Plant Archives Vol. 19, Supplement 2, 2019 pp. 310-312 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

CONCENTRATIONS EFFECT OF SOME SALTS ON GROWTH OF *ASPERGILLUS NIGER* AND *PENICILLIUM OXALICUM*

Ali Ahmed Waheed, Ali Amer Dahham, Ebthal Kadhim Azra, Jawad A. Kamal and Hussein M. Khaeim
College of Agriculture, University of Al-Qadisiyah, Iraq
Email : Hussein.Khaeim@qu.edu.iq; ahmed.guti@gmail.com

Abstract

A laboratory experiment was conducted to study both salts type and concentrations effect on the growth of *Aspergillus niger* and *Penicillium oxalicum*. Four concentrations of NaCl, $MgCl_2$, and $CaCl_2$ (0, 2, 4, and 6 $ds.m^{-1}$) were used. Fungi were grown on PDA medium. The results present that the concentration of (6 $ds.m^{-1}$) decrease the growth of *Aspergillus niger* with viable cells of 2.6 x 10^4 CFU. The lowest viable cells number were with the concentration of (2 $ds.m^{-1}$), which were $10.6x10^4$ CFU. $MgCl_2$ that increases in the viable cells of fungi to $7.35x10^4$ CFU. Viable cells of *Penicillium oxalicum* was decreased with the concentration of (2 ds m^{-1}) of all salts, which was ($4.4x10^4$ CFU) and ($10.3x10^4$ CFU) for *Penicillium oxalicum* fungus at 4 $ds.m^{-1}$. $Mgcl_2$ salts increase the viable cells of *Penicillium oxalicum* to (10.8 x 10^4 CFU).

Keywords: *Aspergillus*, *Penicillium*, salts, Fungi growth

Introduction

Fungal growth affected by many factors in the environmental system such as salinity concentrations, (Al-Shakari, 1991). Salinity limits fungal mycelium growth through the harmful effect of salts, and through reduces carbohydrates availability, (Imshenetskii et al 1983). *Aspergillus niger* fungus is one of the widely spread fungi since it has the ability to form a large number of spores. Moreover, some of its species have sexual stage and stone bodies that are more resistant to inappropriate environmental changes. The reason behind the wide separation of fungus in various environments is its secretion of several enzymes that enable it to the exploitation of different food sources and carrying various environmental conditions. This fungus has the ability to withstand drought and a wide range of wet tension. (Abdullah and Al-Bader 1990), studied different samples of the Iraqi regains including deserts, pastures, forests, marshes, and palm groves. According to their study, *Aspergillus niger* had the highest frequency among the recorded species.

Fungi that live in harsh imperfect environments where fungi cannot grow are characterized as extreme fungi, (Guffnti and Krulwih 1989). The term of Halophila Fungi called for fungi that grow in high saline concentrations, (Satio 1962; and Wafaa Sahib Alawsy 2018). *Aspergillus* and *Penicillium* fungi tolerate high salts concentrations and most of their species are grown at a concentration of 20% of Nal or more, (Rai and Agrawal 1973). Through a study done by (Gashgari and Al-Hzmi, 2006); Five species of *Aspergillus* fungus were isolated and developed in saline concentrations of (10, 20, 30 and 40) of NaCl. Their results show fungal growth decrease as NaCl concentration going up till no fungus growth at a concentration of 40%. (Asghari *et al*., 2008), found that salinity negatively affects the growth of mycelium growth by reducing the carbohydrate availability for the growth of hyphae and mycelium. (Juniper and Abbot, 2006) show that salts inhibit the growth of hyphae and mycelium of fungi. This effect varies depending on fungus age and salt concentration.

In a study conducted by (Abohaila, 1987), on the salinity tolerant fungi of in desert soils, it was found that the most tolerant species are *Aspergillus* and *Penicillium*. *Penicillium* spp shows dominance in desert soils along with *Aspergillus*, which presents their ability to tolerate dehydration and their ability to grow in soils that are poor to organic matters, (Hamad 1998; and Luma A. Alabadi, *et al*., 2018).

The study aims to study the effect of different types of salts on the growth of *Aspergillus niger* and *Penicillium oxalicium* fungi.

Methods and Materials

Potato Dextrose Agar

Potato dextrose agar was prepared by dissolving (39 g) of the powder in 1 liter of distilled water according to the manufacturer's instructions then sterilized at (121 °C) and pressure (15 bar) for 20 minutes. After the sterilization period, vials were left to cool down. 250 mg. L^{-1} of Chloramphenicol was added and refrigerated until use.

Salty Potato Dextrose Agar

It was prepared by adding different saline concentrations (0, 2, 4, and 6 $ds.m^{-1}$) of NaCl, $MgCl_2$, and $CaCl_2$ separately PDA and sterilized at (121 ºC) and pressure (15 bar) for 20 minutes.

The effect of NaCl and $MgCl_2$ and $CaCl_2$ on alive units of *Aspergillus niger* and *Penicillium* fungi

0.5 cm diameter round sample of 1 cm diameter away from the edge of the *Aspergillus niger* and *Penicillium* colony that were grown on the PDA mediums that have different saline concentrations (0, 2, 4 and 6 $ds.m^{-1}$) of NaCl, $Mgcl_2$, and $Cacl_2$, separately.

Several dilutions were made of it to reach the dilution of 10^{-4}, and then (1 ml) of it was taken and transferred to a Petri dish. 20 ml P.D.A medium was added with 3 replications for each concentration. Dishes were slightly shacked for homogeneity. These dishes were incubated at (25 ºC). 1-3 days after, developing colonies were counted in each dish according to the following equation:

The number of colonies forming units = number of colonies x inverted dilution.

Statistical Analysis

This experiment was carried out according to the Complete Randomized Design (C.R.D). Averages were compared by using the least significant difference (L.S.D) at 5% significant level, (Al-Rawi 2000 and Khaeim, 2013).

Results and Discussion

Effect of salt type and concentration on spore density of the alive of *Aspergillus niger*

Table (1) presents that concentration of (6 ds) of the studied salts concentrations reduces the number the spore density of *Aspergillus niger* fungus. The heights alive spore density average was (2.6 x 10^4) colony formation units. On the other hand, the concentration of (2 ds) increases the alive spore density average (10.6 x 10^4). This is consistent with Juniper and Abbot, (2006) study, which confirmed that salinity inhibits both of spore growth and mycelium formation.

Results showed that NaCl salt reduced the number of living colonies of *Aspergillus niger* (3.33 x 10^4), while $MgCl_2$ increases number of living colonies of this fungus (7.35 x 10^4) as compared with NaCl and $CaCl_2$ with (3.33 × 10^4) colony formation units. These results are consistent with (Balkrami and Verna, 1988) study, which indicated that the fungi tolerate magnesium salts concentration more than sodium and calcium salts.

Table 1 : Effect of salt type and concentration on the alive spore density of *Aspergillus niger.*

Salt concentration (ds. m^{-1})	Number of live reproduction units (x10^{-4}) for *Aspergillus niger* fungus in salty mediums			Average
	$CaCl_2$	$MgCl_2$	NaCl	
0	4	4	4	4
2	2.1	17.6	3.6	10.6
4	3.6	4.8	4.4	4.2
6	2.4	3	2.4	2.6
Average	3.33	7.35	3.6	
L.S.D	7.2	5.16	5.8	

Effect of salt type and concentration on the spore density of alive of *Penicillium* fungus

Table (2) shows reduction in the number of alive colonies of *Penicillium* fungus at concentration of (2 ds.m-¹), which are (4.4 x 10^{-4}) colony formation unit, while the concentration of (2 ds.m-¹) increase the number of living units of the fungus (10.3 x 10^{-1}) unit colony formation. This result agrees with the results of (Madigan and Marrs, 1997) who found that the fungi use salts with concentrations of less than 10% in the current of cellular proteins with high levels of sodium chloride in the outer medium in order to achieve the required balance for salt adaptation.

$MgCl_2$ increase in *Penicillium* fungus growth significantly, which was (10.8 x 10^{-4}) colony as compared to NaCl and $CaCl_2$ (4.5 x 10^{-4}) and (6.6 × 10^{-4}) colony formation respectively.

Table 2 : Effect of salt type and concentration on the alive spore density of *Penicillium* fungus

Salt concentration (ds. m^{-1})	Number of live reproduction units (x10^{-4}) for *Aspergillus niger* fungus in salty mediums			Average
	$CaCl_2$	**$MgCl_2$**	**NaCl**	
0	4	4	4	4
2	2.1	17.6	3.6	10.6
4	3.6	4.8	4.4	4.2
6	2.4	3	2.4	2.6
Average	4.5	10.8	6.6	
L.S.D	5.5	6.1	4.7	

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