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Isolation and Characterization of Some Haloalkaliphilic Fungi from Egypt

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Abstract

Aspergillussalinarum, Cladosporiumsphaerospermum, and *Penicillium camemberti* were isolated as haloalkaliphiles from Egyptian soils. The three fungal isolates tolerate pH (5 – 11), and they grew on NaCl to 25% (w/v). They were properly recognized morphologically and genetically. *Aspergillus salinarum* is obligately halophilic fungus required at least 5% (w/v) NaCl and tolerated to 25% (w/v) NaCl at pH 10.0. They prefer KCl on NaCl, the obligate one tolerates 30%(w/v) KCl. Sucrose was preferred on sugar alcohol used followed by glycerol. Increasing glycerol concentration increased the tolerance of these fungal isolates to pH and salt stress. A screen for the ability to produce extracellular enzymes showed that they produce amylase, protease, cellulase and lipase on different conditions of growth. These fungal isolates have many biotechnological applications.

Keywords: Filamentous fungi – Haloalkaliphilic microorganisms – Extracellular enzymes- Polyextremophiles

1. Introduction

Salinity levels are just one of many environmental elements that have an impact on fungus growth [1]. Salinity inhibits the growth of fungal mycelium by reducing the availability of carbohydrates and by the detrimental effects of salts [2]. Extremophiles offer intriguing models for improving our comprehension of the functional evolution of stress adaptation. Their biology broadens our understanding of the variety of terrestrial life, and it has surprised scientists to discover that eukaryotes, as well as prokaryotes, have a remarkable aptitude to adapt to harsh environments. The fungi kingdom contains several successful instances. As a result, specialized fungi have been found in ecosystems that are extremely cold, dry, salty, acidic, and deep sea [3]. Thermoacidophiles and haloalkaliphiles are two examples of polyextremophiles, which are organisms that can adapt to several stresses at once [4]. The haloalkaliphiles have evolved to thrive in conditions of high salinity and alkaline pH. These characteristics make them intriguing systems for basic research and the investigation of biotechnology potential. These fungi, which buffer salinity and alkalinity by absorbing and/or constraining salt ions, produce organic acids and/or macromolecules, secrete macromolecules like cellulose degradation enzymes, and provide biomass that is advantageous for soil health. Haloalkaliphilic fungi are a special group of extremophiles that grow best under conditions of extreme salinity and alkalinity. Haloalkaliphilic fungi are anticipated to be used in biotechnology because they are a significant genetic resource for degradation and resistance genes [5]. According to [6], the development and stabilization of soil aggregates is largely dependent on soil fungus and other microbial activity. The active enzymes that soil-derived fungus make and secrete are tightly linked to the characteristics, types, health, and environmental factors of the soil.[7] isolated 52 unique isolates from lake Magadi in Kenya, the isolates were affiliated to 18 different genera with *Aspergillus, Penicillium, Cladosporium, Phoma* and *Acremonium* being dominant. A screen for the ability to produce extracellular enzymes showed that different isolates could produce proteases,

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chitinases, cellulases, amylases, pectinases and lipases.

Extremozymes often have higher reaction rates, the capability of destroying and/oreliminating xenobiotics (chemical compounds foreign to a given biological system), and the ability to modulate the hyperaccumulation of substances such as heavy metals, pollutants, and radionuclides. Extremozymes such as proteases, lipases, cellulases, and amylases are commercial enzymes that have been used in industry, especially in detergents [8]. [9] studied the haloalkaliphiles hydrolytic enzymes and they decided that they have the potential to be used in the development of new medicinal formulations. Overall, this study illustrates the potential of employing microbes from extreme environments to produce useful bioactive chemicals with a variety of applications in diverse industries.

This study aims at isolation, identification and investigation of haloalkaliphilic fungi from Egyptian soils to ascertain their characteristics and activities.

2. Materials and methods

Soil Samples: Four soil samples were used in this study, three of them were collected from soil located beside the Fertilizer and graphite Factory at Talkha, Dakahlia Governorate, Egypt (Latitude: 31° 03' 14.04" N and Longitude: 31° 22' 40.33" E). The fourth one was collected from Wadi El natrun, Egypt (Latitude: 30° 24' 59.99" N and Longitude: 30° 19' 60.00" E). The samples were collected from 15-25 cm from soil surface and saved in plastic bags sealed and then stored at 20 ºC until used.

3. Culturemedia

3.1. Isolation medium: Czapek'sDox agar medium [10] with some modifications was used for isolation and maintenance of haloalkaliphilic fungi. The pH value of the medium was adjusted at 10 using 2.5 % sodium carbonate solution. Also, the medium was supplemented with different NaCl concentrations (10, 15, and 20 % (w/v)) after autoclaved at 1.5 atm. for 20 minutes. 2.2. Identification media: Beside the medium used in isolation, other media such as Malt extract agar (MEA) [11]; Potato dextrose agar (PDA) [12]; Sabouraud dextrose agar (SDA) [13]; Rose Bengal agar(RBA) [14] ; Oatmeal agar (OA) [15] and Czapek yeast extract agar (CYA) [16], were used in fungal characterization and identification. After autoclaving at 1.5 atm. for 20 minutes, the pH was adjusted at 7 and at 10 (by using 2.5 % sterilized sodium carbonate solution). NaCl concentration was adjusted at 15% of NaCl (w/v) also NaCl free medium was prepared. The purified fungal colonies were identified morphologically using the most documented identification keys and manuals [17-24].

4. Image analysis of fungal isolates: Image analysis was made at Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt by using Olympus microscope X40 and X10.

4.1.Genetic identification of fungal isolates: Genetic identification of haloalkaliphilic fungi was carried by analyzing 18S rRNA full sequencing according to [25] at Macrogen Company for Humanic Genomics by using primers: Forward (NS1);5' (GTA GTC ATA TGC TTG TCT C) 3' and Reverse (NS8) ;5' (TCC GCA GGT TCA CCT ACG GA) 3'.4.2. Phylogenetic analysis: The evolutionary history was inferred using the UPGMA method [26]. The optimal tree with the sum of branch length = 0.03519049 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [27] and are in the units of the number of base substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 476 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [28].

5.Growth parameters of the isolated fungi: In each case triplicate sets of 250 conical flasks were sterilized at 1.5 atm for 20 minutes, the pH of the medium was adjusted at 10, then inoculated with 2 discs of each fungal isolate(separately) and incubated at 28 ± 2ºC for 10-14 days. Cultures were removed after incubation period and the mycelial dry weights were obtained.

5.1-Effect of pH value of the medium on growth of haloalkaliphilicfungal isolates. The fungal isolates were subjected to different pH values by using Dax's liquid medium at 15% sodium chloride(w/v). The pH (5, 7, 8, 9,10,11 and12) was adjusted after autoclaving at 1.5 atm for 20 minutes by using Na_2CO_3 (2.5%) and diluted HCl.

5.2-Effect of different salts on growth of haloalkaliphilic fungal isolates. The following salts, NaCl, $CaCl_2$, KCl, $MgsO_4$, NH_4Cl and NH_4No_3 were

added to Dox liquid media at a concentration of 15% (w/v).

5.3-Influence of potassium chloride concentration on growth of haloalkaliphilic fungal isolates. Dox's liquid media supplemented with different potassium chloride concentration; 0, 5, 10, 20, 25 and 30% (w/v) were used. Additional concentrations of potassium chloride (1, 2, 3, 4%) were made for *Aspergillus salinarum.*

5.4- Influence of different temperatures on growth of haloalkaliphilic fungal isolatesDax's liquid media supplemented 15% with potassium chloride concentration. Triplicate sets of 250 ml conical flasks were sterilized at 1.5 atmosphere, for 20 min, the pH of the medium was adjusted at 10 then inoculated with two discs (of each fungal isolate) and incubated at different temperatures (15 ,20 ,28,30 ,35 ,40 and 45 ±2 °C) for 14 days. The mycelial dry weights were determined.

5.5-Effect of different sugar alcohols on growth of haloalkaliphilic fungal isolates. The following carbon compounds were added separately to the basal medium (carbon free Dox s liquid medium) which supplemented with 15%(w/v) KCl. Erythritol, Xylitol, Inositol, glycerol, mannitol, sorbitol was used, sucrose was used as a control at 20 g/L (w/v) (sugar alcohols concentrations were calculated on the bases of sucrose content in Dox s medium). In the second experiment different concentrations of KCl were used (15 % and 20% (w/v)) with different concentrations: 0 .5, 1.0, 1.5, 2.0, 2.5, 3.0, ,5.0,7.0 %(w/v) of glycerol (to identify the effect of increasing glycerol concentration with different KCl concentration).

5.6. Detection of some extracellular enzymes at different conditions of growth: The fungal isolates were screened for their ability to produce proteases, amylases, lipases and cellulases enzymes. Two conditions were used for fungal growth, the first one was at neutral pH and free salt medium (*Aspergillus salinarum* was at 5% (w/v) KCl) the second condition was at pH 10 and 15 % KCl(w/v). Enzyme production was evaluated on solid medium. To visualize the enzymatic activity a specific substrate of each enzyme was added to the culture medium as a carbon source or nitrogen source. After inoculation and incubation of cultures for 2–5 days depending on the enzyme and growth rate of the isolate, the appearance of a clear halo or precipitation around the fungal growth indicates enzyme production. Amylase activity and Lipase activity [29]. Cellulase activity [30]. Protease activity [31].

Statistical analysis: **Statistical analysis** was carried out using one way Analysis of Variance (ANOVA) with posttest if P< 0.05 and using software GraphPadInStat 3.06 Guide.

3. Results:

Analysis of Soil samples: Table (1) indicates the results of the analysis of the four soil samples, the pH of the soils tends to be alkaline (7.25-8.5). The texture was sandy to sandy clay, they contain many soil anions and cations (Ca^{++}, Mg^{++}, K^{+} and Na^{+}). Large amount of total dissolved salts specially in soil 1 followed by soil 2 and 3 while the least amount was in soil 4.

Table (1): Physical and chemical properties of soil samples used in the isolation of haloalkaliphilic fungi

Soil No.	Physical texture	PH	E.C. Ds/m	(TDS *mg/ l	*Soluble Cationsmeq/L				**Soluble anion meq/L			
					Ca++	Mg++	Na+	K+	CO^{3--}	HCO^{3-}	CL-	SO4
1	Sandy	7.25	2.35	1522	14.691	6.435	1.739	0.204	0.000	1.600	20.2	2.284
2	Sandy gravel	7.98	0.79	494	4.591	1.839	1.696	0.281	0.000	2.240	2.9	3.426
3	Sandy clay	8.02	0.401	235	3.214	0.460	0.435	0.230	0.000	2.240	0.2	1.904
4	Sandy Clay	8.5	8.03	91.59	42.11	12.24	54	1.57	0.00	0.24	74.6	33.9

***TDS=Total dissolved salts **meq/L=ml equivalent /L**

Isolation and identification of haloalkaliphilic fungal isolates

Twenty fungal isolates were isolated from the four soil samples. The most potent three ones were chosen to complete the search.

Culture characterization and identification of the most potent fungal isolates

The three fungal isolates were isolated on modified Czapek's Dox agar medium at pH 10 and tolerated 15 % NaCl. The first fungus has white color on all media used whether at 15 % (w/v) NaCl, at pH 7 and pH 10. This fungus didn't grow on NaCl free medium, it requires at least 5% (w/v) NaCl. It has

velvety growth on Dox, s, PDA and CYA culture media, while it has only substrate mycelium (yeast like) on MEA and RBA media at all pH values. On OMA medium at pH 7 it has only substrate mycelium (yeast like) while it has good velvety white growth on pH 10. There were no exudates or soluble pigments on all media used at different pH values. The morphological structures, represents that, the hyphae grow straight, mostly unbranched. Conidiogenous cells were phialides laterally borne on short unbranched conidiophores. Conidia were globose to sub globose and produced in long chain or occasionally heads. This isolate resembles*Phialosimplx sp.* (Plate 1). This isolate may be *Phialosimplxsalinarum,* that, it couldn't grow on NaCl free medium it required at least 5% (w/v) NaCl. The image analysis revealed that this fungus had conidiophore length of 13.36±2.41 µm and width of 2.50±0.40 µm while the conidium width was 1.85±0.30 µm and length was 2.28±0.68 µm.

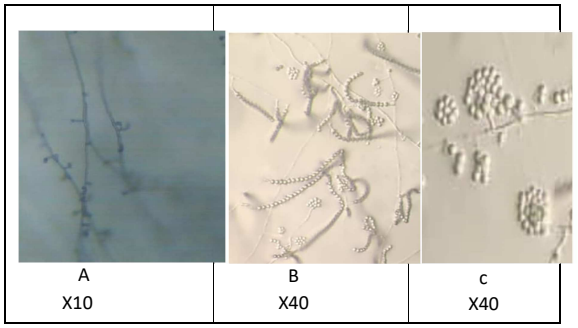

Plate (1): The white fungal isolate. A and B at pH 7 and 5.0% NaCl, and C 20% NaCl and pH 10

The second fungal isolate has velvety olivaceous dark green color colonies on all media used. The growth of the fungus at pH 7 was heavy on PDA medium, moderate on RBA and CYA, low on MEA and OMA media and scarce growth (only substrate mycelium) on SDA medium. The growth at pH 10 was low on all media used. No soluble pigments, no exudates, no sulcation, and the reverse of the colony was dark yellowish on all media. Plate (2) recorded the images of the fungus. The hyphae were septate, erect, pigmented, conidiophores are branched, septate, and dark, long and wide. The structure of the conidiophores was tree-like, conidia dark-pigmented (micro and macro conidia present). This fungus resembles *Cladosporium sp.* the image analysis of this fungus revealed that it had macroconidia of14.86±3.36 µm with width 4.40±0.76 µm and the microconidia had length of 5.28±0.79 µm and width of 3.30±0.71 µm.

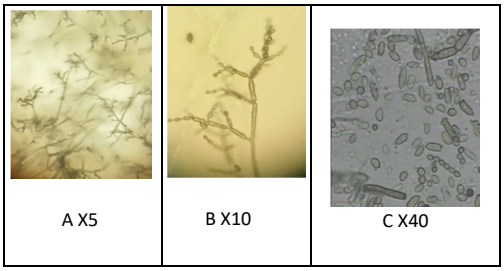

Plate (2): Dark Oily green(olivaceous) fungal isolate – A and B at pH7 and zero NaCl and C at 15% NaCl and pH 10

The third one has green color at pH 7 on all media while the colony color tends to be faint cottony brownish green on all media at pH 10. The growth of the fungus was heavy, green in color on CYA, OMA, MEA and PDA at pH 7 and pH 10. The growth at pH 10 was low on RBA and SDA while it was moderate on modified Dox, s medium. No soluble pigments, no exudates, no sulcation, and the reverse of the colony was yellowish white on all media used. Plate (3) recorded the images of the fungus, the hyphae were septate hyaline, simple or branched conidiophores, metulae, phialides, and conidia are observed. The metulae carry flask-shaped phialides. They form brush-like clusters. The conidia are round, unicellular, and visualized as unbranching chains at the tips of the phialides. This fungusresembles *Penicillium sp.* it has longconidiophores 9.61±1.33 µm with width of 2.79±0.08 µm, 6.52±1.11 µm phialide length with diameter of 1.65±0.24 µm. Conidia were globose with width of 1.48±0.28 µm.

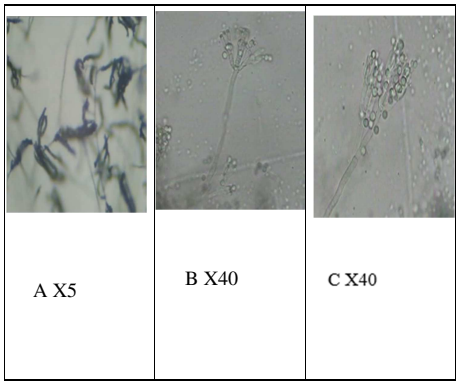

Plate (3): The green fungal isolate images. A&B at pH7 and zero NaCl, and C at 15% NaCl and pH 10

Genetic identification of the haloalkaliphilic fungal isolates**:** Phylogenetic analysis results after sequencing Table (2) showed the BLAST result of the relatives of the three fungal isolates which indicated that, the white isolate was *Aspergillussp*

by identity 99%, the green one was *Penicilliumcamembert* by identity 92 % and the olive dark green fungus was *Cladosporium sphaerospermum* by 99 % identity or may *Cladosporiumcladosporioides* but morphological feature resembles *Cladosporium sphaerospermum* .So, the morphological identification agreed with the genetic identification in the case of the two fungal isolates ,while the white one when applied to the GenBank the data indicated that it is *Aspergillus salinarum* .About *Aspergillus salinarum*, it is an obligate halophilic fungus, because it doesn't grow without salt the concentration needed at least 5% NaCl, while the two other isolates, *Penicilliumcamembert and Cladosporium sphaerospermum* are tolerant halophilic fungi.

Table (2): Blast results of 18S ribosomal RNA gene, partial sequence

Name	AC	Gene	Pct.(%)
H210908-020_I05_1_NS1	HQ188293.1	*Aspergillus*sp NIOCC 1	99
H210908-020_M05_2_NS1	GQ458020	*Penicilliumcamemberti* strain MA09-L	92
H210908-020_A07_3_NS1	JN939021.1	*Cladosporiumcladosporioides*strain DAOM 196948	99
H210908-020_C07_3_NS24	KJ443070.1	*Cladosporium sphaerospermum*	99

All of them tolerate alkaline conditions at pH 10.So, they are haloalkaliphilic fungi. They take new accession numbers recorded in table (3).

Table (3): GenBank accession no of the fungal isolates used in this study

No. of Isolate	GenBank Accession No.	Identification
1	ON970492	*Aspergillus salinarum* isolate H210908-020_I05_1_NS1
2	ON975038	*Penicillium camemberti*
3	ON705117	*Cladosporium sphaerospermum*

The isolates were blasted, and based on their % similarity; the nearest neighbors in the data bank were identified. The isolates were all distributed among three genera: *Aspergillus*, *Penicillium*, and *Cladosporium*, and they are all grouped together within the phylum Ascomycota. The created phylogenetic tree showed the isolates' phylogenetic position (Fig.1). According to the bootstrap (evolutionary distances) values, various isolate strains are closely related to other known strains from the Gene bank. *Penicillium camemberti* is more closely related to*Aspergillussalinrum* than *Cladosporiumsphaerospermum* according to evolutionary distances (Fig. 1).

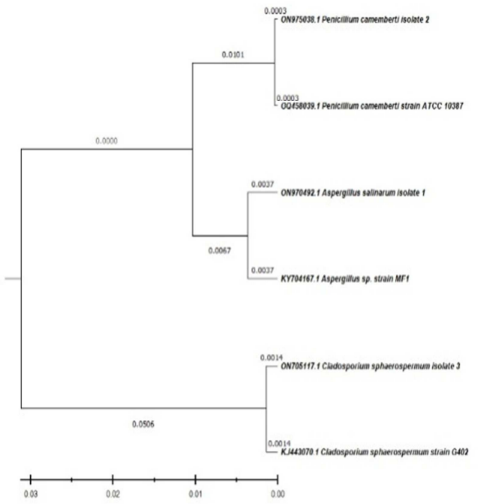

Figure (1): Rooted Phylogenetic tree created using Neighbor-joining method and is based on a comparison of the 18S ribosomal RNA sequences of three fungal isolates and their closest phylogenetic relatives

Growth parameters of the isolated fungi

1-Influence of hydrogen ion concentration on growth haloalkaliphilic fungal isolates.

The pH curve of the three fungal isolates ranging from 5 to 12 (Fig.2) no growth was detected above pH 11. The growth of *A. salinarum* reached its maximum at pH 7 followed by gradual decrease from pH 8 -11. Gradual decrease in the growth of *P. camemberti* and *C. sphaerospermum* by increasing pH from pH 5 to pH 11 maximum growth was at pH 8 (Fig.2). The decrease in the dry weight was about 56.35 % *A.salinarum* ,49.06% *P.camemberti*and 54.84% for *C.spherospermum.* After the incubation period the pH of the medium was calculated, there is no decrease or increase in the pH of the medium from 5-7 while at pH 8, 9, 10 and 11the pH decreased to 6, 6.5 and 7 (respectively) for the three fungal isolates. The three fungal isolates were considered alkali tolerant fungi.

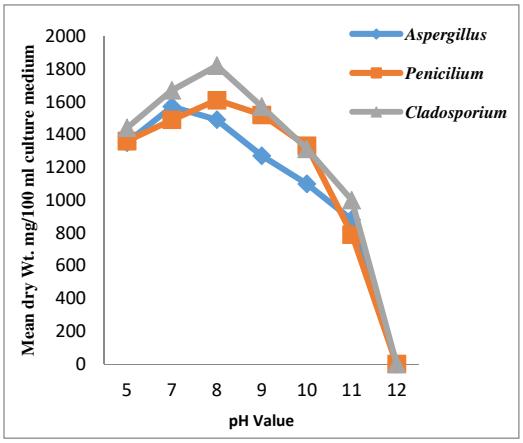

Figure (2): Influence of hydrogen ion concentration on growth of haloalkaliphilic fungal isolates *A. salinarum, P.camemberti* and *C. sphaerospermum* grown on 15 % sodium chloride

2-Effect of different salts on growth of *A. salinarum, P. camemberti* and*C. sphaerospermum* at pH 10.

The fungal isolates grow well on all slates used except that *C.sphaerospermum* didn't grow on NH_4Cl and NH_4NO_3 also *P. camemberti* didn't grow on NH_4NO_3 (figure 3). Using KCl instead of NaClincreased the dry weight of the three fungal isolates compared to NaCl and other salts. Using NH_4Cl and NH_4NO_3instead of NaCl resulted in a decrease in dry weight of *A. salinarum* compared to calcium chloride. $MgSO_4$resulted in an increase in dry weight of *C. sphaerospermum* while itdecreased the dry weight of *A. salinarum* and*P.camemberti*, whereas using calcium chloride resulted in increase in dry weight of *A. salinarum* and *C. sphaerospermum* (slight increase) and decrease in dry weight of *P. camemberti* (fig.3)comparing with the growth on NaCl. So, potassium chloride was chosen for forward experiments. Different concentration reached its maximum at 25% (w/v) KCl, then the growth was decreased by increasing the concentration to 30 %(w/v). As in the case of NaCl this fungus has at least 5% KCl requirement for its growth. The growth of *P. camemberti* and *C. sphaerospermum*was initiated in KCl free medium and increased by increasing KCl concentration to 15%(w/v) KCl, then the growth began to decrease. The growth of *C. sphaerospermum*was significantly decreased by increasing the concentration to 30% w/v) while that of *P. camemberti* stopped completely at 30% (w/v) (Table (4)).

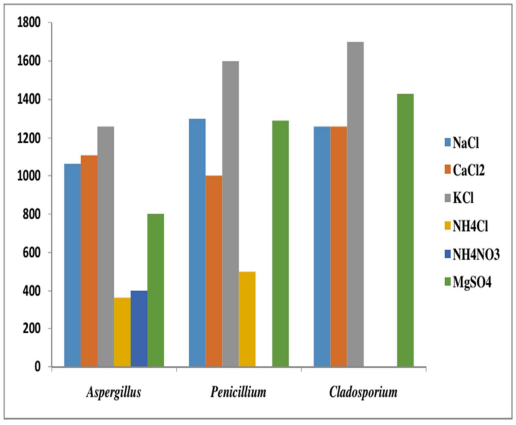

Figure (3) Effect of different salts on growth of haloalkaliphilic fungal isolates *A. salinarum* ,*P. camembertiandC. sphaerospermum* at pH 10

Table (4): Influence of potassium chloride concentration on growth of haloalkaliphilic fungal isolates *Aspergillus salinarum,Penicillium camemberti* and*Cladosporium sphaerospermum*

KCl % (w/v)	Mean mycelia dry Weight (mg/100ml) ±SD		
	Aspergillus salinarum	***Penicilliumcamemberti***	***Cladosporium sphaerospermum***
Zero	No growth	1160±134.54	1193.3 ±92.92
5	910±15.27	1323.3±68.06	1466 ±43.58*
10	1210±106.1*	1440±43.589*	1620 ±25.166*
15	1320±60.8*	1720±77.675*	1700 ±37.58*
20	1430±92.9*	1580±125.83*	1560±104.8*
25	1830±115.2*	1510±104.08*	1490±36.01*
	110±57.7*	No growth	860±28.8*

Significant VS 5% control for *Aspergillussalinarium*- * Significant VS control 0.0% *Penicillium camemberti* - * Significant VS control 0.0% for *Cladosporium sphaerospermum*

3-Influence of different temperatures on growth of haloalkaliphilic fungal isolates *A. salinarum,P. camemberti* and *C. sphaerospermum*.

From figure (4) the optimum temperature of the growth of the three fungal isolates was at 28±2 °C. They tolerate temperatures from 20-35°C and completely inhibited at 40-45 °C.

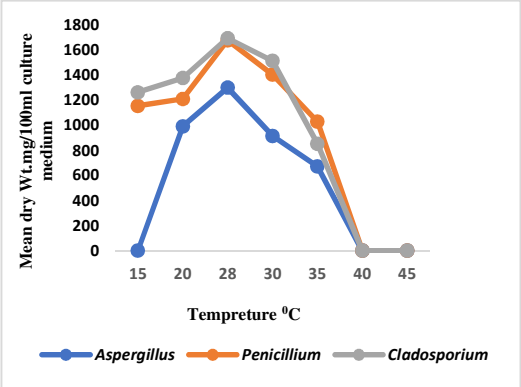

Figure (4): Effect of different incubation temperatures on growth of haloalkaliphilic fungal isolates *A. salinarum, P. camemberti* and *C. sphaerospermum*.

5-Effect of different sugar alcohols on growth and adaptation of haloalkaliphilic fungal isolates *A. salinarum, P. camemberti* and *C.*

Sucrose was examined with some sugar alcohol; it was the best carbon source followed by glycerol (Fig.5). Many literatures appeared that glycerol *sphaerospermum.* has significant role in the adaptation of microorganisms in extreme conditions.So, it is worth identifying the effect of different concentrations of glycerol on the growth of the has significant role in the adaptation of microorganisms in extreme conditions.So, it is worth identifying the effect of different concentrations of glycerol on the growth of the three fungal isolates. At concentration of 15% KCl, the growth of *A. salinarum* increased by increasing glycerol concentration reached its maximum at 3%. At 5% and 7% glycerol there was no significant decrease in the growth comparing to 3%, while by increasing the KCl concentration to 20 % the growth was increased by increasing the glycerol concentration till 2%, then the growth was sharply decreased by increasing the concentration to 5% glycerol (50% only comparing to growth at 2%) (Fig.6 A and B) . The growth of *C. sphaerospermum* increased by increasing glycerol concentration up to 2.5%, at concentration of 15% KCl (Fig.6A), increasing the concentration of glycerol from 3% to 7% (w/v) decreased the growth of the fungus while at 20% KCl the growth of*C.sphaerospermum* increased by increasing glycerol concentration reached its maximum at 2%, then the growth decreased by increasing the concentration to 5% glycerol (Fig.6B). Fig.6(A and B) appeared that the growth of *P. camemberti* at 15% and 20 % (w/v) KCl was increased by increasing glycerol concentration reached its maximum at 3%, then decreased by increasing the concentration to 7% glycerol in the case of 15 % salt while at 20 % KCl no growth was detected. The growth ofthe three fungal isolates was inhibited completely at 7% (w/v).

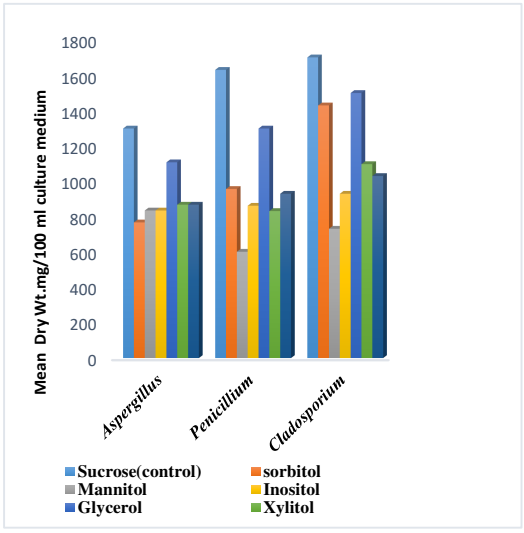

Figure (5): Effect of different sugar alcohols on growth of haloalkaliphilic fungal isolates *A. salinarum,P. camemberti* and*C. sphaerospermum*grown on 15% KCl at pH 10

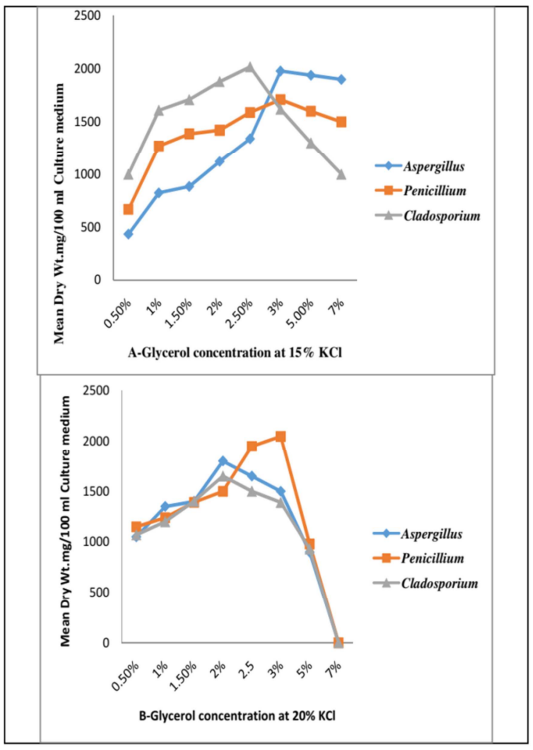

Figure (6): Influence of glycerol concentration on growth of haloalkaliphilic fungal isolates *A.salinarum , P. camemberti* and *C. sphaerospermum*

6-Production of some extracellular enzymes by haloalkaliphilic fungal isolates *A.salinarum* , *P. camemberti* and *C. sphaerospermum* at different conditions.

Figure (7) showed the production of protease, amylase, lipase and cellulase enzymes by *A. salinarum; P. camemberti* and *C. sphaerospermum* at different conditions of growth, at pH7, pH 10, and on medium containing KCl and medium free of KCl (except *Aspergillussalinarum* which grows at 5% and 15%). Neutral and haloalkaliphilic conditions proceed enzymes (protease, amylase and cellulase) production by the three fungal isolates, while haloalkaliphilic conditions did not proceed lipase enzyme production by *P. camemberti* the neutral conditions proceeded its production by high amount.High amounts of cellulase enzyme were secreted by the three fungal isolates at all conditions applied (the clear zone was 40;25 mm for *A. salinarum;* 50;50 for *P. camemberti* and 50;45 for *C. sphaerospermum* at neutral an haloalkaliphilic conditions respectively). The production of amylase and lipase enzymesincreased by *Cladosporium sphaerospermum* at extreme conditions than its production at neutral conditions.

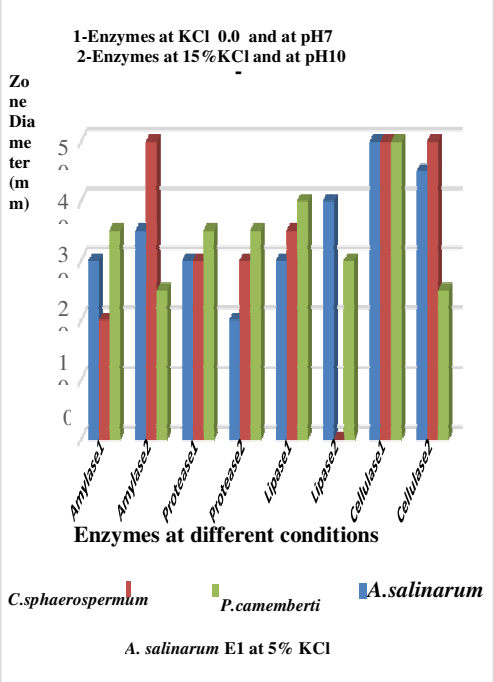

Figure (7): Secretion of extracellular enzymes by haloalkaliphilic fungal isolates *A. salinarum, P. camemberti* and *C. sphaerospermum* at different concentrations of KCl and at different pH values

6. Discussion: Microorganisms have the ability to adapt and survive in harsh abiotic conditions [32]. These types of conditions facilitate numerous modifications and adjustments of specific fungal pathways that allow the creation of various metabolites.[33] reported that polyextremophiles must have developed novel adaptive strategies enabling them to grow and proliferate under multiple extreme conditions. There are several polyextremophile combinations that have not yet been seen on Earth. Extremophiles can thrive in any harsh environment by combining diverse biological survival mechanisms, novel polyextremophiles can be produced [34]. Polyextremophiles, which can survive in a wide range of extreme environments, make excellent models for potential extraterrestrial homes. Twenty fungal isolates were isolated from the four soil samples. The most potent three ones were chosen to complete the search. The three fungal isolates were identified morphologically and genetically as *Aspergillus salinarum*, it is an obligate halophilic fungus, because it doesn't grow without salt the concentration needed at least 5% NaCl, while the two other isolates, *Penicilliumcamembert and Cladosporium sphaerospermum* are tolerant halophilic fungi. They tolerated pH 2-11. The ability of the three fungal isolates in the current study to grow at high pH and high salt concentrations allowed them to be as true polyextremophiles. [35] isolated black yeast-like and related melanized fungi of the genus *Cladosporium* from rock soils, naturally hypersaline environments, salted food, as well as various anamorphic *Aspergillus, Penicillium, teleomorphicEmericella*and*Eurotium species, Wallemia spp.,* as well as various species of non-melanized yeasts. In biotechnology, the capacity to withstand salt stress is a distinctive trait for such species. *Aspergillusbaarnensis*, *Aspergillussalisburgensis*, and *Aspergillusatacamensis* are obligate halophilic fungi that strictly require NaCl from 5 to 10% [36-37]. Other obligatory halophilic fungi include *Wallemiaichthyophaga* and *Wallemiamuriae*.Many researchers isolated obligate halophilic fungi as [38] *Gymnascellamarismortui*, [39] *Trichodermapiluliferum*fs. *Halophila AZ*, [40]*Aspergillus unguis*, and *Aspergilluspenicillioides* [41. [42] Claims that *A. restrictus* develops poorly on NaCl-free media and needs between 15% and 20% (w/v) of NaCl for viability and robust growth. Therefore, it is a halophilic fungus rather than a halotolerant one, as stated by [43-44].The three fungal isolates grow at

pH from 5-11, they have maximum growth at pH 8 so the were considered as alkaliphilic fungi. *Aspergillus glaucus* CCHA, a halophilic fungus that exhibits exceptional salt tolerance with a salinity range of 5-32% necessary for growth (saturation, NaCl), was identified by [45] from air-dried wild plants from the surface perimeter of a solar salt field. Unexpectedly, *A. glaucus* CCHA can survive in a wide range of pH solutions, from 2.0 to 11.5-indicating that it is a haloalkaliphilicfungus.According to research by [46], some genera, including *Cladosporium, Aspergillus, Penicillium, Alternaria,* and *Acremonium*, have species that are either weakly or moderately alkalitolerant. *Aspergillus, Penicillium, Cladosporium, Phoma,* and*Acremonium* were isolated by [7] using soil from Lake Magadi. Haloalkalitolerant and haloalkaliphilic fungi were isolated from Turkey's Acgöl Lake by [47]. They talked about how the isolated fungi withstood varying amounts of salt and pH, tolerating 0 to 20 percent NaCl at pH 8 to 10. The majority of the fungi belonged to the species *Aspergillus* and *Penicillium*, as well as *Cladosporiumacalypha.*It was determined from the pH range that isolates found in Lake Magadi are alkaliphiles and not acidophiles due to the lack of significant fungal development at pH4. This is comparable to the research of alkaliphiles in the Saharan Salt Flat in Southern Tunisia conducted by [48]. According to [7], most of the fungus identified from Lake Magadi are alkaliphiles because the lake's pH ranges from 9 to 10. Other diversification studies on yeasts and filamentous fungi, such as those conducted in the muds of the hypersaline alkaline lakes of Wadi El-Natrun, similarly revealed a mean pH of 9.21 to favor the growth of alkaliphiles [49].The three fungal isolates *A. salinarum,P. camemberti* and*C. sphaerospermum* have optimum temperature at 28±2°C, [7] noted that the majority of the fungi recovered from Lake Magadi did not have a specific temperature requirement.[1] reported that numerous elements of the environmental system, such as salt levels, have an impact on fungal growth. In comparison of NaCl and other utilized salts,thatKCl was preferred for the growth of fungal isolates. Salinity restricts the growth of fungal mycelium due to the detrimental effects of salts and decreases the availability of carbohydrates [2]. According to [50], in conditions of high osmolarity, fungi can prevent water loss by accumulating K^+ ions within their cells, while other organisms can do the same by storing osmolytes, such as polyols, sugars, and amino acids, as suitable organic solutes [51]. A few bacteria belonging to the orders *Natranaerobiales* and *Halanaerobiales*, as well as the well-known halophilic bacterium *Salinibacterruber*, a member of the Bacteroidetes, employ KCl as the dominant osmolyte [52]. The present observation agrees with [53] who reported that the enzymatic activity of ligase N from *Haloferaxvolcanii* is very low in the presence of NaCl and that KCl is needed to increase it. The influence of salt type and concentration on the growth of *Penicilliumoxalicum* and *Aspergillusniger* is studied by [54]. NaCl, $MgCl_2$, and CaCl2 were utilized at four different concentrations. Fungal development is significantly impacted by salt concentration increases, yet they are more tolerant of high magnesium salt concentrations than sodium and calcium salts. On the other hand, *H. werneckii* was examined by [55]. This organism can withstand high NaCl concentrations as well as the chaotropic salts $MgCl_2$ and $CaCl_2$, and it can also be isolated from bitterns that are high in Mg. Due to KCl's lower toxicity and compatibility with their biological processes, halophilic fungi prefer it to NaCl [56]. Additionally, KCl can aid in regulating several enzymes' activity and membrane potential [50]. In order to balance the osmotic pressure, some halophilic fungi, such *Wallemiaichthyophaga*, can accumulate significant amounts of intracellular K^+. This is because K^+ has a stronger affinity for these organisms than Na^+. According to [50] and [56], some halophilic fungi, including *Hortaeawerneckii*, can withstand high NaCl concentrations as well as other salts, like $MgCl_2$. Considering this, the preference of the halophilic fungi for KCl over NaCl may differ depending on their species and the environment.It has been established that polyols function as compatible solutes in *W. ichthyophaga,* much like they do in *H. werneckii*, and that glycerol is the most prevalent of them. Cells reject Na^+ and maintain low intracellular K^+ and Na^+ concentrations even in environments with exceptionally high NaCl concentrations together with glycerol buildup [57-58]. In a study of 32 haloarchaeal genomes, [59] found that 27 of them could use glycerol as a source of carbon and energy. In their discussion of osmotic stress, [60] noted that both halotolerant and halophilic fungi use polyols (mannitol, erythritol, glycerol, and arabitol) as osmotic solutes in order to prevent salt entry into their cytoplasm. They also noted that fungi in extreme environments produce extremolytes and extremozymes. [7] and [9] suggest that in high osmolarity conditions, haloalkaliphilic fungi can accumulate osmolytes (polysols, sugars, and amino acids) as suitable organic solutes to counteract the loss of water. According to [61], 5% glycerol

completely reduced both the growth and cellulolytic activity of *N. frontalis*. *Acinetobacter*, *Haloferax, Halobacterium*, *Halorhabdus*, *Marinococcus*, *Micrococcus*, *Natronococcus*, *Bacillus*, *Halobacillus*, and *Halothermothrix* are just a few of the halophiles that produce extracellular enzymes, according to studies by [62-63]. Alkaliphilic enzymes that are useful for industry as well as improving fungal bio-control activity have received a lot of interest [64-65]. These comprise cellulases, pullulanases, xylanases, lipases, and proteases. In various industrial processes, filamentous fungi are employed to create enzymes and metabolites. Faster manufacturing and easy enzyme modification are only a few of the many benefits that come with the manufacture of enzymes by fungus, in addition to low material costs and high productivity. The enzymes can also be easily recovered from the medium because they are typically extracellular [66] and [65]. [7], have been discovered that, fungi produce extremoliths and extremozymes in saline environments to cope with osmotic stress. The enzymes produced by halophiles, such as amylase, xylanase, protease, cellulase, esterase, amylopullulanase, endo-1, 4-xylanase, etc., were discussed in a report by [67]. According to [68] marine microorganisms are attractive sources of enzymes with industrial uses due to their enormous genetic and metabolic diversity.

4. Conclusion

These fungi are promising sources of haloalkaliphilic enzymes and have many biotechnological applications in industry and agriculture. So further research is needed to identify.

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