Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 9 (6): 287-295, 2014
ISSN 1816-4935 / DOI: 10.3923/jm.2014.287.295

Effect of Salinity on the Fungal Occurance in Al-Shega Area at Al-Qassim, Saudi Arabia

Mona S.S. Al Tamie
Qassim University, College of Science, Saudi Arabia

ABSTRACT

The present study included isolation, identification and evaluation of physiological traits of some halotolerant fungi isolated from soil samples taken from Al-Shega province at Al-Qassim region. The results revealed the existence of seventeen species belonging to ten genera. The most frequent genus was *Aspergillus* from which the isolates were detected. High fungal growth rate was observed at low salt concentrations while at high salt concentrations, the fungal growth was reduced. *Emericilla nidulans* has achieved the first place in the ability to grow and survive in salt concentration of 15%. All isolates vary in their growth rate at pH values of 6.5 and 8, *Acremonium rutilum*, *Fusarium chlamydosporum*, *Aspergillus pulverulentus* and *Fusarium oxysporum* showed the highest growth rate. Results indicated that *Emericilla nidulans*, *Syncephalastrum racemosum*, *Aspergillus fumigates*, *Aspergillus parasiticus* and *Ulocladium atrum* could grow at all degrees of temperatures (30, 40 and 50°C) and also under low and moderate concentrations of NaCl (0, 5 and 10%) but the concentration of 15% NaCl only could not encourage the growth of *Emericilla nidulans* under 30°C. *Cephalosporium humicola*, *Mucor racemosus*, *Aspergillus parasiticus*, *Ulocladium atrum* and *Aspergillus flavus* could grow on the nickel (Ni) concentration of 0.25 and 0.5% but the concentration of 1% stopped fungal growth. For a shot of all the isolated fungi grew on the lead concentrations of 0.25 and 0.5%. All fungal isolates were sensitive to cadmium. All fungus showed allergy sensitivity to cadmium (Cd), since they were not able to grow in the presence of Cd ions.

Key words: Al-Qassim region, NaCl, halotolerant fungi, heavy metals, thermophiles, pH values, high salt

INTRODUCTION

Salt mine or Al-Shega area at Al-Qassim region is considered one of the sabkhas or salt marshes which are generally associated with terrestrial lands that are often subjected to being flooded with rain. This water remains there for a few weeks to several months. As water evaporates from the shallow depressions, it leaves a crest of salt and thereby, forming a substratum for the growth of several salt loving plants (halophytes). The salinity of the salty lands varies from site to site and from season to season depending upon the climate in which these sites are located. The salinity and water level decide the vegetation type of the area.

To the best of our knowledge, there is no enough botanical or microbiological studies concerning the area of Al-Shega. We expect that the fungi existing in this area are tolerant (halophilic and halotolerant fungi) to harsh environmental conditions such as high salinity and high temperature therefore, these fungi may have a high economical values.

Tresner and Hayes (1971) studied the tolerant behavior of some fungi to soil salinity and found that some fungi such as *Aspergillus* sp. and *Penicillium* sp. have the ability to resist salinity stress. They also found that most species have the ability to grow at a concentration of 20% or more of NaCl. Similar results were reported by Kulik and Hanlin (1968) and Rai and Agarwal (1973, 1974).

Many studies were conducted in several Arab countries (Salama *et al.*, 1971; Abdel-Fattah *et al.*, 1977; El-Abyad *et al.*, 1979; Abdel-Hafez *et al.*, 1989; Moubasher *et al.*, 1990; Khodair *et al.*, 1991; El-Mougitah, 1993; Gashgari and Al-Hazmi, 2006) and came up with nearly similar conclusion.

Early investigations approved that fungal growth stopped at high saline conditions and high pH values (Guiraud *et al.*, 1995) while some other studies showed the adaptability of fungi to these conditions where they grow at a saline concentration up to 200 g L^{-1}. In addition, Oren (2002) isolated the *Halothermothrix orenii* living in Tunisian soil under very high temperature (up 86°C). Moreover, halophilic and thermohalophilic fungus were isolated from the desert soil of Makkah region (Abdel-Hafez, 1981, 1982, 1984; Khodair *et al.*, 1991; Gashgari and Al-Hazmi, 2006).

Khiyami (2007) studied halotolerant fungi isolated from the rhizosphere of Sailicorina grown in Sabkha Al-Oshizah at Al-Qassim which is nearly similar to the configuration of the study area. In this preliminary study of Sabkha Al-Oshizah, halophilic and hoalotolerant fungi has been isolated and the majority of known strains were found to be grown in the presence of high concentrations of NaCl. Because of the importance of halotlerant or thermohalophilic fungi and the paucity of information in this field in Saudi Arabia, this study was conducted to isolate, identify some halotlerant fungi and study their physiological attributes in order to determine the ability of these fungi to withstand the harmful effects of salinity, unsuitable pH and high temperature.

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 (26°23'1"N 43°51'21"E). Each soil sample was collected in plastic sealed bags for the proper use and was brought together in August, 2012. The other part of the soil samples were devoted to the identification and isolation of fungi.

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 were employed by Moubasher *et al.* (1975).

This medium consists of $NaNO_3$, 2 g L^{-1}; $MgSO_4.7H_2O$, 0.5 g L^{-1}; KCl, 0.5 g L^{-1}; KH_2PO_4 1 g L^{-1}; $FeSO_4.7H_2O$ in traces; sucrose, 20 g L^{-1}; agar, 20 g L^{-1}. All these components were added to 1 L distilled water. The pH was set to 7 and then the medium was sterilized at 1.5 atm for 20 min.

Serial dilutions and direct spreading technique of soil techniques were used in the isolation of halophilic fungi. Fungal colonies that appeared on the medium were picked up and transferred to a new medium of the same components. Single fungal colony which developed on the agar surface of the isolation medium was transferred on a slant medium of the same components. The isolated fungi were cultivated on Dox's agar medium at 30°C for 10 days.

Fungal isolates were defined microscopically by Scanning Electron Microscope in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

To study the effect of salinity, temperature and pH, fungi were grown on Dox's agar medium supplemented with different concentrations of sodium chloride, 0, 5, 10, 15 and 20% (w/v) and pH values of 6.5, 7 and 8. All media, then, was inoculated with two discs of each fungal isolate and incubated at 28±2°C for 7 days.

Inoculated dishes were incubated at different temperatures 30, 40 and 50°C at control free NaCl and with different sodium chloride treatments.

Size of each colony was measured in 3 replicates and measurements were recorded.

The ability of fungal isolates to resist the effect of heavy metals by adding salts of nickel, lead and cadmium to the Dox's agar medim was also studied. Width of the colonies was measured and the average growth of each three dishes was recorded.

RESULTS AND DISCUSSION

Table 1 shows the chemical and mechanical analysis of the soil samples. Soils appeared to be mostly clay, poor in the organic substances and high in the sodium chlorides.

Results in the Table 1 indicates clearly that the coefficient of soil electric conductivity is equal to 9.62 ds m^{-1} which is an acceptable value for soil medium salinity of the ground water to the arid and semi-arid areas where this value is 12-15 dS m^{-1}. The proportion of total dissolved salts in the soil was about 666.4 ppm.

Table 2 shows the concentration of heavy metals in soil samples. It is clear from the data in the table that nickel (Ni) was the highest element concentration found in the soil followed by lead (Pb) and cadmium (Cd) concentrations. It was also clear that soil was lacking zinc (Zn) and copper (Cu).

Table 1: Chemical analysis and mechanical analysis of the soil samples

	Locations				
Test	1	2	3	4	5
Chemical analysis					
Na	45.00	43.20	43.40	44.00	42.10
Cl	39.40	38.10	39.20	40.50	39.00
Ca	3.10	3.80	3.50	1.90	3.90
Mg	0.72	0.70	0.74	0.69	0.65
EC (dS m^{-1}*)	1.44	1.61	1.59	1.57	1.51
TDS (ppm*)	966.40	1030.40	1017.60	1004.80	966.40
Mechanical analysis					
Soil samplesec (dS $msec^{-1}$)					Values
Clay#					59.65
Salt#					35.16
Sand#					5.19
Analysis of soil sample					
EC (dS $msec^{-1}$)**					9.62
TDS (ppm)**					966.40

*1 g of slat dissolved in 1 L of water to estimate salinity level, **1 g of soil in 10 mL distilled water (1: 10), #By traingle taxture clay siol

Table 2: Estimation of heavy elements of the soil samples

	Locations				
Heavy metals (ppm)	1	2	3	4	5
Cu	-	-	-	-	-
Zn	-	-	-	-	-
Ni	40.0	37.3	45.6	37.6	43.7
Pb	37.2	32.4	33.2	27.2	18.2
Cd	1.68	0.58	0.80	0.32	0.32

Table 3: List of fungi isolated from soil samples

Sr. No.	Isolated fungi	Sr. No.	Isolated fungi
1	*Aspergillus niger*	10	*Aspergillus fumigates*
2	*Emericilla nidulans*	11	*Fusarium chlamydosporum*
3	*Acremonium rutilum*	12	*Aspergillus pulverulentus*
4	*Cephalosporium humicola*	13	*Fusarium oxysporum*
5	*Fusarium* sp.	14	*Alternaria chlamydospora*
6	*Mucor racemosus*	15	*Aspergillus parasiticus*
7	*Alternaria pluriseptata*	16	*Ulocladium atrum*
8	*Penicillium canescens*	17	*Aspergillus flavus*
9	*Syncephalastrum racemosum*		

In this study seventeen fungal isolates were isolated and identified from soil samples of Al-Shega Area at Al-Qassim region in Saudi Aribia (Table 3).

Figure 1 shows the growth rate of fungal strains by adding various concentrations of NaCl. The results indicated that the high growth rate of isolates was observed at low salt concentrations while high salt concentrations reduced fungal growth. Concentration of 5% NaCl was suitable for the growth of all fungal isolates. At this low salt concentration, an increase in the growth of isolates was observed for *Emericilla nidulans*, *Penicillium canescens, Syncephalastrum racemosum, Aspergillus parasiticus* and Mucor racemosus, whereas the growth of isolates was stopped at 20% NaCl.

These results were consistent with results of many other researches (Abdel-Hafez, 1981, 1982, 1984; Khodair *et al.*, 1991; Gunde-Cimerman *et al.*, 2000, 2006, 2009; Butinar *et al.*, 2005a, b; Gashgari and Al-Hazmi, 2006; Khiyami, 2007; Vaupotic *et al.*, 2008; Yadav *et al.*, 2011; Nayak *et al.*, 2012).

Emericilla nidulans has achieved first place in the ability to grow and survive in salt concentration of 15% and *Penicillium canescens*, *Syncephalastrum racemosum*, *Aspergillus parasiticus*, *Mucor racemosus*. So we can know that they are halotolerant fungus moderately but not a halophilic, where they were able to grow and survive in 10% concentration of NaCl.

Acremonium rutilum, *Cephalosporium humicola*, *Fusarium* sp., *Alternaria pluriseptata*, *Aspergillus fumigates*, *Fusarium chlamydosporum*, *Aspergillus pulverulentus*, *Fusarium oxysporum*, *Alternaria chlamydospora*, *Ulocladium atrum* and *Aspergillus flavus* are weak halotolerant isolates, they were able to grow and survive in 5% concentration of NaCl only.

Figure 2 shows the growth rate of fungal strains with different pH values (6.5 and 8). The study showed the ability of fungal isolates to grow at pH ranged from 6.5-8 and their growth was even high. This fact was observed for *Acremonium rutilum*, *Fusarium chlamydosporum*, *Aspergillus pulverulentus* and *Fusarium oxysporum*.

The results of the effect of temperature on the growth of fungal strains in the presence and absence of NaCl was recorded in Fig. 3. All genus have a fungal growth at 30°C with concentration of 5 and 10% of NaCl, respectively, it has been observed that a tractor and a high salt concentration started declining growth until faded.

Results indicated that *Emericilla nidulans*, *Syncephalastrum racemosum*, *Aspergillus fumigates*, *Aspergillus parasiticus* and *Ulocladium atrum* could grow at all degrees of temperature (30, 40 and 50°C) and also under low and moderate concentrations of NaCl (0, 5 and 10%) while at 15% (w/v) NaCl, only *E. nidulans* was encouraged at 30°C (Fig. 3).

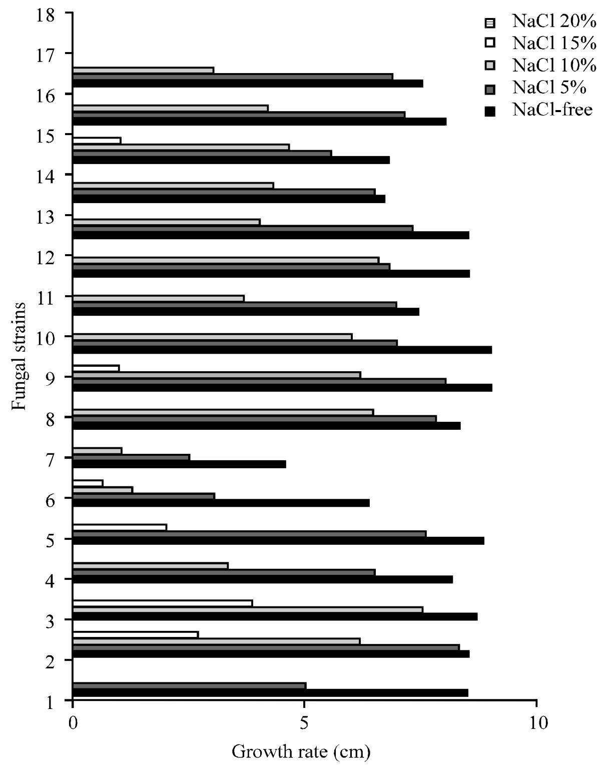

Fig. 1: Growth rate of fungal strains by adding various concentrations of NaCl

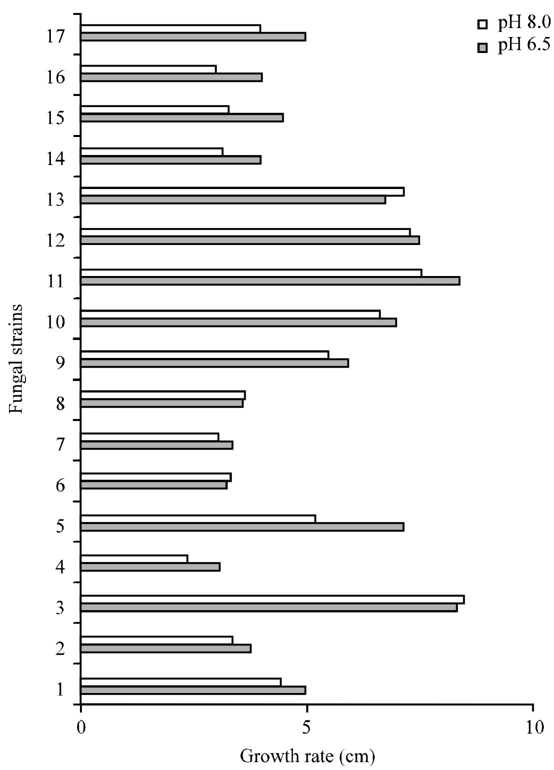

Fig. 2: Growth rate of fungal strains with different pH (6.5, 8)

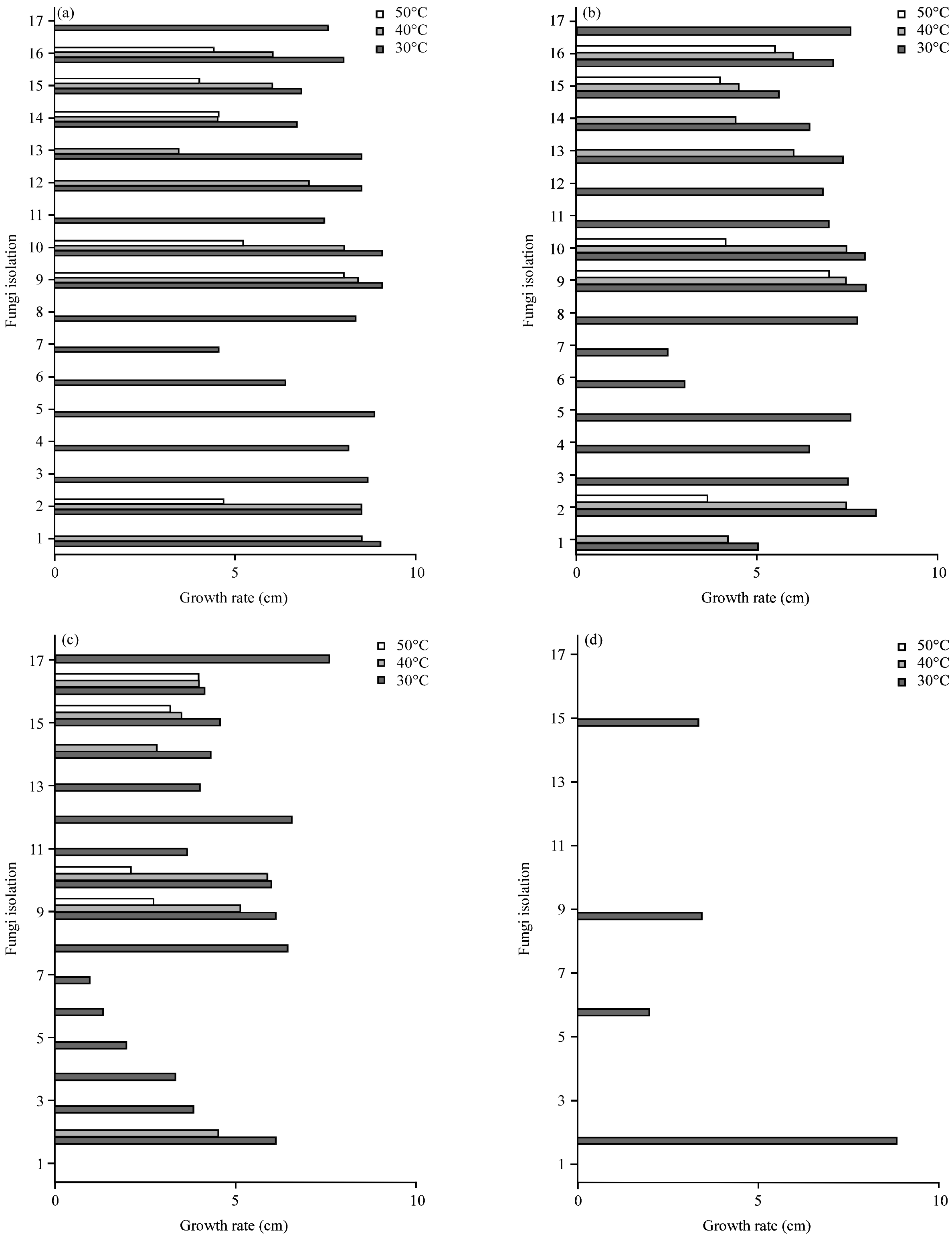

Fig. 3(a-d): Growth rate of fungal strains with different temperatures and concentrations of NaCl (a) NaCl-free, (b) 5% NaCl, (c) 10% NaCl and (d) 15% NaCl

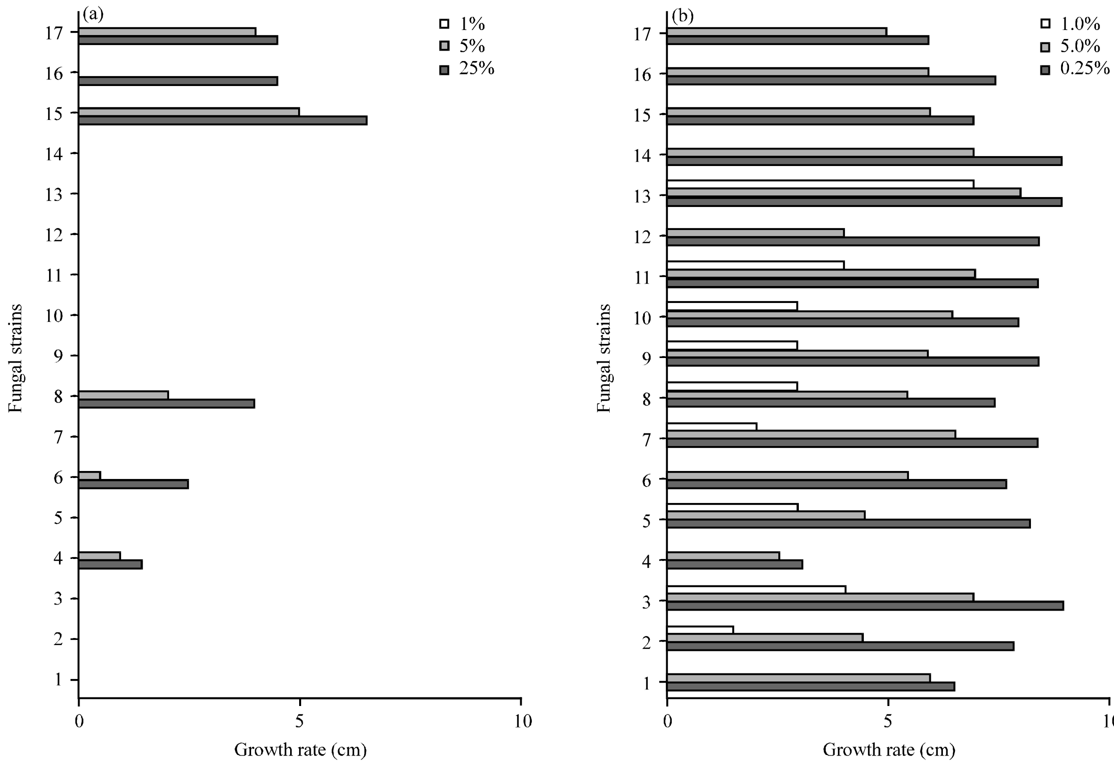

Fig. 4(a-b): Growth rate of fungal strains at different concentration of heavy metals, (a) Nickel and (b) Lead

Figure 1 shows the growth rate of fungal strains with adding various concentrations of NaCl. The results indicated that the high growth rate of isolates was observed at low salt concentrations while high salt concentrations reduced fungal growth. Concentration of 5% NaCl was suitable for the growth of all fungal isolates. At this low salt concentration, an increase in the growth of isolates was observed for *Emericilla nidulans*, *Penicillium canescens*, *Syncephalastrum racemosum*, *Aspergillus parasiticus*, *Mucor racemosus* and whereas the growth of isolates was stopped at 20% NaCl. These results were consistent with results of many other researches (Bokhary, 1986; Al-Fassi *et al*., 1994; Khiyami, 2007; Yadav *et al*., 2011).

Table 2 shows the effect of the concentration of heavy metals on the growth of soil fungal isolates. It has been found that the isolates were resistance to Zn, Pb and Cd.

Figure 4 shows the growth rate of fungal strains with different concentration of heavy metals nickel, zinc and cadmium (0.25, 0.50 and 1.0%).

Cephalosporium humicola, *Mucor racemosus*, *Aspergillus parasiticus*, *Ulocladium atrum* and *Aspergillus flavus* could grow on the nickel concentration of 0.25 and 0.5% but concentration of 1% stopped growth.

For a shot of all the isolated fungus grew on the lead concentrations of 0.25, 0.5 and 1.0%, the fungus *Emericilla nidulans*, *Acremonium rutilum*, *Fusarium* sp., *Alternaria pluriseptata*, *Alternaria pluriseptata*, *Penicillium canescens*, *Syncephalastrum racemosum*, *Aspergillus fumigates*, *Fusarium chlamydosporum* and *Fusarium oxysporum* could grow.

It seems that this fungus rectifier for a shot, where growth is in the presence of all concentrations. All fungus showed allergy to element cadmium, it could not grow is in the presence of Cd concentrations.

CONCLUSION

This is the first study on fungicides in Al-Shega area at Al-Qassim region. The study has shown that alkaline and clay soil of the region contains many of heavy metals and many saprophytes fungi, the fungi is adapted to saline condition and it is not obligate halophilic fungus. These fungi also endured the heat of this environment that may reach 50°C in summer seasons. The isolated fungi can be defined as thermophiles.

REFERENCES

Abdel-Fattah, H.M., A.H. Moubasher and S.I.I. Abdel-Hafez, 1977. Studies on mycoflora of salt marshes in Egypt. I. Sugar fungi. Mycopathologia, 61: 19-26.

Abdel-Hafez, S.I.I., 1981. Halophilic fungi of desert soils in Saudi Arabia. Mycopathologia, 75: 75-80.

Abdel-Hafez, S.I.I., 1982. Survey of the mycoflora of desert soils in Saudi Arabia. Mycopathology, 80: 3-8.

Abdel-Hafez, S.I.I., 1984. Survey of airborne fungus spores at Taif, Saudi Arabia. Mycopathology, 88: 39-44.

Abdel-Hafez, S.I.I., A.H. Moubasher, M.A. Bagy and M.A. Abdel Sater, 1989. Seasonal fluctuations of halophilic and halotolerant soil fungi in upper Egypet-extreme arid region. Bull. Faculty Assiut Univ., 18: 13-25.

Al-Fassi, F.A., A.A. Malibari and M.A. Moustafa, 1994. Physiological studies on ten thermophilic and thermotolerant fungi isolated from different locations in the Western Region of Saudi Arabia. Arab Gulf J. Sci. Res., 12: 321-340.

Bokhary, E.E.M., 1986. Studies on thermophilic and thermotolerent fungi in the soils of Kingdom of Saudi Arabia. M.Sc. Thesis, Girls Collage of Education, Jeddah, Saudi Arabia.

Butinar, L., P. Zalar, J.C. Frisvad and N. Gunde-Cimerman, 2005a. The genus *Eurotium*-members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol. Ecol., 51: 155-166.

Butinar, L., S. Sonjak, P. Zalar, A. Plemenitas and N. Gunde-Cimerman, 2005b. Melanized halophilic fungi are eukaryotic members of microbial communities in hypersaline waters of solar salterns. Bot. Marina, 48: 73-79.

El-Abyad, M.S., I.K. Ismail and M.A. Rizk, 1979. Ecological Studies of the Microflora of Saline Egyptian Soils. In: Arid Land Plant Resources: Proceedings of the International Arid Lands Conference on Plant Resources, Goodin, J.R. (Ed.). International Center for Arid and Semiarid Land Studies, Texas Tech University, USA., pp: 582-597.

El-Mougitah, A.A., 1993. The Effect of Salinity on Some Halophilic Fungi. In: Towards the Rational use of High Salinity Tolerant Plants, Helmut, L. and A.A. Al-Masoom (Eds.). Vol. 1, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp: 473-477.

Gashgari, R. and N. Al-Hazmi, 2006. The effect of salinity on the fungal occurrence of the Red Sea coastal Qunfiddh city, Saudi Arabia. Assiut Univ. Bull. Environ. Res., 9: 27-37.

Guiraud, P., R. Steiman, F. Seigle-Murandi and L. Sage, 1995. Mycoflora of soil around the Dead sea II-deuteromycetes (except *Aspergillus* and *Penicillium*). Syst. Applied Microbiol., 18: 318-322.

Gunde-Cimerman, N., J. Ramos and A. Plemenitas, 2009. Halotolerant and halophilic fungi. Mycol. Res., 113: 1231-1241.

Gunde-Cimerman, N., L. Butinar, S. Sonjak, M. Turk, V. Ursic, P. Zalar and A. Plemenitas, 2006. Halotolerant and Halophilic Fungi from Coastal Environments in the Arctics. In: Adaptation to Life at High Salt Concentrations in Archaea, Bacteria and Eukarya, Gunde-Cimerman, N., A. Oren and A. Plemenitas (Eds.). Springer, New York, USA., ISBN: 9781402036330, pp: 397-423.

Gunde-Cimerman, N., P. Zalar, S. de Hoog and A. Plemenitas, 2000. Hypersaline waters in salterns: Natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol., 32: 235-240.

Johnson, L.F., E.A. Curl, J.H. Bond and H.A. Fribourg, 1959. Methods for Studying Soil Microflora-Plant Disease Relationships. Burgess Publishing Company, Minneapolis, MN., USA., pages: 178.

Khiyami, M.A., 2007. Ecological and physiological study of moderate halotolerant fungi isolated in Sabkha AL-Oshizah at Al-Qassim region in Saudi Arabia. Saudi J. Biol. Sci., 14: 115-123.

Khodair, A.A., A.S. Ramadani and A.M. Aggab, 1991. Occurrence and density of alkalophilic bacteria and fungi in saline soils of Makkah district, Saudi Arabia. Arab Gulf J. Sci. Res., 9: 119-132.

Kulik, M.M. and R.T. Hanlin, 1968. Osmophilic strains of some *Aspergillus* species. Mycologia, 60: 961-964.

Moubasher, A.H., F.T. El-Hissy and M.I.A. Abdel-Kader, 1975. Mucorales in Egyptian soils. Egypt. J. Bot., 18: 115-124.

Moubasher, A.H., S.I.I. Abdel-Hafez, M.M.K. Bagy and M.A. Abdel-Satar, 1990. Halophilic and halotolerant fungi in cultivated desert and salt marsh soils from Egypt. Acta Mycol., 26: 65-81.

Nayak, S.S., V. Gonsalves and S.W. Nazareth, 2012. Isolation and salt tolerance of halophilic fungi from mangroves and solar salterns in Goa-India. Indian J. Geomarine Sci., 41: 164-172.

Oren, A., 2002. Diversity of halophilic microorganisms: Environments, phylogeny, physiology and applications. J. Ind. Microbiol. Biotechnol., 28: 56-63.

Rai, J.N. and S.C. Agarwal, 1973. Salinity optima as affected by temperature for some Usar soil Aspergilli. Mycopathologia Mycologia Applicata, 50: 307-312.

Rai, J.N. and S.C. Agarwal, 1974. Increased osmotic tolerance of some Aspergilli isolated from Usar (alkaline) soils: A possible indication of ecological specialization. Mycopathologia Mycologia Applicata, 52: 299-305.

Salama, A.M., K. El-Batanoni and M.I. Ali, 1971. Studies on the fungal flora of Egyptian soils. I. Western Mediterranean coast and Lybian desert. UAR J. Bot., 14: 99-114.

Tresner, H.D. and J.A. Hayes, 1971. Sodium chloride tolerance of terrestrial fungi. Applied Environ. Microbiol., 22: 210-213.

Vaupotic, T., P. Veranic, U. Petrovic, N. Gunde-Cimerman and A. Plemenitas, 2008. HMG-CoA reductase is regulated by environmental salinity and its activity is essential for halotolerance in halophilic fungi. Stud. Mycol., 61: 61-66.

Yadav, J., J.P. Verma, S.K. Yadav and K.N. Tiwari, 2011. Effect of salt concentration and pH on soil inhabiting fungus *Penicillium citrinum* Thom. for solubilization of tricalcium phosphate. Microbiol. J., 1: 25-32.