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Original Article **Open Access**

Morphological and molecular characterization of haloalkaliphilic fungal isolates from hot-spring shores of Lake Magadi, Kenya

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Abstract:

Background: The extensive saline-alkaline ecosystem is increasingly gaining recognition as a rich source of haloalkaliphilic fungal biota whose ecological roles remain less explored. Equally, hot water and saline releasing lakes are being appreciated as untapped natural sources of fungal extrolites bearing unique chemical bioactivities. Existing literature reveals scanty evidence on fungal biodiversity in East African Rift-valley soda lakes. This study sought to bio-prospect Lake Magadi in Kenya as a source of extremophilic fungal microbial diversity.
Methodology: A laboratory-based experimental study was conducted using simple random sampling to select 9 mark-points in the lake for establishment of fungal morphological and molecular characterization. Wet sediments, surface water and soil samples were collected from each sampling site in triplicate and cultured on Sabouraud's Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) plates at 25^0C and 41^0C for 1-3 weeks, to isolate and identify fungi by conventional macro and microscopic morphology. The ITS-1 and ITS-4 universal fungal primer pairs were used to amplify extracted fungal DNA from isolates, followed by Sanger sequencing. The NCBI BLASTn was used for molecular identification and evolutionary relationships were inferred using neighbor-joining method with 1000 bootstrap support. The salinity, pH, electrical conductivity (EC) and total dissolved solids (TDS) of the samples were measured onsite using portable pH meter and double interface electrical-chemical analyzer.
Results: A total of 30 fungal isolates were recovered from the samples using culture based and molecular techniques. Fungal sporulation was observed at pH 8–9 and salinity of 9.6–10.8 ppm. Growth was more evident at 25^0C (n=25) compared to 41^0C (n=5), and further supported on SDA (n=16) relative to MEA (n=10) and PDA (n=4). Wet sediments accrued highest diversity of fungal biota (n=15). Phylogenetically, isolates were affiliated to 9 genera with *Cladosporium* (n=8), *Aspergillus* (n=5) and *Penicillium* (n=4) displaying dominance. The genera *Aspergillus* and *Penicillium* alongside *Fusarium* and *Sarocladium* exhibited close evolutionary affiliation as sister groups with overlapping clades.
Conclusion: The findings of this study validate existence of diverse fungal community species discerned through observed distinct phenotypic features vis-a-vis molecular identities. Diversity and taxonomic richness are significantly influenced by pH, salinity, temperature and variable cultivation media types. Metagenomic tools including DNA pyrosequencing and whole genome sequencing can enhance heightened detection of diverse microbial biota across East African saline-alkaline ecosystems.

Keywords: Haloalkaliphilic, Characterization, Saline-alkaline, Extremophiles, Hot-spring, Kenya

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Caractérisation morphologique et moléculaire d'isolats fongiques haloalcaliphiles provenant des rives des sources chaudes du lac Magadi, au Kenya

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Résumé:

Contexte: Le vaste écosystème salin-alcalin est de plus en plus reconnu comme une riche source de biote fongique haloalcaliphile dont les rôles écologiques restent peu explorés. De même, les lacs libérant de l'eau chaude et de l'eau salée sont considérés comme des sources naturelles inexploitées d'extrolites fongiques présentant des bioactivités chimiques uniques. La littérature existante révèle peu de données sur la biodiversité fongique dans les lacs sodiques de la vallée du Rift est-africain. Cette étude visait à bioprospecter le lac Magadi, au Kenya, comme source de diversité microbienne fongique extrémophile.
Méthodologie: Une étude expérimentale en laboratoire a été menée par échantillonnage aléatoire simple afin de sélectionner 9 points de repère dans le lac pour établir une caractérisation morphologique et moléculaire des champignons. Des échantillons de sédiments humides, d'eau de surface et de sol ont été prélevés en triple sur chaque site d'échantillonnage et mis en culture sur des plaques de gélose dextrose de Sabouraud (SDA), de gélose dextrose de pomme de terre (PDA) et de gélose à l'extrait de malt (MEA) à 25°C et 41°C pendant 1 à 3 semaines, afin d'isoler et d'identifier les champignons par morphologie macro et microscopique conventionnelle. Les paires d'amorces fongiques universelles ITS-1 et ITS-4 ont été utilisées pour amplifier l'ADN fongique extrait des isolats, puis le séquençage Sanger a été réalisé. Le NCBI BLASTn a été utilisé pour l'identification moléculaire et les relations évolutives ont été déduites par la méthode de «jointure entre voisins» avec un support bootstrap de 1000. La salinité, le pH, la conductivité électrique (CE) et les solides dissous totaux (SDT) des échantillons ont été mesurés sur site à l'aide d'un pH-mètre portable et d'un analyseur électrochimique à double interface.
Résultats: Au total, 30 isolats fongiques ont été récupérés à partir des échantillons par des techniques de culture et moléculaires. La sporulation fongique a été observée à un pH de 8 à 9 et une salinité de 9,6 à 10,8 ppm. La croissance était plus marquée à 25°C (n=25) qu'à 41°C (n=5), et a été confirmée par la SDA (n=16) par rapport à la MEA (n=10) et à la PDA (n=4). Les sédiments humides ont présenté la plus grande diversité de biotes fongiques (n=15). Phylogénétiquement, les isolats étaient affiliés à neuf genres, *Cladosporium* (n=8), *Aspergillus* (n=5) et *Penicillium* (n=4) étant dominants. Les genres *Aspergillus* et *Penicillium*, ainsi que *Fusarium* et *Sarocladium*, présentaient une étroite filiation évolutive en tant que groupes frères avec des clades se chevauchant.
Conclusion: Les résultats de cette étude valident l'existence d'espèces fongiques diverses, distinguées par des caractéristiques phénotypiques distinctes observées par rapport aux identités moléculaires. La diversité et la richesse taxonomique sont significativement influencées par le pH, la salinité, la température et les différents types de milieux de culture. Les outils métagénomiques, notamment le pyroséquençage de l'ADN et le séquençage du génome entier, peuvent améliorer la détection de biotes microbiens diversifiés dans les écosystèmes salins-alcalins d'Afrique de l'Est.

Mots-clés: haloalcalin, caractérisation, salin-alcalin, extrémophiles, source chaude, Kenya

Introduction:

Saline-alkaline soda lakes within Kenyan Rift-valley comprise of Lake Magadi, Bogoria, Elementaita, Nakuru, and Sonachi (1). Besides exclusive topographical and geological alignments, large deposits of sodium carbonate (Na_2CO_3) complexed with insoluble Ca^{2+} and Mg^{3+} salts facilitate existence of varied microbial biota under alkaline conditions (2,3). Likewise, substantial saline deposits in these ecosystems favors thriving of extremophile species displaying halotolerant and alkaliphilic attributes (4). The pH levels of Lake Magadi range between 8–12, whereas total salts are estimated between 5-10% (5). Among widely profiled hypersaline and alkaliphilic lake catchments comprise of Solar Salterns (Puerto Rico), *Chott* Melghir (Algeria), Caspian Sea, Great Salt Lake (Utah), Wadi Natrun (Egypt) and Lake Bogoria (Kenya) (6-10).

Halotolerant and alkaliphilic fungal species are ecologically defined rather than taxonomically classified, with further categorization as either obligate or facultative (11, 12). They are isolated by diverse cultivation methods using specific or modified fungal media, however, does not distinguish between facultative strains and terrestrial habitat contaminants (13). Yet still their isolation including cultivation from natural ecosystems requires careful picking of the fungal host or supporting material such as sponges, algae, sea grasses, sediments, soils and water media (14).

Growth of obligate extremophilic fungal biota ensues entirely within hypersaline ecosystems, whereas their facultative counterparts exhibit sporulative cycles, based primarily on their physiological adaptive mechanisms which confers tolerance to abiotic stressors inclusive of high salinity, hydrostatic pressures, elevated temperatures and pH fluctuations (7,9). They produce substantial amounts of extremozymes and extremolytes that enhances their tolerability (15).

Regardless of the harsh saline-alkaline ecosystem, appreciable fungal biodiversity produces a repertoire of natural products

with varied bioactivities. Bio-prospecting Kenya's marine-like ecological setting through morphological and molecular characterization provides crucial evidence-based material and knowledge for critical research founded decision-making practices in the country's conservancy and natural protected sites. Profiling of fungal diversity within East-African soda lakes also forms a steady basis for supplementary exploration of potent natural bioactive compounds from extremophilic fungal species that may reveal capacity for potential medicinal applicability.

Materials and method:

Study site, design and sampling technique:

This was a laboratory-based experimental study using random sampling in 9 mark-points at study site in Lake Magadi, Kenya. It covers an estimated 100 square km in size and lies 2° 00' 0'' S and 36° 00' 0'' E of the equator at an elevation of 600 meters (16). Saline emitting hot-springs (heating up to 86°C) rest along the North-western and Southern shore-lines and release into alkaline lagoons along the lake margins (3). Saline deposits also enhance thriving of a fish species (*Cichlid alcolapia graham)* that occupies the lake's basin (17).

Ethical consideration:

This study was approved by Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMU/COR: 403012 vol-2). Consent for participation was not required for this study.

Physico-chemical indices of the lake sampling points:

The pH levels were measured onsite using portable digital pH meter (Oakton® pH 110) and universal indicator strips (range 1-14) (Merk®, USA). The *in-situ* and sampling points temperature was obtained using digitized water thermometer (Hanna; model HI-98509, Europe). The total dissolved solids (TDS), electrical conductivity (EC) and salinity levels were assessed using double interphase electrical-chemical analyzer (Jenway™-3405 series, England). Geographical positionning of site sampling locations with regards to elevation, latitude and longitude were determined using global positioning system (Garmin, eTrex 22x®, USA).

Sample collection and processing:

About 30g each of wet sediments and soil material were collected into zip-lock bags, while 50ml surface water was aspirated using sterile syringes and transferred into dry, leak-proof screw capped bottles. All samples were obtained in triplicates, transported under dry ice and stored at -20^{0}C overnight until processing.

One gram each of the pooled sediment and soil samples were suspended in sterile universal bottles containing 10ml distilled water. Contents were vortexed for 5 min and allowed to settle before undergoing a ten-fold (1:10) dilution sequence. 100µl of highest dilution (10^{-1}) from each sample was inoculated by spread plate method into sterile Sabouraud's Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) plates.

Fungal media preparation and fungi isolation:

Varied agar media types (SDA, PDA, MEA) were used for fungal isolation with reconstitution based on pH levels measured at sampling site, while adhering to manufacturer's directions for culture media preparation. Media contents were sterilized at 121^{0}C, 15 lbs for 15 min. Streptomycin and carbenicillin (Duchefa Biochemie®) stock solutions were prepared at equal ratios (0.1g), with 100µl being pipetted into growth medium cooled to molten state at 50^{0}C. Contents were swirled gently before plating onto 9mm plates.

Approximately 100µl of the sample aliquots were pipetted into respective molten agar plates and allowed to solidify before incubating in an inverted position aerobically at room temperature (RT; 25^{0}C) and 41^{0}C for 1-3 weeks, with evaluation of mycelial growth. Resultant pure culture isolates were aseptically cut as agar plugs and seeded onto freshly prepared media. Mycelial growth duration was evaluated as mean growth rate (colony diameter; mm), while recording initial and final growth duration in days.

Morphological identification of fungal isolates:

Pure fungal isolates were identified up to genera level on the basis of visualized discrete morphological characteristics. Macroscopic features including colour, shape, pigmentation, texture, ridges, consistency, elevation, margins and opacity were carefully assessed and recorded. Microscopic properties were ascertained after lactophenol cotton blue (LPCB) staining. Micrographs were generated using mounted digital camera at 100x magnification.

Macro and micro-morphological properties of isolates were compared against fungal identification keys and reference illustration tables (18), aided by MycoBank fungal stock descriptions (19). Microscopic identification features comprised of conidial shape, hyphae, aerial mycelia, vesicles and phialides.

Molecular characterization of fungal isolates:

DNA extraction:

Genomic DNA was extracted using Solarbio® kit (Solarbio Life Sciences©, Beijing,

China) consistent with the manufacturer protocol. Briefly, 100mg fungal mycelium was resuspended in 200µl DNAse-free water and contents crushed. 200µl of solution A (50mM Tris pH 8.5; 5mM EDTA pH 8.0 and 25% sucrose) and 2µl of RNase A were pipetted into mixture containing 100mg bashing beads. Contents were vortexed at high speed and incubated for 30min at RT for proper lysis. 20µl proteinase K was added and digestion done in a 55^{0}C pre-heated water bath for 30 min.

Tube contents were centrifuged at 12,000rpm for 2min and the supernatant transferred. 200µl solution B (10mM Tris pH 8.5; 5mM EDTA pH 8 and 1% sodium dodecyl sulfate) was added with further incubation. 200µl of anhydrous ethanol followed by 600µl wash buffer was added to adsorption column with centrifugation at 12,000rpm for 2min while discarding the filtrate. 50µl elution buffer was then pipetted into adsorption column matrix and centrifugation repeated at 12,000 rpm for 2min. DNA pellet deposited at bottom of tube was stored at -20^{0}C until purity assessment.

DNA quantification and purity assessment:

Nucleic acids exhibit maximal spectral absorbance at 260 nm, whereas proteins and residual extraction salts absorb at 280 nm (20). Absorbance ratios at 260/280 nm, 260/230 nm and DNA yield (ng/µl), were quantified using Nanodrop One© (Thermo-Fisher™, USA). Briefly, 2µl blanking solution was pipetted onto the Nanodrop sensor and 30 sec allowed for optical density reading. Sample pedestal was then cleaned before loading 2µl genomic DNA samples in successive order. DNA bands quality control check was done using 3µl template on 1.5% agarose gel under UV visualization.

PCR amplification:

PCR amplification targeted the ITS gene region using primer pairs ITS-1 (5'-TCC GTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCC TCCGCTTATTGATATGC-*3'*) as previously described (21). Briefly, 25µl reaction volume contained 5 µl Taq 5x (New England Biolabs™, USA), 1 µl ITS-1 forward primer, 1 µl ITS-4 reverse primer, 15 µl molecular-grade water and 3 µl DNA template. The control lacked DNA template. The Realplex thermal cycler was configured at 1 cycle initial denaturation (2 min; 95^{0}C), 34 cycles final denaturation (30 sec; 95^{0}C), annealing (1 min; 56^{0}C), initial extension (1 min; 68^{0}C) and final extension at (7 min; 68^{0}c).

Gel electrophoresis and DNA cleaning:

About 5µl of amplicons volume each were carefully loaded onto 1.5% agarose gel and run at 100 volts for 45 min. DNA bands were stained with SYBR™ green, with 100 base pair (bp) ladder used as size standard. Image visualization utilized UVIDOC HD6 (Cleaver Scientific®, England). Target fragments were purified from the gel slice using Pure Link™ DNA clean-up kit (Thermo-Fisher™, USA) as per manufacturer's guidelines, followed by repeated quality-check electrophoresis prior to submission for sequencing.

Sequencing and phylogenetic analysis:

The PCR products were sequenced using Sanger sequencing (Macrogen®, Europe) on Applied Biosystems genomics platform. Multiple sequences were aligned with ClustalW (Bio Edit v7.2) while MEGA v11 performed phylogenetic analysis. Aligned sequences were deposited in NCBI database for accession numbers generation. Sequences with >90% similarity using BLASTn progressed to phylogenetic analysis, with 1000 bootstrap support. Evolutionary relationships were inferred using neighbor-joining method following Kimura two-parameter with Gamma distribution (K2+G). Interactive Tree of Life (iTOL) conducted tree visualization.

Results:

Physico-chemical parameters of the sampling sites:

The physico-chemical parameters of the lake and sampling sites are summarized in Table 1. Temperatures at sampling points ranged from 76.9 (MP-08) to 83.1 ^{0}C (MP-05) while *in situ* recordings fluctuated between 34.1 and 35.2 ^{0}C for MP-09 and MP-01, respectively. The pH levels were moderately alkaline varying between 8.3 (MP-09) to 9.1 (MP-01). The EC readings ranged between 32.0 (MP-08) and 34.8 (MP-01) mS/cm and TDS between 17.8 and 19.7 ppm for MP-08 and MP-05 mark-points. Salinity values ranged from 8.7 (MP-08) to 10.8 (MP-01) ppm. Site elevation was spread across 612 (MP-02) and 645 (MP-01) meters.

Characteristic fungal isolation profiles in varying culture media types:

A total of 30 isolates grew on variable media types, SDA (n=16) showed higher isolation capacity relative to PDA (n=4) and MEA (n=10). Optimal growth occurred at RT of 25^{0}C (n=25) compared to 41^{0}C (n=5). Wet sediments (n=15) had higher isolates recovery compared to soils (n=13) and surface water (n=2). Sampling points MP-04 (n=9) and MP-08 (n=1) recorded highest and least growth of isolates, respectively. Results are summarized in Table 2 and Supplementary Table (https://afrjcem.org/supplementary-materials/)

Table 1: Physico-chemical parameters of sampling sites in Lake Magadi, Kenya

Sampling Points (Mark point)	Elevation (Metres, M)	Temperature (^{0}c)		pH	EC (mS/cm)	TDS (PPM)	Salinity (NaCl) (PPM)
		Environment (*In situ*)	Sample points				
MP-01	645	35.2	82.1	9.1	34.8	19.4	10.8
MP-02	612	34.8	81.8	8.7	33.9	18.1	92
MP-03	613	34.8	80.5	8.9	33.6	18.9	10.1
MP-04	615	34.6	76.1	8.6	32.1	18.5	9.4
MP-05	617	34.7	83.1	8.8	34.1	19.7	10.5
MP-06	618	34.9	81.7	8.7	32.7	18.4	9.3
MP-07	618	34.4	77.6	8.5	32.3	18.8	9.9
MP-08	620	34.5	76.9	8.5	32.0	17.8	8.7
MP-09	621	34.1	77.3	8.3	32.4	18.6	9.6

Mark-point (MP); Total dissolved solids (TDS); Electrical conductivity (EC); milli Siemens per cm (mS/cm); Potential hydrogen (pH); Degree Celsius (^{0}c); Meters (M); Parts per million (PPM)

Table 2: Characteristic fungal isolation profiles on growth media types

Sampling Points	Sample Type	Sample Isolate	Growth Temp Range (°C) RT (25°C); 41°C	Growth Media (SDA, PDA, MEA)
MP-01	Sediment	-		
	Soil	-		
	Water	-		
MP-02	Sediment	**P17**, 41°C, SDA		
	Soil	-		
	Water	**P21**, 41°C, SDA		
MP-03	Sediment	**P5**, RT, PDA; **P10**, RT, MEA; **P12**, RT, SDA; **P14**, RT, MEA; **P27**, RT, SDA; **P28**, RT, SDA		
	Soil	-		
	Water	-		
MP-04	Sediment	-		
	Soil	**P2**, RT, PDA; **P4**, RT, SDA; **P9**, RT, SDA; **P11**, RT, SDA; **P15**, RT, SDA; **P20**, RT, MEA; **P26**, RT, PDA; **P30**, RT, MEA		
	Water	**P19**, RT, SDA		
MP-05	Sediment	**P6,**41°C, SDA		
	Soil	**P13**, 41°C, SDA		
	Water	-		
MP-06	Sediment	-		
	Soil	**P7**, RT, MEA; **P8**, RT, MEA; **P18**, RT, MEA; **P24**, RT, MEA		
	Water	-		
MP-07	Sediment	-		
	Soil	-		
	Water	-		
MP-08	Sediment	**P29**, 41°C, SDA		
	Soil	-		
	Water	-		
MP-09	Sediment	**P1**, RT, PDA; **P3**, RT, MEA; **P16**, RT, SDA; **P22**, RT, SDA; **P23**, RT, SDA; **P25**, RT, MEA		
	Soil	-		
	Water	-		

Mark-point (MP), Sabouraud's dextrose agar (SDA), Potato dextrose agar (PDA), Malt extract agar (MEA), Degrees Celsius (°C), Temperature (Temp), Room temperature (RT), Fungal isolate code number (P)

Morphological identification of fungal isolates:

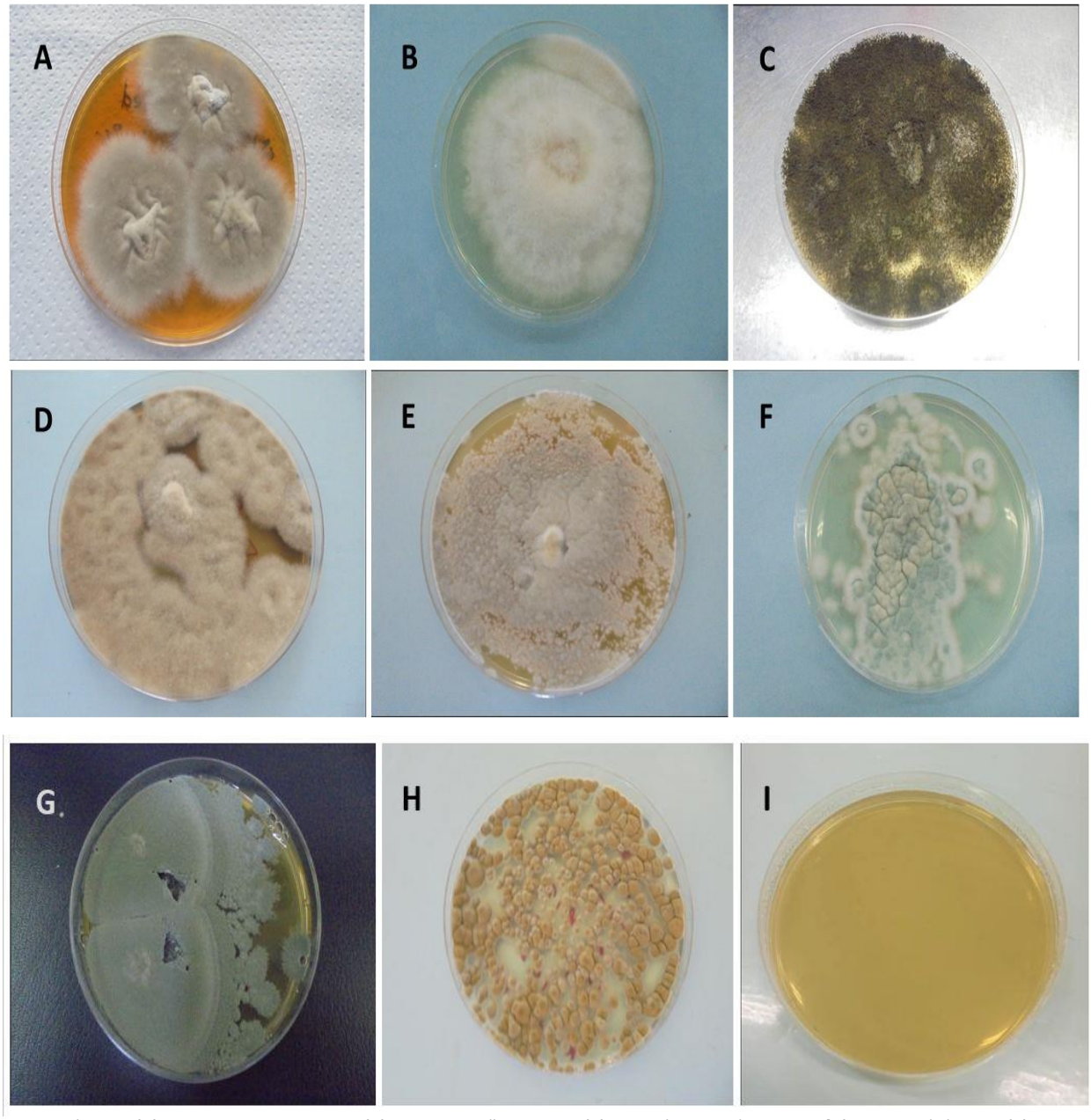
Macroscopic morphological description:

Colonial features varied from front and reverse angle view in 30 culturable isolates. Fungal isolate P19 (*Alternaria alternata*) exhibited raised brown velvet-like mycelia with round shape, entire margins, smooth non-powdery texture with opaque opacity (Fig 1). Sample isolate P21 (*Aspergillus niger*) displayed brownish black filamentous mycelia with filiform margins, rough texture, non-powdery consistency and opaque opacity. Isolate P12 (*Fusarium equiseti*) appeared filamentous with cotton white mycelia, filiform margins, rough texture, non-powdery consistency and translucent opacity.

Microscopic features of fungal isolates:

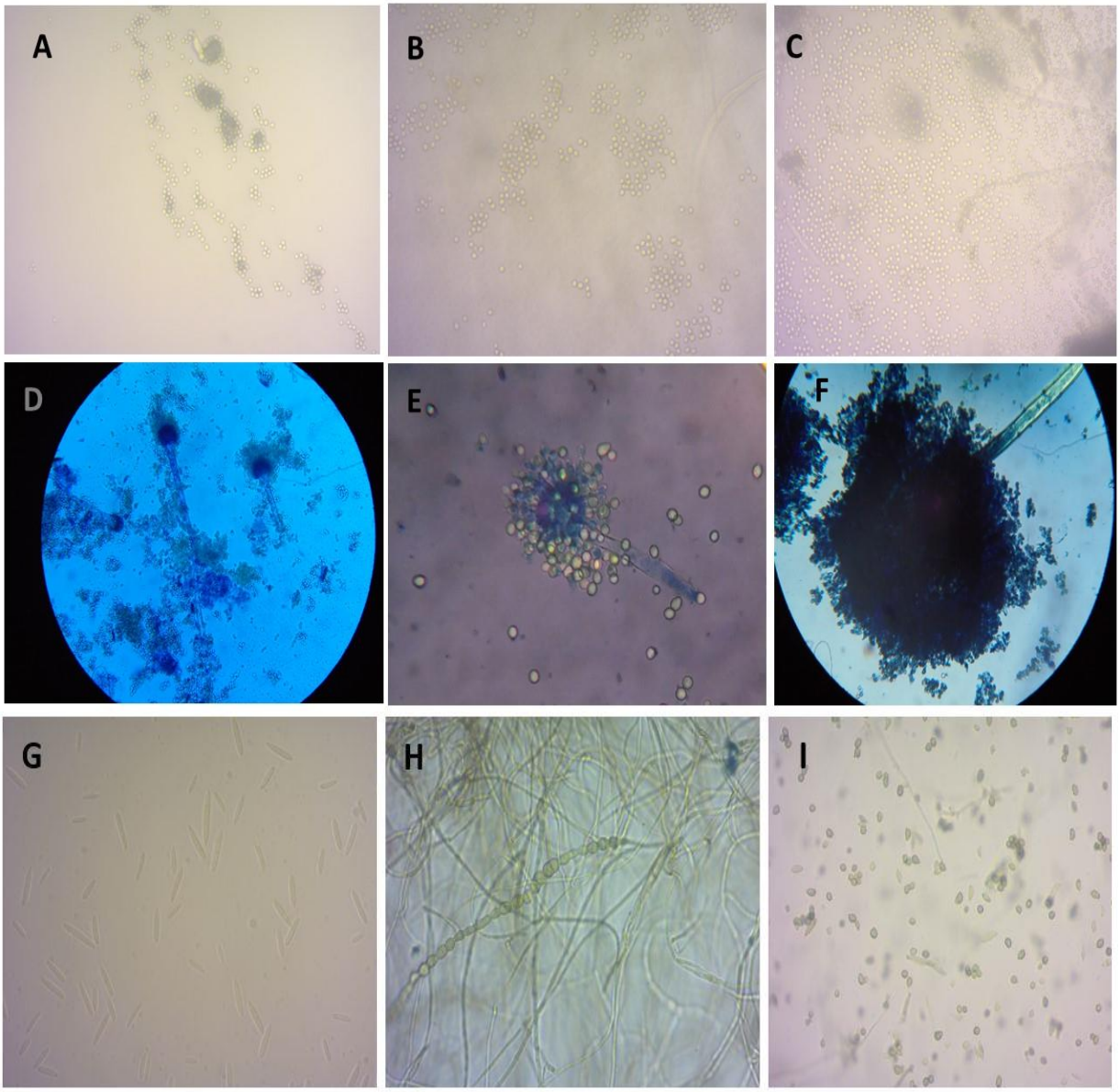
Isolate P7 (*Fusarium incarnatum*) displayed phenotypic features of elongated slightly curved tapering apical cells with 3-5

septate, numerous hyaline macroconidia and phialoconidium (Fig 2). Isolate P26 (*Penicillium expansum* presented brush-shaped conidiophores with multiple conidia in chains and septate mycelial hyphae that appeared hyaline. Isolate P16 (*Aspergillus versicolor*) exhibited long septate hyphae, pale brown upright and loosely radiating conidiophores with mature vesicles bearing biseriate phialides. Isolate P13 (*Aspergillus fumigatus*) showed spiked conidiophores with greenish conidia arranged in column chains and supported by phialide structures with clavate vesicles (Fig 2).



P29, *Bipolaris* sp. (**A**); P12, *Fusarium equiseti* sp. (**B**); P21, *Aspergillus niger* sp. (**C**); P19, *Alternaria alternata* sp. **(D**); P9, *Sarocladium* sp. (**E**); 26, *Penicillium expansum* sp. (**F**); P30, *Penicillium chrysogenum* sp. (**G**); P3, *Aspiosporina morbosa* sp. (**H**); Negative Control **(I**).

Fig 1: Macroscopic morphological properties of fungal isolates from Lake Magadi, Kenya



Magnification at x100. P2, *Penicillium chrysogenum* sp. strain EG 13-1 (**A**); P6, *Penicillium chrysogenum* sp. isolate KP-114 (**B**); P26, *Penicillium expansum* sp. (**C**); P13, Aspergillus *fumigatus* sp. (**D**); P16, *Aspergillus versicolor* sp. (**E**); P21, *Aspergillus niger* sp. (**F**); P7, *Fusarium incarnatum* sp. (**G**); P19, *Alternaria alternata* sp. (**H**); P20, *Cladosporium cladosporioides* sp. (**I**).

Fig 2: Microscopic features of study isolates following lactophenol cotton blue (LPCB) staining

Molecular characterization of fungal isolates:

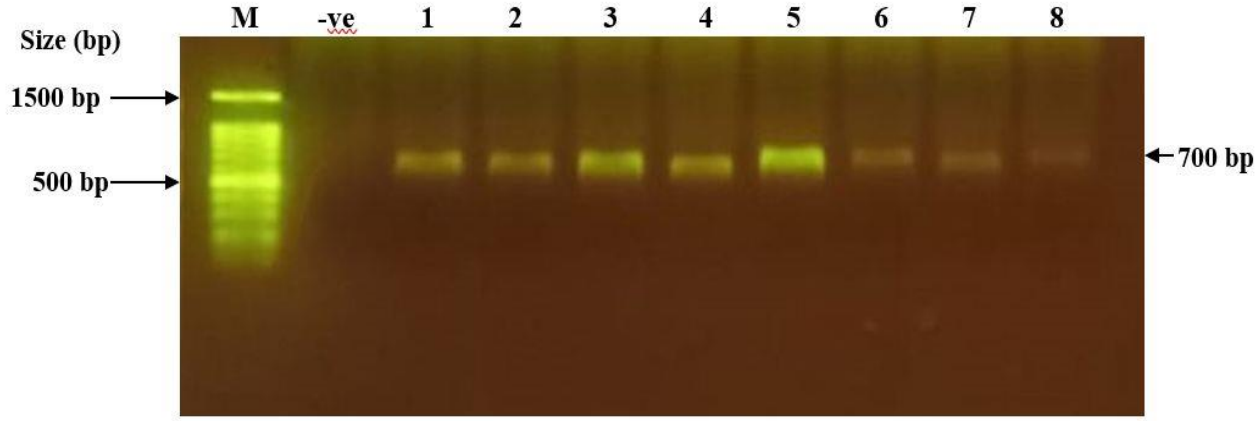
ITS gene sequence analysis and phylogenetic diversity of study isolates:

Amplified ITS genes produced band fragments of approximately 700 base pairs (bp) for representative isolates P7, P12, P14, P18, P19, P21, P27 and P29 alongside 100 bp molecular ladder as shown in Fig 3. DNA yield ranged between 101.5-446.6 ng/µl, while purity assessment readings averaged 1.8-2.0 for bulk of samples. Sequence data detected fungal clusters with high percentage identity (≥90%) classified into 9 clades (*Cladosporium, Aspergillus, Fusarium, Penicillium, Sarocladium, Curvularia, Bipolaris, Alternaria*, and *Dothideomycetes*) (Table 3 and Fig 4).

The genera *Cladosporium, Aspergillus, Penicillium* and *Fusarium* dominated, with numerous individual strains. For instance, *C. cladosporioides, C. oryzae*, *C. parahalotolerans*, *C. oxysporum* and *C. perangustum* were affiliated to genus *Cladosporium,* whereas *A. fumigatus*, *A. niger* and *A. versicolor* were linked to genera *Aspergillus.* The genera *Fusarium* and *Penicillium* strains included *F. incarnatum*, *F. oxysporum* and *F. equiseti* alongside *P. chrysogenum* and *P. expansum*, respectively. Minor strains comprised *Sarocladium* sp. voucher GJB; *Phoma* sp. Sin 154W03 and *Curvularia mebaldsii* Bi22.

Evolutionary relationship of taxa with

good bootstrap support of 1000 replicates showed genera *Aspergillus* and *Penicillium; Curvularia*, *Alternaria* and *Dothideomycetes as well as Fusarium* and *Sarocladium* as sister groups with overlapping clades that bear close evolutionary affiliation.



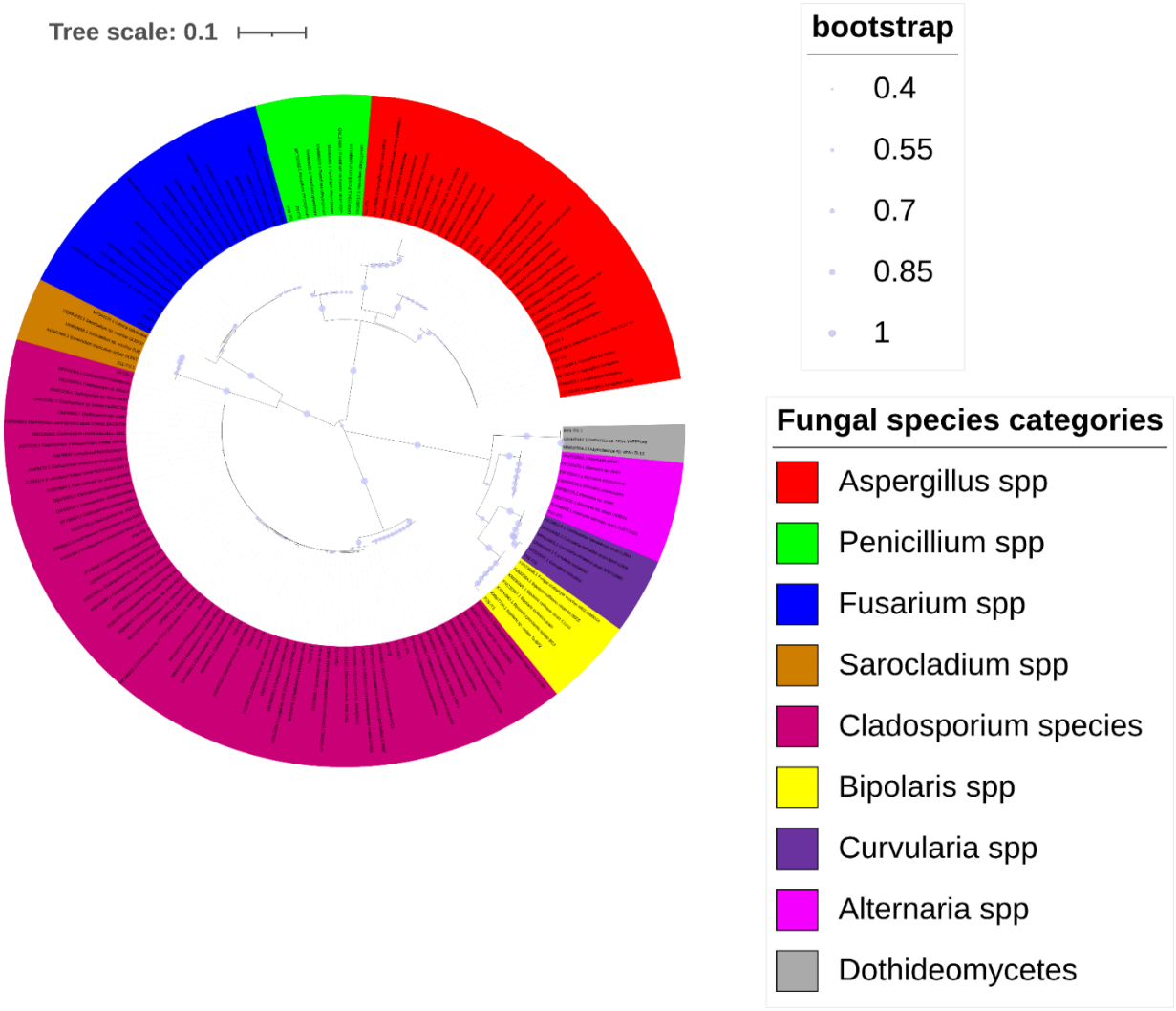
700 bp fragments targeting ITS region. Lane M; 100 bp ladder. -ve; negative control. Lanes 1 – 8; isolates P7, P12, P14, P18, P19, P21, P27 and P29. Base pairs (bp).

Fig 3: Agarose gel with PCR products of study isolates

Table 3: ITS gene sequence analysis results of fungal isolates using BLASTn

Sample ID (Query Sequence)	Sequence Accession Number	Percentage (%) Identity	Fungal Species Identified
P1	MK140706.1	99.79	*Cladosporium oxysporum* isolate MN 12
P2	MT730080.1	97.81	*Penicillium chrysogenum* strain EG 13-1
P3	AF493982.1	93.99	*Aspiosporina morbosa* strain PFC1 ITS
P4	LN834380.1	99.58	*Cladosporium perangustum*
P5	KX387685.1	99.79	*Cladosporium* sp. XQ3-13 isolate J29
P6	OQ826664.1	100.00	*Penicillium chrysogenum* isolate KP-114
P7	KX523892.1	95.70	*Fusarium incarnatum* isolate F31
P8	KF914438.1	100.00	*Fusarium oxysporum* sp. *lycopersici* strain FO43
P9	MH890606.1	99.43	*Sarocladium* sp. voucher GJB
P10	KT192295.1	99.81	*Aspergillus fumigatus* strain 40a3-2
P11	MH890606.1	99.43	*Sarocladium* sp. voucher GJB
P12	KY436192.1	91.84	*Fusarium equiseti* isolate ATS37
P13	MT994683.1	91.89	*Aspergillus fumigatus* isolate AA
P14	MT786364.1	97.62	*Cladosporium cladosporioides* strain JZY1-25
P15	MG018196.1	100.00	*Phoma* sp. 1KM-2017 isolate Sin154W03
P16	PP076803.1	99.42	*Aspergillus versicolor* isolate E42
P17	OW984430.1	99.61	*Aspergillus fumigatus*
P18	MG385089.1	98.96	*Cladosporium oryzae* isolate ihWWF158
P19	KJ410043.1	100.00	*Alternaria alternata strain* DUCC5021
P20	AF455442.1	99.79	*Cladosporium cladosporioides* isolate wb413
P21	MK840965.1	99.04	*Aspergillus niger* isolate F5
P22	MT229245.1	100.00	*Curvularia mebaldsii* strain Bi22
P23	MN859971.1	99.19	*Cladosporium halotolerans* isolate DC03
P24	MF688676.1	99.15	*Cladosporium cladosporioides* isolate VGCC15-3
P25	MN837894.1	99.78	*Didymellaceae* sp. strain SL13
P26	MK907949.1	97.85	*Penicillium expansum* isolate EF171
P27	OP237121.1	98.94	*Cladosporium parahalotolerans* strain 299N1
P28	MN837894.1	99.78	*Didymellaceae* sp. strain SL13
P29	KX867789.1	99.00	*Bipolaris* sp. isolate Ta-BP2
P30	MT730080.1	97.63	*Penicillium chrysogenum* strain EG 13-1

Internal transcribed spacer (**ITS**); Nucleotide basic local alignment search tool (**BLASTn**).



Google Drive Link: https://drive.google.com/file/d/12DYGu_B1ZqzQ1RAtj7YyuiSO6pDXrEe/view?usp=drive_link

Fig 4: Tree diagram of fungal species targeting ITS gene region

Discussion:

Physico-chemical indices are formerly shown to impact on survivability of diverse microbial biota including extremophilic fungi in hypersaline ecosystems (22). In particular, salinity levels (range 8.7–10.8 ppm) in our study conform with earlier studies reporting mean levels of 10 mg/L (4,5). Sporulation and metabolites production are propagated in sodium chloride and glucose enriched catchments (23). Our isolates were predominantly alkaliphiles which portrays adaptive resilience towards abiotic stressors (5). The sampling site temperatures (range 76.1–83.1^{0}C) in our study parallel previous studies in hot-springs of Rift-valley soda lakes recording measurements between 77.7–84.6^{0}C (6).

Regarding *in situ* temperatures, the bulk of haloalkaliphiles exhibit optimal growth range between 20–45^{0}C (9,24), which coincides with our study, with a range of 34.1-35.2^{0}C. Lower TDS values averaging 0.64–0.65 mg/l were documented from Lake Bogoria sampling points compared to our study findings of 17.8–19.7 ppm. Lake Magadi is supplied by run-off waters bearing eroded material from higher adjacent ground areas which consequently propels and deposits large number of dissolved solids in form of silt into hot-spring rivulets (25).

Additionally, our study agrees with previous research using culture-based techniques and assorted media types for fungal isolation (7,26). Cultures of related fungal genera thrive best on media types with comparable growth requirements which enhances cultivation from their natural ecosystems (27). SDA showed highest suitability for fungal isolation among profiled media types, attributable to differences in concentration of growth nutrient composition as constituted by media manufacturer brands.

The fungal isolates in our study were phylogenetically clustered into 9 genera, maj-

ority of which have formerly been isolated in hypersaline habitats sharing ecological preference to extreme conditions. In particular, genus *Penicillium, Aspergillus, Cladosporium, Fusarium, Chaetomium, Phoma, Talaromyces, Ectophoma, Emericella, Phaeosphaeria* and *Acremonium* have reported isolation from sediments in Rift valley halotolerant ecosystems (3). Importantly, genera *Aspergillus, Penicillium* and *Cladosporium* exhibit higher colonization rates among *Ascomycota* compared to other minor classes (8,28). Consistent pattern is experienced from hypersaline waters of *Chott* Melghir (Algeria), Solar Salterns (Puerto Rico) and Caspian Sea especially involving numerous strains of *Aspergillus, Penicillium* and *Cladosporium* (9,10). Phylogenetic and molecular diversity observations in our study concurs with previous studies from solar salterns that reported genera *Aspergillus* and *Penicillium* as sister groups with joint evolutionary ancestry (10,29). Likewise, genus *Curvularia* and *Alternaria* reveal close relatedness and shared ancestry in *Pleosporaceae* family (30).

Nonetheless, our study has some limitations. The study did not critically evaluate seasonal variability patterns that may positively impact on total fungal community from the study setting. Morphological and molecular identities of study isolates may not represent entire fungal biota across Kenyan saline-alkaline ecosystems. DNA pyrosequencing and whole genome sequencing were not incorporated for heightened detection of fungal species in the catchment setting.

Conclusion:

Notwithstanding we observed significant difference in morphological and molecular identification among the isolates in our study that was discerned through observed distinct phenotypic features relative to molecular identities. Importantly, *C. cladosporioides* and *A. fumigatus* were most predominant species among all profiled isolates. Fungal diversity and taxonomic richness are significantly influenced by salinity levels, pH, temperature and variable culture media types.

There is need to design studies that will optimize culture growth conditions for numerous fungal species due to lack of an independent growth medium ideal for cultivation of all fungal groups. Adoption of next generation sequencing to enhance characterization scope of halotolerant and alkaliphilic fungal species in Kenyan extreme environments.

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Contributions of authors:

NK, TW and MPN conceived and designed the study. NK, CA and BM performed the laboratory experiments and statistical analyses. NK, TW, and DM drafted the manuscript. TW, DM and MPN critically reviewed, edited and validated the manuscript. All authors read and approved the final manuscript version.

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Conflict of interest:

The authors have no known competing financial or personal interests to declare.

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