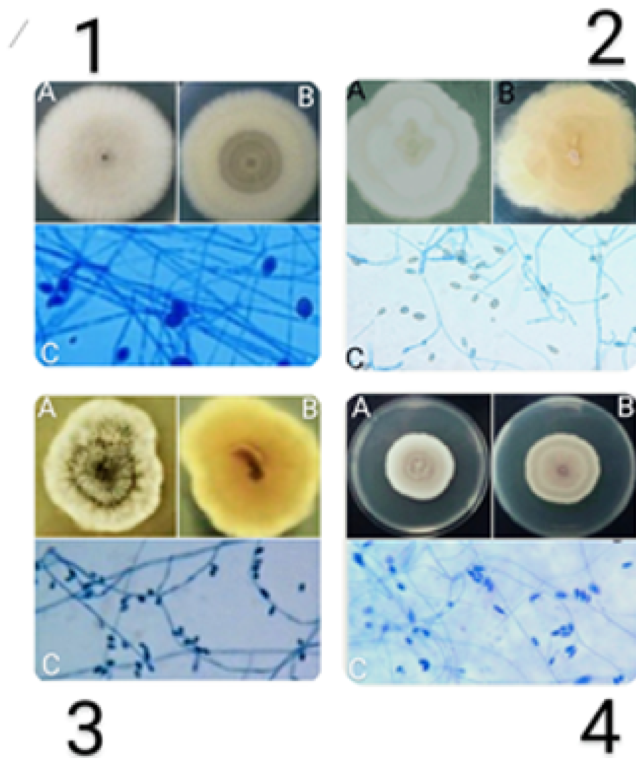

Figure 3

1: *Scedosporium. boydii*: A., Colonies on PDA after 7 days incubation from Surface side and B from reverse side C. hyaline and septate hyaphae and . unicellular conidia formed singly from short annellidic necks arising directly from the hyphae;2: *Scedosporium apiospermum* A., Colonies on PDA after 7 days incubation from Surface side and B from reverse side C Hyphae hyaline with ellipsoidal conidia.;3: *Scedosporium aurantiacum*: A., Colonies on PDA after 7 days incubation from Surface side and B from reverse side C. Conidia borne directly from undifferentiated hyphae abundant, sessile or on short protrusions, brown, usually obovoidal thick-walled.;4: *Scedosporium dehoogii* A., Colonies on PDA after 7 days incubation from Surface side and B from reverse side C. Conidiogenous cells arising from undifferentiated hyphae, cylindrical to flask-shaped, producing slimy heads of 1-celled, smooth-walled, subhyaline ovoidal conidia.

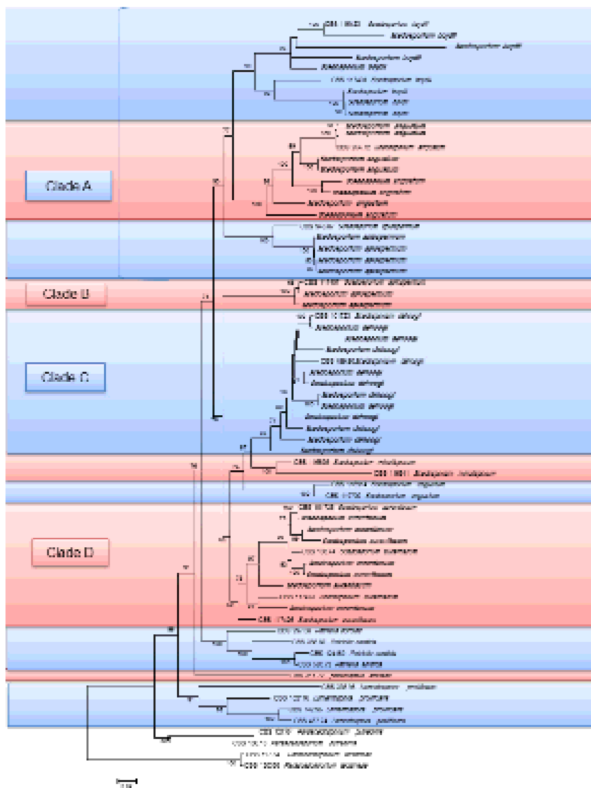

Figure 4

Phylogenetic tree of *Scedosporium/Pseudallescheria* species with closely related taxa from GenBank and their accession numbers generated from maximum likelihood (ML) analysis based on the combined dataset of ITS, partial LSU, tub2 and partial rpb2 sequences. with *Parascedosporium tectoneae* (CBS 120338) as outgroup. Bootstrap values > 50% (1000 replicates)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- IsolationofScedosporiumspeciesfromsoilandwaterusingsequences.docx
- Primersusedinthestudy.docx