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Research Article

Studies on the effect of salinity on some *aspergilli* of usar and fertile soil to assess possible ecological specialization

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Abstract: A comparative salt (Viz., Sodium Sulphate, Sodium Chloride, Magnesium Sulphate) tolerance tests conducted in relation to usar (=Alkaline Soil) and fertile soil *Aspergilli*, revealed that over all, usar isolates showed more growth and tolerance on increased salt concentrations than their fertile counterparts. Among the three salts put to test, sodium chloride has shown pronounced toxic or inhibitory effect on Aspergilli at higher concentrations. The maximum tolerance has been exhibited by *Aspergillus fumigatus* as it grew up to twenty four percent concentration, however none of the other *Aspergilli* including both usar and fertile type showed such type of sustainability. Of the salts tested, Magnesium sulphate seemed to be the salt of more utility to both types of isolates as exhibited by their growth at different concentration levels and further all the Aspergilli tested showed more physical growth at different concentrations of this salt in comparison to other two salts tested. Salinity also affected the sporulation of different *Aspergilli*. Visual observations of culture of different *Aspergilli* revealed that usar islotaes comparatively showed more tolerance even in sporulation. Usar isolates showed the alkaliphilic nature by showing more salt tolerance and can be recognized as Haloalkaliphiles as they have attained ecological specialization to quite an extent.

Keywords: Salinity, *Aspergilli*, Usar soil, Ecological specialization.

Introduction

Soil support an abundant and extremely diverse population of micro organisms with a number of biotic and abiotic factors influencing either singly or in combination, the existence of microbes including fungi. Ecologically, every living organisms requires a set of physical condition for the optimal growth.

"Usar" soil widely occur in many part of Uttar Pradesh and sustain extreme ecological conditions in having high ph, high degree of salinity, comparatively low moisture content and are exposed to high temperature in summers, thus are subjected to relatively high degree of solar radiation. During the present studies (Soni, 2004). It has been observed that large number of fungal forms representing different fungal groups do occur in active state in these alkaline soils. A continuous and prolonged effect of the extremes of such conditions as mentioned above, appear likely to develop some degree of eco logical specialization in microorganism native to the soil and with this objective in view, investigations were undertaken to study the impact of some ecological conditions prevailing in these soils on such microorganisms, specially *Aspergilli* which constituted major proportion of mycoflora in summers during which rigorous condition prevail.

In the present paper, studies on the affect on salinity tolerance on ten species of *Aspergilli* have been carried out and result have been presented.

Materials and Methods

For studying the effect of salinity or salt tolerance the cultures were grown on Czapek-Dox liquid medium (in which different concentrations of salts viz., sodium sulphate, sodium chloride and magnesium sulphate were added accordingly). The concentration of different salts taken up during the studies were 3,6,9,12,15,18,21,24 (%) and the respective control treatments were maintained on normal Czapek's broth medium. The ph of the medium, initially was adjusted at 7.0 before steam autoclaving.

For finding out the comparative growth of the mycelial mat, 50 ml of the medium was taken in Erlenmeyer flasks. After inoculation with spore suspensions of different species these flasks were incubated 28+-2degree Celsius for seven days. Later the fungal mats were filtered on a previously dried and weighed filter paper (Whatman no.1), which were dried for 24 hours at 60 degree Celsius and weighed again for obtaining the final dry weight of fungal mycelial mat. For each treatment three replicates were taken and average of these replicates have been presented as final readings.

Results

The data regarding the comparative growth on different salts(sodium sulphate, sodium chloride,and magnesium sulphate) and the effect of their different concentration of on 'usar' soil aspergilli isolates and their fertile soil counterparts have been tabulated in [Table (1),(2),(3)]. On normal Czapek's broth usar and fertile isolates initially showed almost similar growth. With the increase in the salt concentrations, the fertile isolates showed less growth, while usar isolates comparatively showed enhanced growth, however, even usar isolates showed retarded growth when the concentration of salt further increased gradually in the medium. Over all, usar isolates showed more growth on increased salt concentrations than fertile soil counterparts thus exhibiting considerable more tolerance to different salts. This is quite convincing indication to the fact that the usar soil *Aspergilli* have far

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more tolerance to salt concentration(s) in them and consequently, mycofloral forms inhabiting these alkaline soils have developed more adaptability to such conditions.

All the usar soil isolates in relation to all the three salts tested showed maximum growth at 3 percent salt level. Among the three salts tested sodium chloride has shown pronounced toxic or inhibitory effect on the fungal growth at higher concentration (s).

In general, both type of isolates exhibited maximum growth at three percent level of sodium sulphate. However, the growth of both types of isolates showed a decline in growth with the increase of the salt concentration in the medium but as usual, usar isolates showed more tolerance, as exhibited by their growth up to twenty one (21%) percent level. The maximum tolerance has been exhibited by *A.fumigatus* as it grew up to twenty-four percent level, however none of the other isolate including both usar and fertile type, showed such type of sustainability. Apart from *A.fumigatus*, among other usar isolates *A.amstelodami* and *A.chevolieri*, showed good tolerance while rest of the *Aspergilli* showed average level of tolerance to sodium sulphate.

Salt sodium chloride, a potent electrolyte, showed pronounced inhibitory effect as both type of isolated showed very poor growth at 21% and 24%. None of the usar and fertile isolates showed any perceptible growth at 24% concentration. All the fertile isolates grew only upto 18% percent level while, they showed almost no or little growth on twenty one (21%) percent level and twenty four (24%) percent level. While usar isolates like *Aspergillus amstelodami, A.flavus, A.fumigatus, A.niger, A.terreus* and *A.ustus* showed tolerance upto 24% and therefore, they can be considered as true osmophilic salt tolerant forms to quite an extent. *Emericella nidulans* a cleistothecial Aspergilli, showed marked inhibition in cleistothecia formation even at 12 percent level, in both types of isolates. Among the three salts tested magnesium sulphate seems to be salt of more utility to both types of isolates as exhibited by their growth at different concentration levels. In this case also alkaline or usar soil isolates showed more tolerance than their counterparts upto 18 percent level, as both fertile and usar isolates showed quite perceptible growth, but at further higher concentration growth of both types of isolates declined to quite an extent. Like two other salts, tolerance test experiments in this case also, usar isolates like *A.amstelodami, A.chevalieri, A.flavus, A.fumigatus, A.niger* and *A.ruber* showed alkaliphilic nature by exhibiting their salt tolerance up to higher levels, i.e., 24%. If amount of growth is considered almost all the fungal isolates(both usar and fertile isolate), showed more physical growth at different concentration of this salt, in comparison to other two salts tested in the present studies.

Salinity affected the sporulation of different *Aspergilli*. With the gradual increase in salt concentration the sporulation declined and with further gradual increase in percentage of salt concentration the sporulation looked to be almost absent or negligible. Among the three salt tried, sodium chloride affected the sporulation quite appreciably as the effect on sporulation started appearing even after 3% level. Visual observation revealed that over all usar isolates comparatively showed more tolerance even in sporulation.

Discussion

Salinity has been reported to affect the existence and growth of fungi in soil. In this connection some work on marine fungi has been done by (Barghoorn and Lender, 1944; Ritchie, 1930; Jones and Jennings, 1964; Omar *et al.,* 1994). The picture is still more complex in soil environment where the conditions are very drastic such as in usar soils where high slat concentrations accompanied by high soil reaction, among other factors, might be proving a real hazard, if one may put it so to microbial life where they have to be really trying to survive. The present study is only a beginning in the process of understanding some of these intricate events. It has been found that fungi, *Aspergilli* in particular, can tolerate appreciably high concentrations of salt found in these soils. However, the degree of tolerance varies from species to species.

Table 1. Showing the effect of different % of sodium sulphate on the growth of Usar and Fertile isolates of *Aspergilli* (Diameter in mm)

% of	*A. amstelodami*		*A. chevalieri*		*A. citroporous*		*A. flavus*		*A. fumigatus*		*A. niger*		*A. ruber*		*A. terreus*		*A. ustus*		*E.nidulans*	
Na_2SO_4	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U
0 (N)	71	69	71	69	68	65	84	7	93	91	79	77	71	69	69	67	63	63	91	90
3	79	80	77	80	76	78	101	97	90	94	68	86	79	81	83	82	58	55	90	95
6	57	67	57	63	55	57	79	87	57	64	50	57	57	61	71	77	42	43	59	65
9	41	61	41	60	39	45	55	62	45	54	37	51	43	64	45	62	33	35	44	55
12	30	41	29	45	27	31	29	37	35	41	27	35	33	49	37	46	29	32	36	42
15	17	35	17	33	19	27	20	28	23	28	21	31	17	35	25	38	14	23	20	28
18	14	30	14	36	12	22	12	25	13	21	14	22	11	27	14	30	11	20	10	21
21	10	23	13	30	8	19	-	15	-	37	-	20	9	18	*	15	8	13	*	15
24	*	17	*	17	*	14	-	12	-	28	-	14	*	*	*	10	*	*	*	*

*= Negligible or no growth

Table 2. Showing the effect of different % of sodium chloride on the growth of 'Usar' and 'Fertile' isolates of *Aspergilli*

% of	*A.amstelodami*		*A.chevalieri*		*A.citroporous*		*A.flavus*		*A.fumigatus*		*A.niger*		*A.ruber*		*A.terreus*		*A.ustus*		*E.nidulans*	
NaCl	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U
0 (N)	70	68	72	72	63	60	60	77	70	86	84	72	70	64	62	60	64	64	84	83
3	72	73	70	73	71	72	94	90	83	87	61	79	72	73	76	75	53	58	83	88
6	50	60	50	56	50	52	72	80	50	57	43	50	50	54	64	70	50	56	52	58
9	34	54	34	53	34	40	48	55	38	47	30	44	36	57	38	55	38	48	37	48
12	23	34	22	37	22	26	22	30	28	34	20	28	26	42	30	39	24	29	29	35
15	10	28	10	26	14	16	13	21	16	21	14	24	10	28	18	31	9	18	13	21
18	8	20	7	19	4	10	5	18	6	14	7	15	*	19	7	23	*	12	7	14
21	*	18	*	17	*	5	-	8	-	10	-	13	*	15	-	8	*	8	*	8
24	*	9	*	*	*	*	*	6	*	8	*	8	*	*	*	7	*	9	*	*

*= Negligible or no growth

Table 3. Showing the effect of different % of magnesium sulphate on the growth of 'Usar' and 'Fertile' isolates of *Aspergilli*

% of	*A.amstelodami*		*A.chevalieri*		*A.citroporous*		*A.flavus*		*A.fumigatus*		*A.niger*		*A.ruber*		*A.terreus*		*A.ustus*		*E.nidulans*	
$MgSO_4$	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U
0(N)	75	73	70	70	66	65	82	82	91	89	77	75	68	65	67	65	62	61	89	88
3	83	84	79	83	78	81	99	95	88	92	66	84	81	84	81	80	60	58	88	93
6	61	72	59	66	57	60	77	85	55	62	48	55	59	64	69	75	44	46	57	63
9	45	65	43	63	41	48	53	60	43	52	35	49	45	67	43	60	35	38	42	53
12	34	45	31	48	29	34	27	35	33	39	25	33	35	52	35	44	31	35	34	40
15	21	31	19	36	21	30	18	26	21	26	19	29	19	38	23	36	16	26	18	26
18	12	27	16	39	14	23	10	23	11	21	12	20	13	30	12	28	13	23	-	19
21	8	18	15	33	10	21	-	20	-	18	-	18	11	21	-	13	10	16	-	13
24	*	12	10	22	*	11	-	13	-	26	*	12	*	13	*	*	*	*	-	-

*= Negligible or no growth

Of the three salt tried during the present studies, the species of *Aspergilli* tested showed comparatively less tolerance to sodium chloride in comparison to magnesium sulphate and sodium sulphate. Among the three salt tested sodium chloride has shown pronounced toxic or inhibitory effect on the fungal growth at higher concentration. Though the tolerance to sodium chloride have been shown by usar isolates of Aspergilli upto twenty one percent but the maximum tolerance upto twenty four percent has been shown by *A. amstelodami, A. flavus, A.fumigatus, A.niger, A.terreus* and *A.ustus*.

Above studies in relation to salinity, sodium sulphate tolerance wise showed intermediate type of effect but here also usar isolates of *Aspergilli* showed comparatively more tolerance than normal or fertile soil counterparts. In comparison to sodium sulpahte, the both types of aspergilla isolates showed better growth on the media supplemented with magnesium sulphate. However, both types of isolates of *Aspergilli* in lieu showed better growth on the media supplemented with sodium chloride.

In case of sodium sulphate and magnesium sulpahte *A. fumigatus* showed better growth in comparison to other usar isolates of *Aspergilli* upto twenty four percent. However, none other including both usar and fertile types showed such types of sustainability. Such salinity tolerant organisms have been categorized by Grant and Jones (2000) as haloalkaliphiles. Thus the *Aspergilli* isolates of usar origin can be recognized as real Haloalkaliphiles as they seem to have attained ecological specialization to quite an extent.

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