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Sodium Chloride Stress Induced Morphological Changes in Some Halotolerant Fungi

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ABSTRACT

Materials and methods: Nine fungal isolates namely *Emericill anidulans, Mucor racemosus , Alternaria pluriseptata Penicillium canescens, Syncephalastrum racemosum, Aspergillus fumigatus, Alternaria chlamydospora, Aspergillus parasiticus* and *Ulocladium atrum* were isolated from AL – SHEGA area at AL- QASSIM region. **Results:** The influence of different sodium chloride concentrations on the growth rate, morphological and ultrastructure were studied. Considerable differences in their growth rate and morphology were detected on medium containing different concentrations of sodium chloride (NaCl). Low growth rates were obseved on high NaCl concentrations . At 15 % NaCl, low growth of *Emericill anidulans, Penicillium canescens, Syncephalastrum racemosum, Aspergillus parasiticus* and *Mucor racemosus* was detected , whereas all fungal isolates were failed to grow at 20% NaCl. Scanning Electron Microscope (SEM) revealed that all fungal asexual reproduction organs were metamorphosed at higher NaCl concentration, fungal heads and sporangia were speculated or elongated. Sporangiophores and conidiophores were shortened and dwarfed ,little number of conidia or spores were detected.

Key words : halotolerant fungi ,salt stress ,SEM.sodium chloride, morphology

INTRODUTION

Follow fungi in desert region the strategy of fungus strain and representing growth of stressful environments, lack of food and low humidity, drought and high temperature or osmophilic [1]. Organisms living in environments with high concentrations of salts are challenged by osmotic stress and by the toxicity of specific ions [2].

Most fungi differ from the majority of halophilic prokaryotes .[3] they tend to be extremely halotolerant rather than halophilic and do not require salt to the survival, .[4] and especially the growth of microorganisms in highly saline environments requires numerous adaptations, [5].

The fungal cell wall is a supramolecular structure that offers strength to the cells, determines their shape, protects them against mechanical damage and mediates the cell-to-cell communication and their interaction with the environment. Physical and biological properties of fungal cell walls are determined by the composition and the arrangement of their structural components.[6]

The fungal cell wall is the first line of defense against environmental stress; therefore, adaptation at the cell wall level is expected to have one of the most important roles for successful growth at a highly saline [7]

The cell wall is essential for maintaining the osmotic homeostasis of cells, since it protects them against mechanical damage as well as high concentrations of salts.[8] Its balance between a rigid and a dynamic structure influences the shape of cells .[9] and enables growth and hyphal branching .[10]

Some studies have focused on the role of the fungal cell wall in chemical sensing and processing of environmental signals that control growth and cell morphology of microorganisms and synthesis and secretion of extracellular enzymes.,[11], [12], & [13]

Little is known about their mechanisms of adaptation to high salinity. To investigate the effects of low and high NaCl concentrations on cell morphology, with particular emphasis on cell wall ultrastructure.[14]

The dominant representatives and the most thoroughly investigated halophilic fungi in hypersaline waters of the salterns are the black yeasts, [15] and particularly the model organism *Hortaeawerneckii*.[16]

An important level of adaptation of the black yeasts to high salinity is seen in their extremophilicecotype , which is characterized by a special meristematic morphology and changes in cell wall structure and pigmentation .[17]

This work aims at investigating the effect of salt stress on growth of some halotolerant fungal isolates, also representing the effect of salt stress on their morphology by (SEM).

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MATERIALS AND METHODS

The soil samples used for isolation of halophilic fungi were collected from different localities representing different saline habitats at AL – SHEGA area at AL- QASSIM region

Organisms and Cultivation

Fungal isolates were isolated and identified [18] .

These fungi were grown on Dox's agar medium was used for the isolation of halophilic fungi , dilution plate method was employed for the estimation of soil fungi as described by Johnson et al ,1959,but with some modification as employed by Moubasher *et. al.* and his co-workers (1957) .

This medium consists of (g / L): $NaNO_3$, 2; $MgSO_4.7H_2O$, 0.5; Kcl, 0.5; KH_2PO_4 , 1; $FeSO_4.7H_2O$, traces; sucrose , 20; Agar, 20. All these components were added to 1L distilled water. The pH was set to 7 and then the medium was sterilized at 1.5 atm. for 20 min salt

Dox's agar medium supplemented with different concentrations of sodium chloride, 0, 5, 10, 15 and 20% (W/ V) . Each plate was inoculated with one disc of fungal isolate and incubated at 28± 2°C for 7 days, three replicates were made for each treatment also control plates(medium free of sodium chloride) were prepared. Colony size of each fungal isolates was measured.

Image Analysis System

The fungal isolates were subjected for certain morphological studies by an Image Analysis System at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt .

Scanning Electron Microscope (SEM)

Coated specimens of each fungal isolate were examined by using (SEM) model of JEOL JSM-5500LVat the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt .

RESULTS

Table 1 & 2 represented the description of **a** growth characteristics and image analysis examination of nine fungal isolates (*Emericilla nidulans*; *Mucor racemosus* ; *Alternaria pluriseptata* ; *Penicillium canescens* ; *Syncephalastrum racemosum* ; *Aspergillus fumigatus* ; *Alternaria chlamydospora* ; *Aspergillus parasiticus* and *Ulocladium atrum*) isolated from soil samples of AL- SHEGA area at AL- QASSIM region.

Influence of different NaCl concentrations on the growth of fungal isolates

Figure (1) shows the growth rate of all investigated fungal isolates cultivated in medium supplemented with various concentrations of NaCl. The results indicate that the isolates exhibited good growth rate at low salt concentration while all the investigated fungi were found to decrease their growth at high salt concentration. The most suitable concentrations for well fungal growth was at 5% NaCl. At 15 % NaCl only low growth of *Emericilla nidulans*, *Penicillium canescens*, *Syncephalastru mracemosum* , *Aspergillus parasitic* and *Mucor racemosus* was detected , whereas the investigated fungi failed to grow at 20% NaCl.

As shown in figure 1 heavy fungal growth attained at low NaCl concentration and decreased by increasing the salt concentration, so these fungal isolates are considered to be halotolerant fungi. The most tolerant ones were *Emericilla nidulans* (No.1) ; *Penicillium canescens* (No. 4); *Syncephalastru mracemosum* (No. 5) and *Aspergillus parasiticus* (No.8) (in descending order).

Table 2 shows Morphological characteristics of fungal isolates by Image Analysis System, In this study Nine fungal isolates were isolated and identified from soil samples they are as follows : *Emericilla nidulans* (No.1) ; *Mucor racemosus* (No.2) ; *Alternaria pluriseptata* (No. 3) ; *Penicillium canescens* (No. 4); *Syncephalastru mracemosum* (No. 5); *Aspergillus fumigatus* (No.6) ; *Alternaria chlamydospora* (No.7) ; *Aspergillu sparasiticus* (N.o 8) and *Ulocladium atrum* (No.9).

To study the effect of salinity on Morphological characteristics of the isolated fungi , they were grown on Dox's agar medium supplemented with different concentrations of sodium chloride, 0, 5and 10% (W/ V)).

The most halotolerant fungal isolates were used to identify the effect of different concentrations of sodium chloride on them by using S E M.

Table 3 shows that No 1 *: Emericilla nidulans*as at NaCl free medium have globosely heads surrounded completely by conidia. As this fungal was subjected to 5% NaCl ; the globosely heads shrunken appeared spatulas' to some extent the globosely conidia changed and reduced in number .At 10 % heads completely shrunken became rode in shape, little conidia appeared.

Number 2*:* *Mucor racemosus* at NaCl free medium sporangiophores and sporangia appeared normally , columella appeared obovate. At 5 % sporangia are shrunken, spores reduced in number. ; increasing NaCl to 10 % sporangiophores were dwarfed , sporangia were shrunken, and spores were rare.

Number 3: *Alternaria pluriseptata* this fungus was characterized by long conidiophores with chains of conidia has terminal peach .When *Alternaria pluriseptata* subjected to 5 % NaCl long conidiophores became shorter with chains of swollen conidia with rounded terminal. By increasing NaCl to 10% conidiophores became drastic with clusters of swollen conidia with rounded terminal or one swollen conidium.

Number 4: *Penicillium canescens* this fungus was characterized by heavily branching conidiophores carrying metulae and phialieds which have chains of conidia. At 5% and 10 % NaCl the fungus has the same appearance to some extent; conidiophores was less branching to some extent metulae and phialieds are carrying smaller number of conidia.

Number 5 : *Syncephalastrum racemosum* this fungus has long sporangiophores with terminal vesicle which forms merosporangia all over its surface on NaCl free medium while on NaCl medium sporangia are smaller in size carrying smaller No of spores to some extent; sporangiospores were less than before; drastic action appeared on sporangiophores.

Number 6: *Aspergillus fumigates* the halotolerant *A. fumigatus* has flask-shaped vesicle carrying sterigmata with long chains of conidia .Sodium chloride affected this fungus as the flask-shaped vesicle became longitudinal with swollen sterigmata carrying little No of conidia. By increasing NaCl concentration to 10 %, vesicles became longitudinal and drastic with drastic (to some extent) sterigmata carrying little number of shrunken conidia .

Number 7 : *Alternaria chlamydospora* at sodium chloride free medium conidia and conidiophores are appeared normally with some chlamydospores. At 5 % NaCl conidia and conidiophores were enlarged clusters of conidia appeared while at 10% NaCl conidia are enlarged ; conidiophores were appeared fine covered with sheath of salt, conidia reduced in number.

Number 8: *Aspergillus parasiticus* flask shape heads covered with sterigmata on long conidiophores were appeared at NaCl free medium. In presence of NaCl flask shape heads became triangle covered with sterigmata on long conidiophores carrying smaller No of conidia. At 10 % NaCl flask shaped heads became triangle or rounded covered with sterigmata or naked carrying smaller No of conidia . All the fungus covered with sheath of salt in presence of all NaCl concentrations.

Number 9 *: Ulocladium atrum* conidia were varicose, sometimes ellipsoidal with one or more transverse and longitudinal septa but most common spherical or subspherical. In presence of NaCl spherical and ellipsoidal smooth conidia were without septa, by increasing salt concentration ellipsoidal and longitudinal conidia were without septa .Hyphae covered with sheath of salt.

DISCUSSION

The investigated fungal isolates showed distinctly improved growth on different NaCl concentrations as the solute and these results were consistent with results of many other researches, As Vaupotič *et.al.* [19,] Yadav, *etal.* [20] and Nayak *et.al.*[21,] . Their growth across increasing NaCl concentration ranges shown here confirm these previous findings of Zalar *et al*. [22]

Emericilla nidulans has achieved first place in the ability to grow and survive at salt concentration of 15 % , also *Penicillium canescens*, *Syncephalastrum racemosum* , *Aspergillus parasiticus* and *Mucor racemosus* . So all these fungal isolates are moderately halotolerant .

Alternaria pluriseptata, *Aspergillus fumigates*, *Alternaria chlamydospora and Ulocladium atrum* are weak halotolerant, they able to grow and survive at 5 % NaCl and failed to grow at salt concentration of 15% .

Growth at high salinities requires the adaptation of cellular metabolism. The reductions in the final biomass yields indicate the high energy demands of life at high NaCl concentrations.[23]

The morphological characteristic of investigated fungi grow at different salt concentrations were consistent with results of Marjetka KraljKuncˇicˇ*et al*. [14] who demonstrated that NaCl concentrations have an impact on the cell morphology of the *Wallemia spp.* At a high salinity, the hyphal compartments in both *W. muriae and W. sebi* were thicker and shorter compared to what have been observed at the low salinity. Such changes of the cell morphology resulted in changed size and shape of the mycelial pellets. The pellets of both *W. muriae* and *W. sebi* were small and regularly shaped at the low salinity, whereas at the high salinity, they were larger and variable in shape.

Shortening and thickening of the hyphal compartments and similar changes in pellet morphology have also been seen in less salt-adapted fungi, such as in the halotolerant *Aspergillu srepens* when grown on medium with 12% NaCl (W/V) .[24] This showed distinctly improved growth with NaCl as the solute , Their growth across increasing NaCl concentration ranges shown here confirm these previous findings of Zalar*et al*. [22] Growth at high salinities requires the adaptation of cellular metabolism. The reductions in the final biomass yields indicate the high energy demands of life at high NaCl concentrations, even in such well-adapted species.[23] The common feature of black yeasts at the high salinities, is hypothesized to enhance the ability to survive under conditions of stress,[25] such as a high concentration of NaCl .[26]

Halophilic and halotolerant fungi showed a surprisingly rich diversity and abundance in natural saline environments . [27], This rich surprisingly and abundance is the result of the adjustment and adaptation of fungi in the salty environment and the impact on the fungal cell in one way or another.

At the impact of higher salinity in the elongated hyphal cells were shorter and thicker, which resulted in a changed surface-to-volume ratio. The decreased surface-to-volume ratio was mainly a consequence of decreased surface area, since the volume of the hyphal cells remained unchanged , but the thickening of the cell walls resulted in decreased functional volume of the cells. The thick cell wall might be important as an armor against changes of osmotic pressure, since it provides a mechanical protection against hyposaline stress at dilution conditions.**[14]**

The sphere has a minimal surface-to-volume ratio, and this cell shape could be one of the important adaptations for growth of halotolerant fungi in saturated NaCl. At high salinities, Similar multicellular structures have also been seen at high salinities in the phylogenetically distant, extremely halotolerant ascomycetous black yeasts, such as Hortaeawerneckii (T. Kogej and N. Gunde-Cimerman, unpublished data), Phaeothecatriangularis.[28] and *Trimmatostroma salinum* .[17] phylogenetically distant from *Wallemia* spp., all grow meristematically and have spherical cells ,[14] a finding which supports this conclusion. The overall morphological changes seen in *Wallemia spp*. correspond to the phylogenetics based on the ITS ribosomal DNA gene.[22].

Through the results of this study and which are consistent with results of many other researches, it was concluded that the acquisition of fungi recipe halophilic and halotolerant is affected by the result of change and deformation in the form of cell adapt to these fungi in saline environment.

CONCLUSION

The Scanning Electron Microscope (SEM), and Image Analysis System had demonstrated various morphological changes at high salinity in Nine fungal isolates . At high salinities, the common morphological feature was decreasing in the growth of fungal isolates by increasing NaCl concentration which means that all these fungi are halotolerant fungi.

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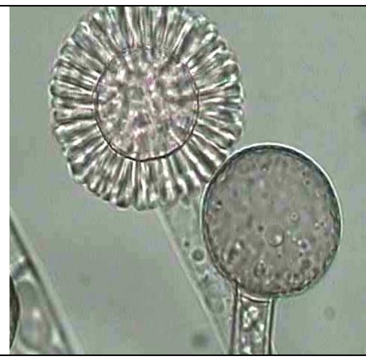
Table 1: A growth characteristics and image analysis examination of nine fungal isolates from soil samples of AL- SHEGA area at AL- QASSIM region.

No.	Isolated fungi	Character: *Culture Exam.:	* Microscopic Exam.:					
1	***Emericellanidulands var. aurantiobrunnea***	**Growth Characteristics**	**Conidial heads:**	**Conidiophores:**	**Vesicles**	**Sterigmata**	**Conidia**	**Hűll cells and Cleistothecia**
		Colonies attending 3-4 cm in 7 days at 25°C on both Czapek-dox and malt agar media, central initiate white and velvety and become cream to buff and granular or powdery golden browed reverse	Columnar, 43 µm in diameter	6.5 µm in diameter	10.6 µm in diameter	Sterigmata in two series. Primary 7.0X3.0 X 6.2X2.5 µm.	Globose to subglobose 3.5µm in diameter	Hűll cells abundant and cleistothecia usually in margin.
2	***Mucorracemosus***	**Growth Characteristics**	**Sporangia**	**Collumella**		**Sporangiospores**	**Chlamydospores**	
		Colonies white become brownish – grey; reaching 6 cm colony diameter in 4 days.	65.3 µm in diameter, wall spinulose	Collumellaobovoid, ellipsoidal, slightly pyriform (4 µm in diam).		7.5 X 5.3 µm in diameter	Chlamydospore numerous in sporangiophores	
3	***Alternariapluriseptata***	**Growth Characteristics**	**Conidiophores:**	**Conidia:**				
		Colonies grow rapidly, effuse, black.	Conidiophores 4.3µm thick.	Conidia obclavate, golden brown in colour, smooth 32.0X15.5 µm, with a short, pale beak 4.0 µm thick, with 2-7 transverse septa				
4	***Penicillimcanescens***	**Growth Characteristics**	**Penicillus type**	**Rami**	**Metulae**	**Phialides**	**Conidia**	
		Light yellow to light orange colonies on CYA with yellow to yellowish brown reverse. Micro colonies at 5°C and 37 °C.	Biverticillate, sometimes terverticellate	17.5X4.1 µm	14.2X3.0µm.	5.8X2.4µm.	Conidia globose , smooth walled 3.0 µm	
5	***Syncephlastrumracemosum***	**Growth characteristics**	**Sporangiophore**	**Vesicle**	**Merosporangia**	**Merospores**		
		Colonies on white becoming grey with age, 5-6 cm in 7 days.	Sporangiophore with curved lateral branches each bearing a terminal vesicle which forums	Vesicle globose; 40.0 µm.	Merosporangia cylindrical grey 25.0X3.0 µm.	Merosporess ubglobose 5.0 µm.		

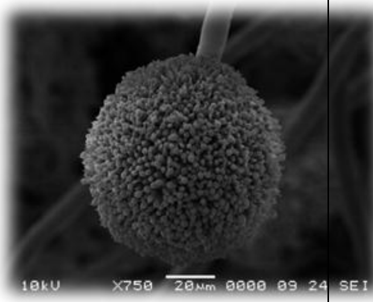
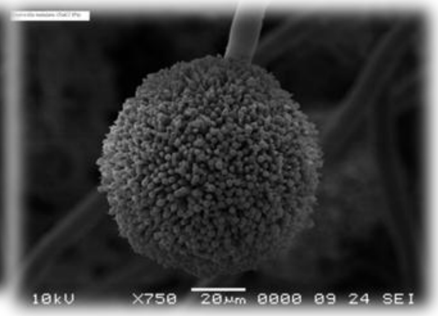
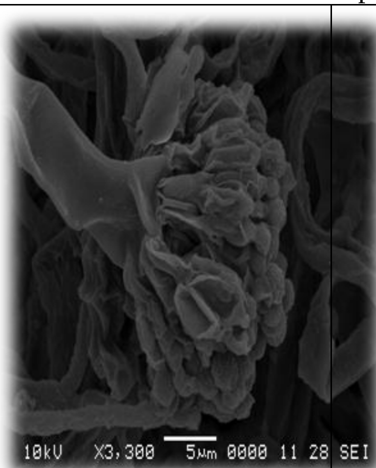
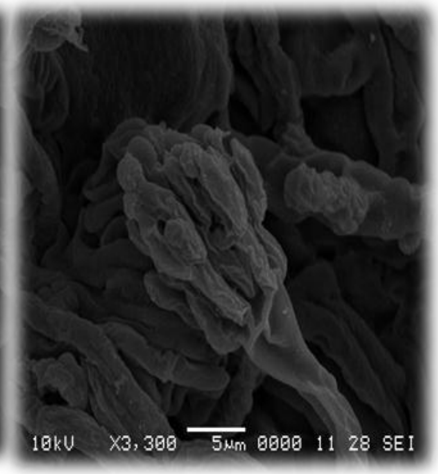
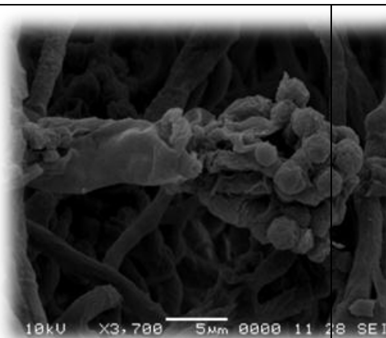
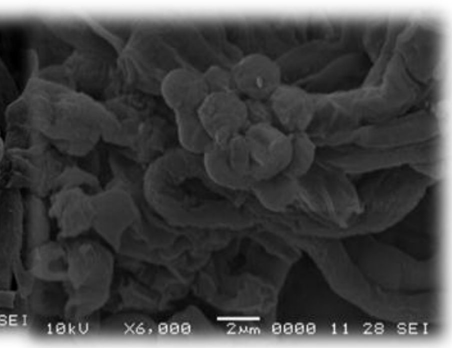
			merosporangia all over its surface.				
6	***Aspergillus fumigatus***	**Growth characteristics**	**Conidiophores**	**Conidial heads**	**Vesicles**	**Sterigmata**	**Conidia**
		Colonies spreeding rapidly on Czapek's agar and MEA at 25°C.	Smooth-walled conidiophore, 200µm in length &6.0 µm in diameter, The conidiophore gradually enlarged upward to give flask-shaped vesicle.	Columnar, 180 µm X 30 µm.	25 µm in diameter, fertile over the upper half only.	Sterigmata in one series, 6.0x2.2 µm.	Globose, echinulate, green-coloured, 2.8 µm in diameter
7	***Alternariachla mydospora***	**Growth characteristics**	**Conidiophores**	**Conidia**			**Chlamydos pores**
		Colonies floccose, dark olive to blackish brown with numerous chlamydospores.	Conidiophores 5.0 µm thick.	Conidia obclavate than swelling brown in colour, smooth 32.0X12.5 µm, with a short, pale beak 4.0 µm thick, with 2-5 transverse septa			Abundant
8	***Aspergillusp arasiticus***	**Growth characteristics**	**Conidiophores**	**Vesicles**	**Sterigmata**	**Conidia**	
		Colonies fast-growing, reaching 5-7cm diameters in four days at 25˚C on malt media; usually consist of a dense felt of yellowish green mycelia, with pale yellow reverse	Conidiophores are coarsely roughened, geenish yellow in color , 10.4 µm in diam.	Subglobose, 24.5 µm in diam.,	Sterigmata in one series, 8.6 – 3.33.4 µm.	Conidia globose, 3.5 µm in diam. Yellow-green, conspicuously rough-walled.	
9	***Ulocladiuma trum***	**Growth characteristics**	**Conidiophore**	**Conidia**			
		Colonies on MEA growing rapidly, golden brown to black. Black reverse.	5.5 µm. In diameter.	Conidia golden brown, or dark reddish brown, verrucose, sometimes ellipsoidal 25.6 X 15.8 with 1 – 3 transverse and 1 or more longitudinal septa but most common spherical or subspherical.			

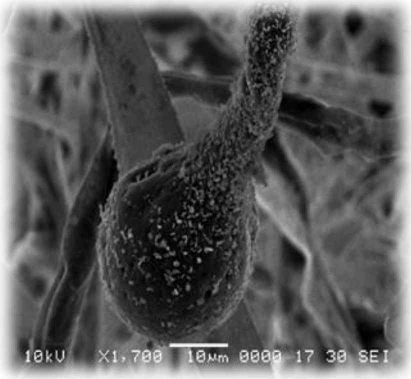
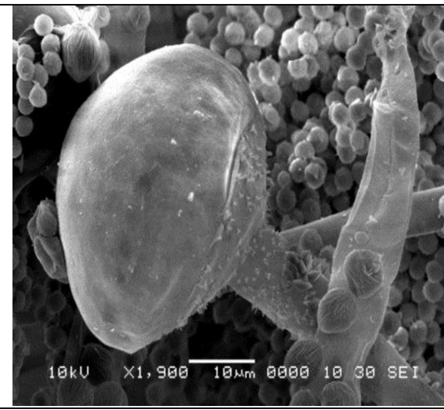
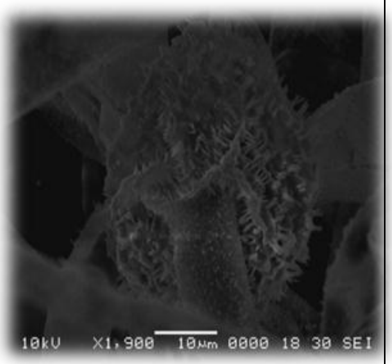
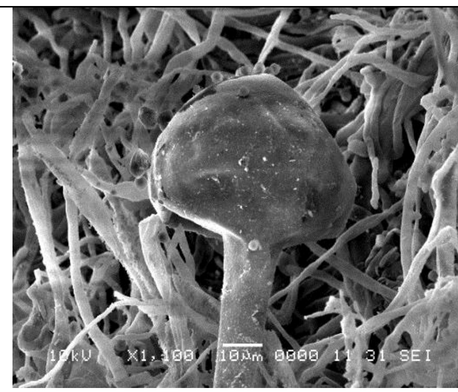
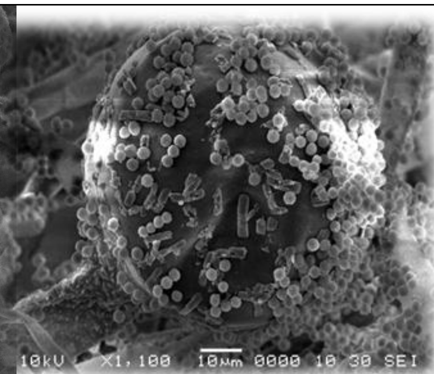
Table 2: Morphological characteristics of fungal isolates by Image Analysis System**.**

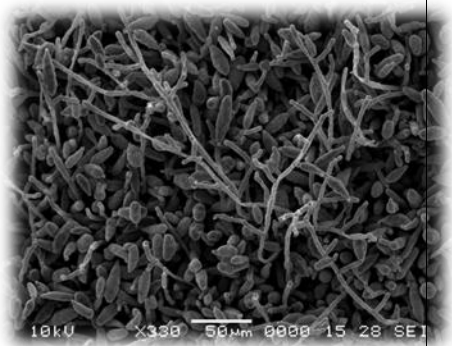
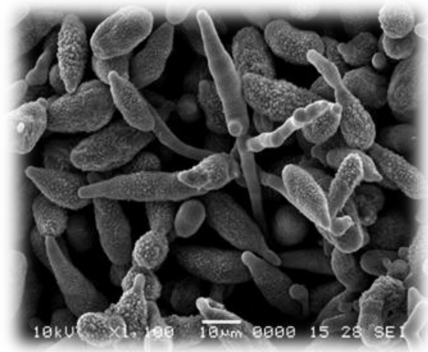
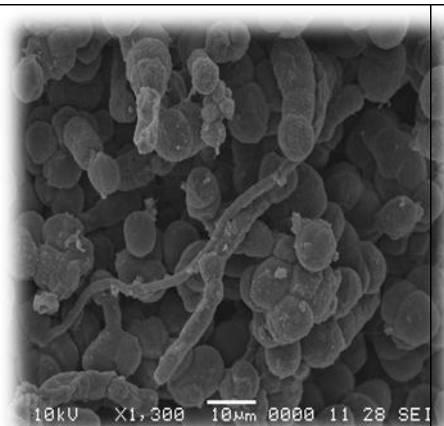
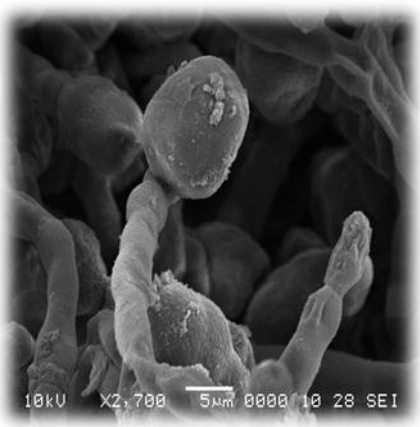
1)Emericell anidulands var. aurantiobrunnea	***2) Mucor racemosus***	***3) Alternaria pluriseptata***
		
4) Penicillium canescens	***5) Syncephlastrum racemosum***	***6) Aspergillus fumigatus***
		
7) Alternaria chlamydospora	***8) Aspergillus parasiticus***	***9) Ulocladium atrum***
		

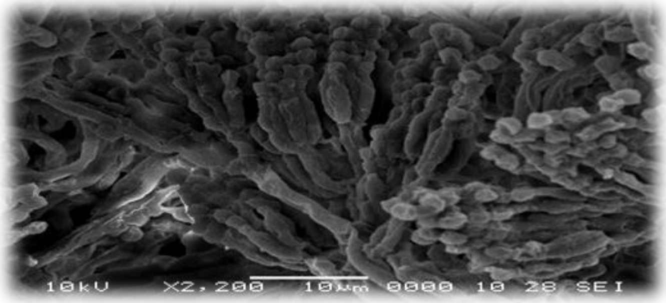
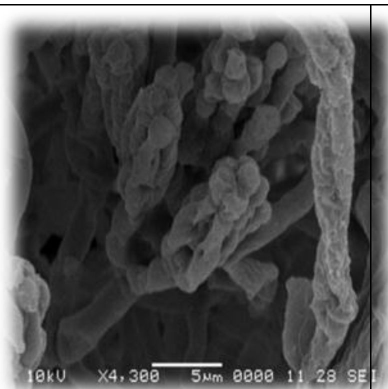
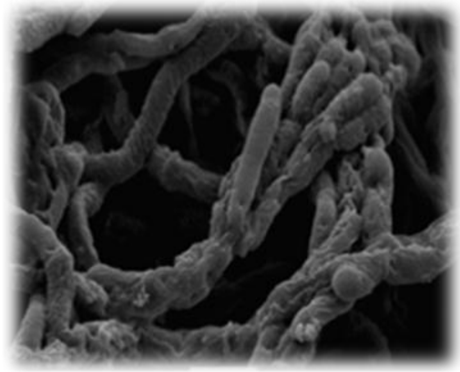
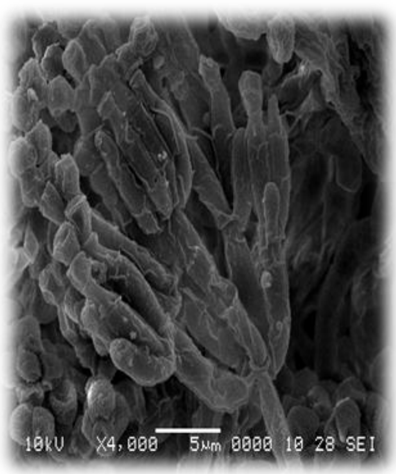
Morphological characteristics of fungal isolates by Image Analysis System, they are as follows : *Emericilla nidulans* (No.1) ; *Mucor racemosus* (No.2) ; *Alternaria pluriseptata* (No. 3) ; *Penicillium canescens* (No. 4); *Syncephalastru mracemosum* (No. 5); *Aspergillus fumigatus* (No.6) ; *Alternaria chlamydospora* (No.7) ; *Aspergillu sparasiticus* (N.o 8) and *Ulocladium atrum* (No.9).

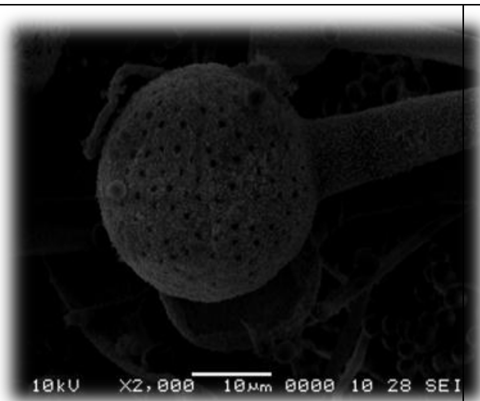
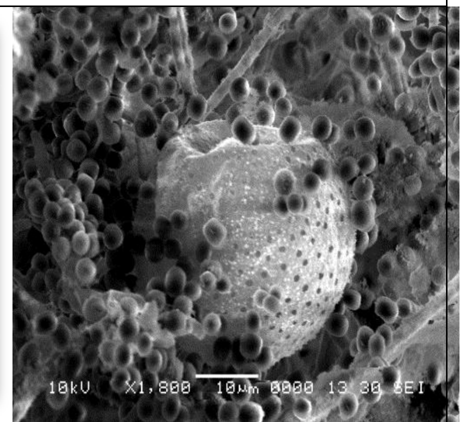
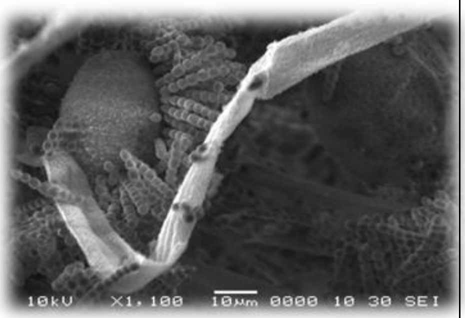
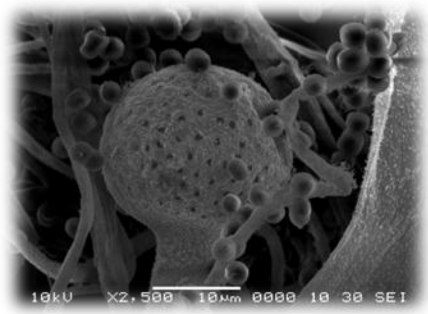
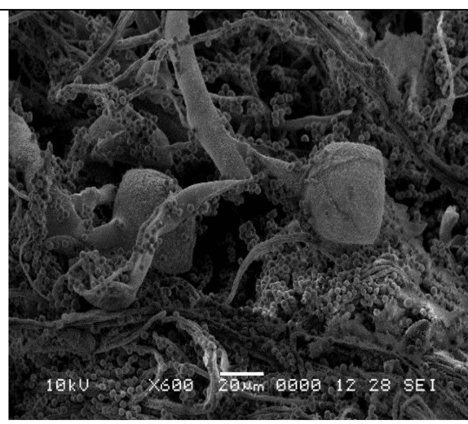
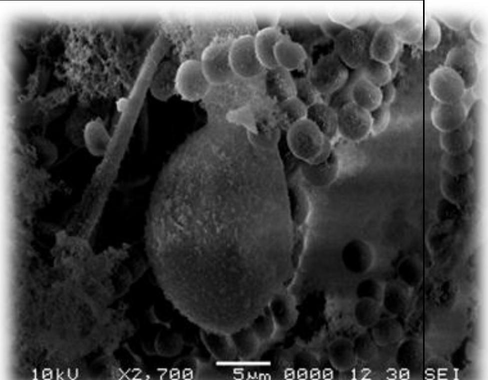
Table 3: Scanning Electron Microscope of isolated fungi at different concentrations of sodium chloride and on sodium chloride free medium.

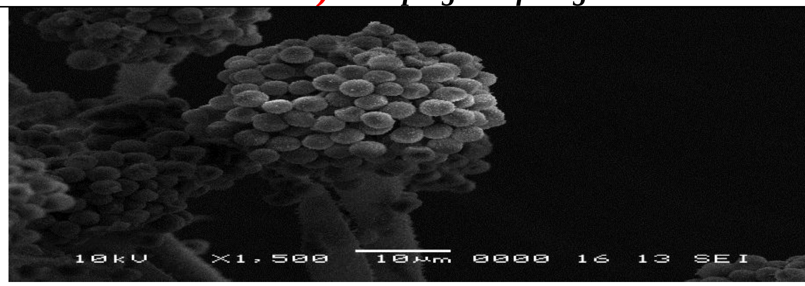
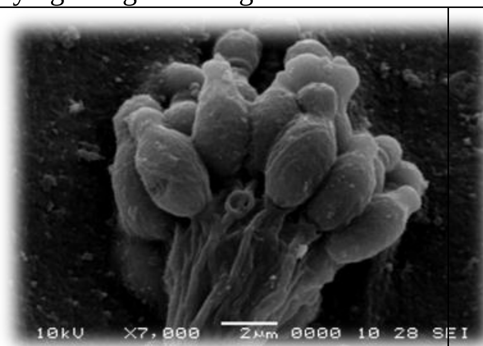
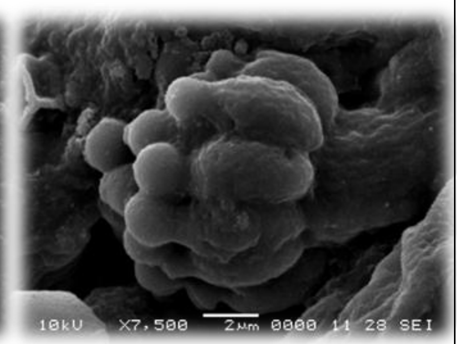
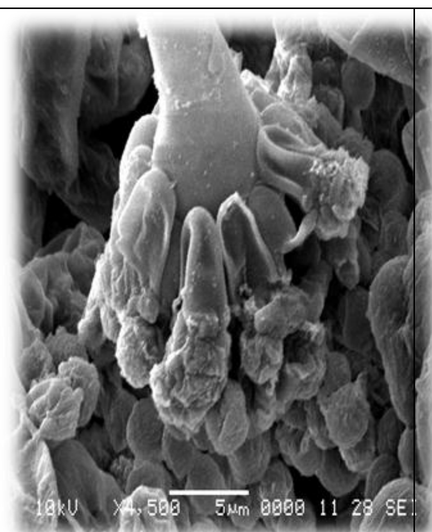
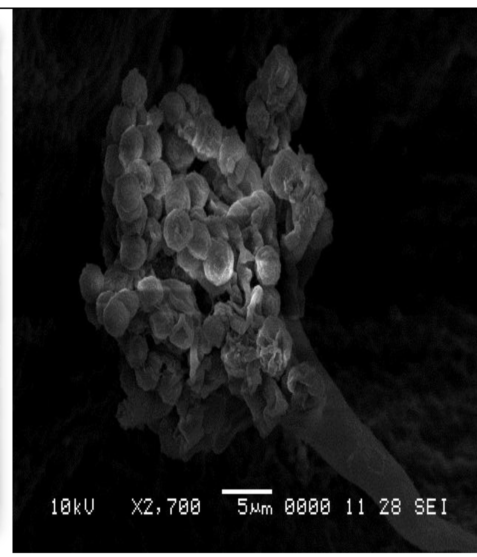
concentrations of NaCl % (w/v)	**Scanning Electron Microscope of isolated fungi**	
	1) ***Emericillanidulans***	
0 % NaCl		
Heads are surrounded completely by conidia .		
5% NaCl		
The globosely heads shrunken appeared spatulas to some extent the globosely conidia changed and reduced in No.		
10% NaCl		
Heads completely shrunken became rode in shape, little conidia appeared		

	2) *Mucor racemosus*	
0 % NaCl		
Sporangiophores and sporangia appearednormally. Columella appeared obovate		
5% NaCl		
Sporangia are shrunken , spores rare		
10% NaCl		
Sporangiophoreswere dwarfed. Sporangia are shrunken, spores rare		

<table>
<tr><td rowspan="2">0 % NaCl</td><td colspan="2">3) Alternaria pluriseptata</td></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td colspan="3">Long conidiophores with chains of conidia with terminal peach</td></tr>
<tr><td>5% NaCl</td><td>
</td><td>
</td></tr>
<tr><td colspan="3">Long conidiophores became shorter with chains of swollen conidia with rounded terminal</td></tr>
<tr><td>10% NaCl</td><td>
</td><td>
</td></tr>
<tr><td colspan="3">Conidiophores became drastic with clusters of swollen conidia with rounded terminal or one swollen conidium</td></tr>
</table>

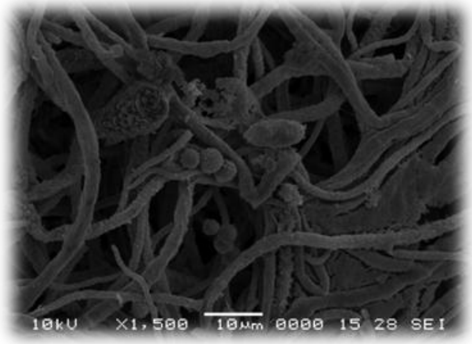
	4) *Penicillium canescens*	
0 % NaCl		
Conidiophores heavily branching,metulae and phialieds carrying chains of conidia		
5% NaCl		
Branching of conidiophores was less to some extent metulae and phialieds are carrying smaller No. of conidia		
10% NaCl		
Branching of conidiophores was less to some extent metulae and phialieds are carrying smaller No. of conidia or no conidia		

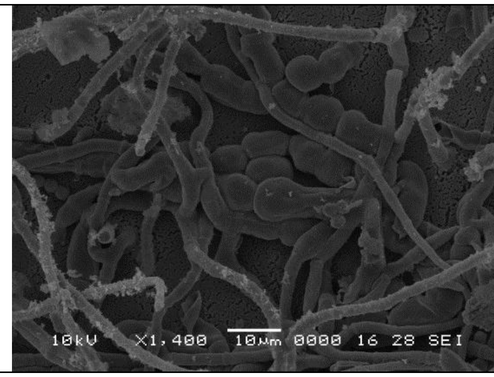
	5) ***Syncephalastrum racemosum***	
0 % NaCl		
Long sporangiophores with terminal vesicle which forms merosporangia all over its surface.		
5% NaCl		
Sporangia are smaller in size carrying smaller No of spores to some extent		
10% NaCl		
Sporangia size were very small sporangiospores were less than before. Drastic action appeared on sporangiophores		

<table>
<tr><td></td><td colspan="2">6) Aspergillus fumigates</td></tr>
<tr><td>0 % NaCl</td><td colspan="2">
</td></tr>
<tr><td colspan="3">Flask-shaped vesicle carrying sterigmata long chains of conidia.</td></tr>
<tr><td>5% NaCl</td><td>
</td><td>
</td></tr>
<tr><td colspan="3">Flask-shaped vesicle became longitudinal with swollen sterigmata carrying little No of conidia</td></tr>
<tr><td>10% NaCl</td><td>
</td><td>
</td></tr>
<tr><td colspan="3">Vesicles became longitudinal and drastic with drastic (to some extent) sterigmata carrying little No of shrunken conidia</td></tr>
</table>

7) ***Alternaria chlamydospora***

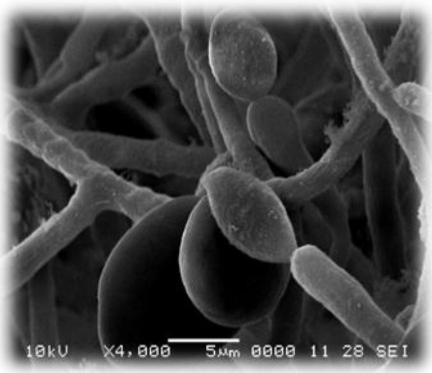
0% NaCl





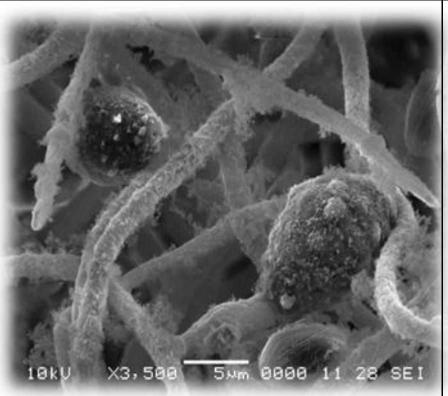
Conidia and conidiophores are appeared normally with some chlamydospores

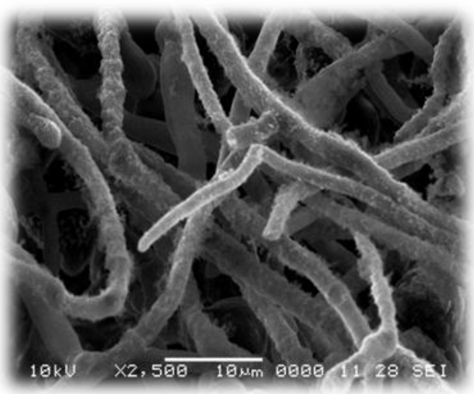
5% NaCl



Conidia and conidiophores are enlarged clusters of conidia appeared.

10% NaCl

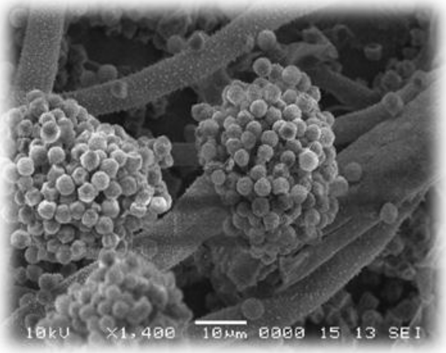


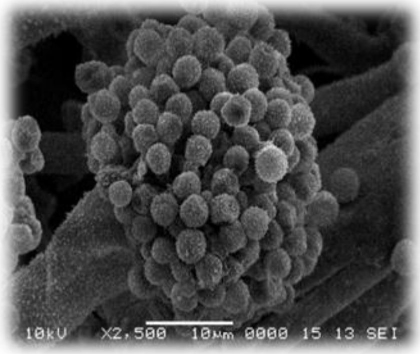


Conidia are enlarged while conidiophores are appeared fine covered with sheath of salt, conidia reduced in No.

8) *Aspergillus parasiticus*

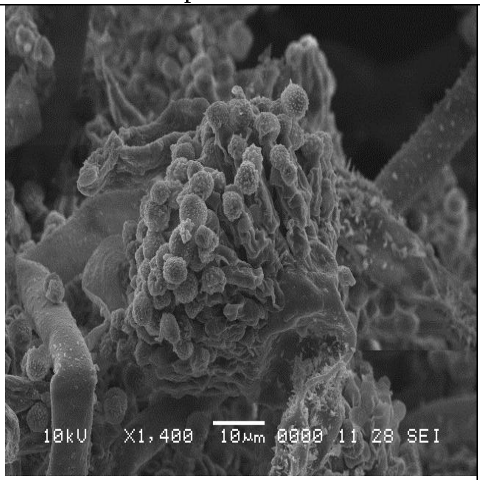
0 % NaCl

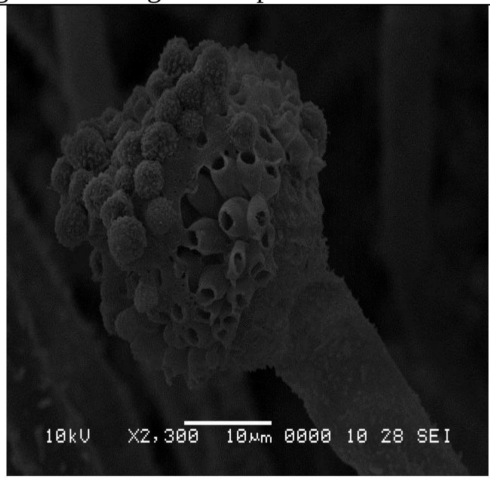




Flask shape heads covered with sterigmata on long conidiophores

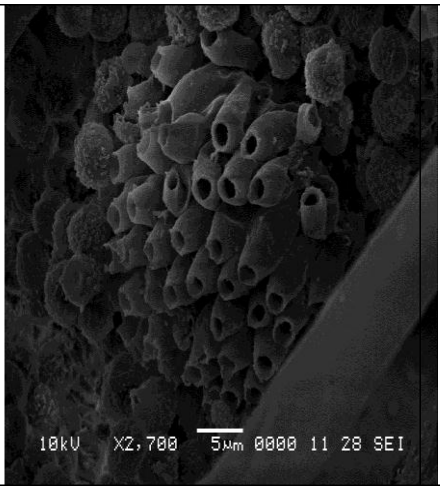
5% NaCl

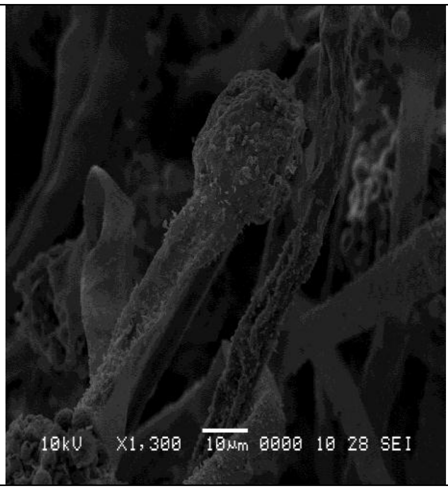




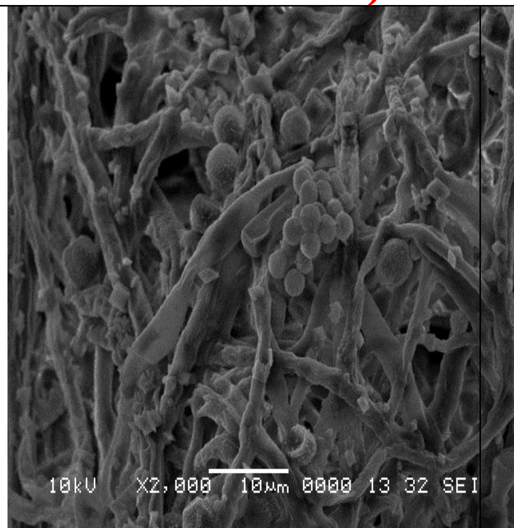
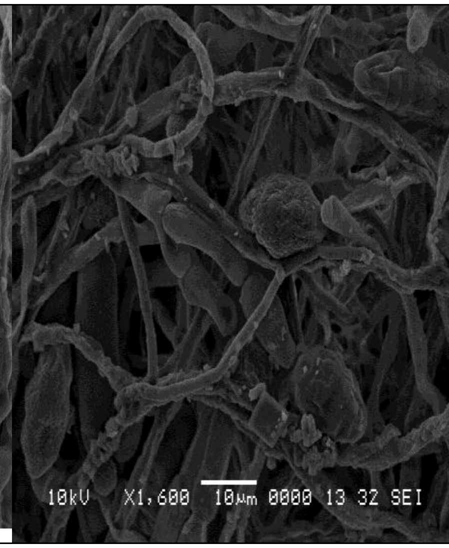
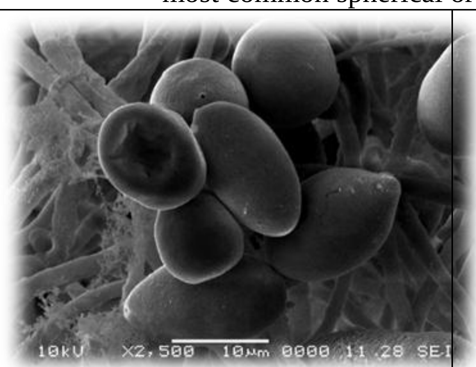
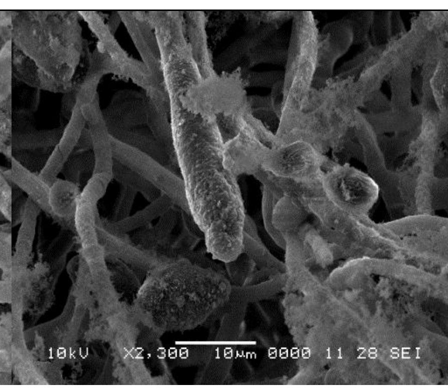
Flask shape heads became triangle covered with sterigmata on long conidiophores carrying smaller No of conidia. All the fungus covered with sheath of salt

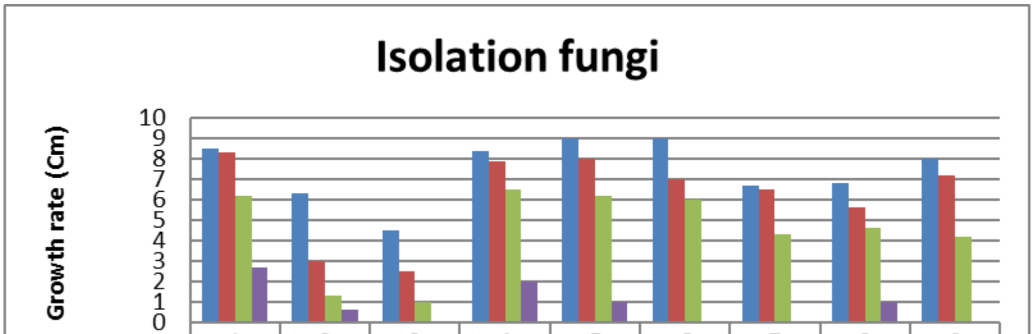
10% NaCl





Flask shape heads became triangle or rounded covered with sterigmata or naked carrying smaller No of conidia .All the fungus covered with sheath of salt.

	9) *Ulocladium atrum*
0% NaCl	 
Conidia varicose, sometimes ellipsoidal with one or more transverse and longitudinal septa but most common spherical or subspherical	
5% NaCl	 
Spherical and ellipsoidal smooth conidia without septa	
10% NaCl	 
Ellipsoidal and longitudinal conidia without septa.Hyphae covered with sheath of salt	



	1	2	3	4	5	6	7	8	9
NaCl-free	8.5	6.33	4.5	8.33	9	9	6.7	6.8	8
5% NaCl	8.3	3	2.5	7.83	8	7	6.5	5.6	7.16
10% NaCl	6.16	1.33	1	6.5	6.16	6	4.3	4.6	4.16
15% NaCl	2.66	0.6	0	2	1	0	0	1	0

Figure1: Influence of different NaCl concentrations on the growth of fungal isolates

The fungal isolates as follow: *Emericilla nidulans* (No.1) ; *Mucor racemosus* (No.2) ; *Alternaria pluriseptata* (No. 3) ; *Penicillium canescens* (No. 4); *Syncephalastru mracemosum* (No. 5); *Aspergillus fumigatus* (No.6) ; *Alternaria chlamydospora* (No.7) ; *Aspergillu sparasiticus* (N.o 8) and *Ulocladium atrum* (No.9)