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In-vitro studies on salinity stress on the growth of aspergillus oryzae

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ABSTRACT

The effect of Fungi under salinity condition was studied. Different concentration of Sodium chloride as 0 (control), 0.5% (low), 4.5% (medium) and 17.5% (high) were employed for salinity stress. It was seemed that fungi colonization was differ in different saline condition. Weight of fungal mat were higher than control in 7.5% saline concentration .incubation period was 15 days .Intervals of 7th and 15th day of incubated sample tested for protein estimation and amino acid test.

KEYWORDS

Aspergillus oryzae;
Salinity;
Growth;
Fungal mat.

INTRODUCTION

All living cells respond to unfavourable environmental conditions i.e. chemical and physical agents, by synthesizing a variety of low-molecular weight compounds as protective responses[6]. The production of low molecular mass hydrophilic compounds, such as aminoacids, sugar alcohols, soluble sugars and proteins as a response of the exposure to salt stress, reduce the osmotic potential of the intracellular solution to achieve osmotic equilibrium with the surrounding medium[5]. Several investigators have studied the response of microorganism to UV-lights and gamma irradiation under salinity stress condition. Most studies dealt with the radiosensitifying action of Salt, the damages caused by irradiation in the presence of Salt in macromolecular components of living cells and how such effect was abolished by mutations or by the addition of certain solutes at the time of irradiation[4].

S.A.Diyaolu and L.O.Adebajo[3]. These two biologists studied about effect of Sodium chloride and relative humidity on growth and sporulation of mould is isolated from cured fish. A.R.Biggs et al.[2]. As per they studied on .effects of calcium salts on growth polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola*.. H.S.H. Attaby[1] studied, the influence of salinity stress on the growth, biochemical changes of *Pencillium chrysogenum*.

The *A.niger, A.flavus, A.oryzae* and *A.chevalieri* shows slight inhibition of mycelial growth and for sporulation was recorded when they were cultured in basal medium containing 5% Sodium chloride. The extent of inhibition increased with increasing salt concentrations and at 25% level all the species had their growth completely inhibited. Similarly calcium salts also shows inhibitory mechanism on fungi, Calcium Pantothenate and dibasic calcium phosphate reduced the growth of fungal mycelia on PDB by approximately 65% compared with the control. The activity of calcium salts was not affection by pH.

These investigations elucidate the influence of salinity stress, induced by different concentration of salt

Regular Paper

TABLE 1: Dry mass of fungal mat at various concentration of salt

Sl. no.	% Of Nacl	Weight of filter paper	Weight of paper +Org.	Weight of organism	Average
1	Control	910 mg	1.050 g	0.63 g	0.44g
	Control	970 mg	1.550 g	0.58 g	
2.	0.5%	945 mg	1.200 g	0.255 g	0.267g
	0.5%	940 mg	1.220 g	0.280 g	
3.	1.0%	920 mg	1.250 g	0.33 g	0.34 g
	1.0%	920 mg	1.270 g	0.35 g	
4.	1.5%	910 mg	1.295 g	0.385 g	0.387 g
	1.5%	910 mg	1.300 g	0.390 g	
5.	2.0%	840 mg	1.280 g	0.44 g	0.45 g
	2.0%	810 mg	1.280 g	0.47 g	
6.	2.5%	820 mg	1.300 g	0.48 g	0.48 g
	2.5%	850 mg	1.340 g	0.49 g	
7.	3.0%	810 mg	1.350 g	0.54 g	0.51 g
	3.0%	830 mg	1.320 g	0.54 g	
8.	3.5%	850 mg	1.440 g	0.59 g	0.54 g
	3.5%	810 mg	1.300 g	0.49 g	
9.	4.0%	640 mg	1.240 g	0.60 g	0.61 g
	4.0%	790 mg	1.400 g	0.61 g	
10.	4.5%	800 mg	1.450 g	0.65 g	0.67 g
	4.5%	720 mg	1.410 g	0.69 g	
11.	5.0%	890 mg	1.590 g	0.70 g	0.71 g
	5.0%	1.100 g	1.810 g	0.71 g	
12.	5.5%	1.070 g	1.770 g	0.70 g	0.72 g
	5.5%	0.720 g	1.460 g	0.74 g	
13.	6.0%	1.050 g	1.820 g	0.77 g	0.72 g
	6.0%	.850 g	1.530 g	0.68 g	
14.	6.5%	.900 g	1.660 g	0.76 g	0.71 g
	6.5%	.910 g	1.570 g	0.66 g	
15.	7.0%	.960 g	1.720 g	0.77 g	0.755 g
	7.0%	.900 g	1.640 g	0.74 g	
16.	7.5%	.930 g	1.800 g	0.87 g	0.87 g
	7.5%	.920 g	1.790 g	0.87 g	
17.	10.0%	1.070 g	1.700 g	0.63 g	0.62 g
	10.0%	1.060 g	1.67 g	0.61 g	
18.	12.5%	1.100 g	1.48 g	0.38 g	0.40 g
	12.5%	1.330 g	1.75 g	0.42 g	
19.	15.0%	1.250 g	1.50 g	0.25 g	0.23 g
	15.0%	1.430 g	1.64 g	0.21 g	
20.	17.5%	1.380 g	1.40 g	0.02 g	0.02 g
	17.5%	1.230 g	1.25 g	0.02 g	

TABLE 2: Estimation of protein by Biuret method (7 days of incubation)

Sl.no.	Volume of std [protein soln. in ml	Volume of H_2O in ml	Biuret reagent		Conc. of protein in µg	OD at 540 nm
1	0	3	4ml	Incubate at room temperature for 30 Min.	0000	0
2	0.2	1.8	4ml		1000	0.051
3.	0.4	1.6	4ml		2000	0.094
4.	0.6	1.4	4ml		3000	0.140
5.	0.8	1.2	4ml		4000	0.166
6	1	1	4ml		5000	0.220
C		1.8	4ml		100	0.005
0.5%		1.8	4ml		100	0.003
1%		1.8	4ml		1200	0.057
1.5%		1.8	4ml		1200	0.058
2%		1.8	4ml		1200	0.057
3%		1.8	4ml		1200	0.055
3.5%		1.8	4ml		1200	0.056
4%		1.8	4ml		1300	0.059
4.5%		1.8	4ml		1300	0.062
5%		1.8	4ml		1200	0.055
Estimation of protein by biuret method (15 days incubation)						
B	0.0	1.0	4ml.	Incubate at room temperature for 30 minutes	0	0
S_1	0.2	1.8			1000	0.051
S_2	0.4	1.6			2000	0.094
S_3	0.6	1.4			3000	0.140
S_4	0.8	1.2			4000	0.166
S_5	1.0	1.0			5000	0.220
C		1.8			2400	0.11
0.5%		1.8			2700	0.126
1.0%		1.8			2500	0.115
1.5%		1.8			2500	0.117
2.0%		1.8			2500	0.116
2.5%		1.8			2400	0.111
3.0%		1.8			2400	0.113
3.5%		1.8			2500	0.117
4.0%		1.8			2600	0.119
4.5%		1.8			2600	0.124
5.0%		1.8			2400	0.110

and calcium salts, on the growth of fungi and the physiological changes involved in its possible salinity tolerance and the response of the fungus to gamma radiation in presence and absence of salinity stress.

MATERIALS AND METHODS

Preparation of fungal suspension

The spores of *Aspergillus oryzae* NCIM1212 were transferred into sterile Tween 20 solutions. This homogeneous solution of fungal suspension was transferred aseptically by using inoculation loop to the respective conical flasks containing media (PDB) of different concentration of salt. The conical flasks were well shaken for the uniform mixing of the fungal spores and were incubated at room temperature for 7 to 15 days.

Determination of fungal biomass

The biomass of the fungal mass was expressed as dry weight. A1 filter paper was used to get fungal mass and it was dried in hot air oven for 100^0C for 30 Min. The dry weights of fungal biomass were calculated.

Biochemical analysis

The filtrate was used for estimation of protein by

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TABLE 3 : Estimation of amino acid (proline) by acid ninhydrin (7 days)

Sl. no.	Vol.of std soln. In ml	Vol.of sulphuric acid in Ml	Vol.of glacial acetic acid	Vol. of acid ninhy drin	Vol. of toulene		Conc. of amino acid in µg	OD
1	0	2.0					0	0.0
2	0.2	1.8					20	0.056
3.	0.4	1.6					40	0.078
4.	0.6	1.4					60	0.157
5.	0.8	1.2					80	0.195
6	1.0	1.0					100	0.250
C		1.8					0	0.003
0.5%		1.8					1	0.016
1%		1.8					1	0.013
1.5%		1.8					1	0.012
2%		1.8					1	0.013
2.5%		1.8					2	0.017
3%		1.8					6	0.021
3.5%		1.8					6	0.020
4%		1.8					2	0.014
4.5%		1.8					6	0.020
5%		1.8					1	0.008

Estimation of amino acid (proline) by acid ninhydrin (15 days)

1	0	2.0					0	0.0
2	0.2	1.8					20	0.056
3.	0.4	1.6					40	0.078
4.	0.6	1.4					60	0.157
5.	0.8	1.2					80	0.195
6	1	1.0					100	0.250
C		1.8					1	0.014
0.5%		1.8					2	0.017
1%		1.8					10	0.032
1.5%		1.8					10	0.023
2%		1.8					15	0.040
2.5%		1.8					14	0.036
3%		1.8					15	0.038
3.5%		1.8					6	0.022
4%		1.8					2	0.018
4.5%		1.8					2	0.016
5%		1.8					1	0.010

Biuret method and estimation of amino acid (Proline) by Ninhydrin method.

RESULT AND DISCUSSION

It was observed that the fungal biomass was highest 8.75g at 7.5% concentration and lowest mass 0.023 at 17.5% concentrations. Under salt stress *A.oryzae* shows highest protein concentration in 0.5% and least concentration of protein in 5% of NaCl and it shows highest amino acid concentration in 3% NaCl and lowest concentration of amino acid in control in 7 days of incubation period and 2% NaCl in 15 days of incubation period. The lowest concentration of Amino acid is control in 7 days of incubation period and 5% in 15 days of incubation period between the concentrations of 0.5%-5%.

CONCLUSION

There are many types of stress in nature on plants and fungus, which includes drought water stress, salinity stress, etc. Salinity stress is due to high concentration of salts. It is important to study that which fungus tolerate how much concentration of salt that is ability of organism to tolerate salinity stress *A.oryzae* can tolerate upto 15% salt concentration. By studying the pathogenic organisms. ability to tolerate stress we can inhibit their growth by adding more salts and thus we can prevent many diseases like root rot and related diseases.

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