Increased pH Tolerance of some Aspergilli isolated from 'Usar' (Alkaline) soils — A possible Indication of ecological Specialization

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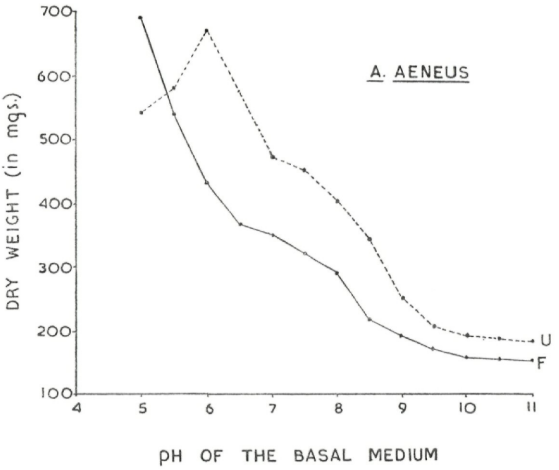
Introduction

'Usar' (alkaline) soils, that occur widely in certain parts of U. P. and Bihar, exhibit extreme ecological conditions in characteristically having a high pH, high degree of salinity and low moisture content during dry weather. Lying either almost barren being devoid of any vegetation or, being sometimes covered only with a poor patchy grassy growth these soils, during summer months, are also subjected to high temperature and relatively higher degree of solar radiation. A study of soil microflora, particularly the fungi, occurring in 'Usar' soils, has



been going on in this laboratory for some time past in which, a large number of fungi have been isolated (Rai et al., 1968 a, b & c; 1970 a & b). Some of these, specially those obtained from soils with high moisture content, occur in active phase. A continuous and prolonged

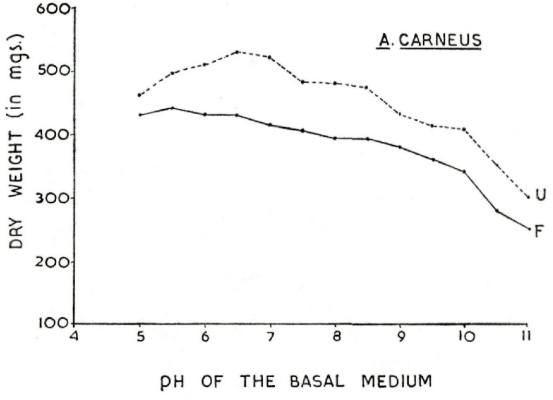
effect of the extremes of such conditions as mentioned above, appear likely to develop some degree of ecological specialization in micro-organisms native to these soils and with this object in view, investigations were undertaken to study the impact of various ecological conditions prevailing in these soils on such microorganisms.

pH of the surrounding medium is one such important factor that is widely known to have a marked influence on the growth, sporulation, pigmentation and various other life-processes of the organisms. There is some work to show that the same species isolated from alkaline soils possesses a higher pH optimum for its growth than the corresponding strains from other habitats especially with regard to Actinomycetes (T a b e r, 1960) and Bacteria (S e n, 1963) but very little work in this direction has been done in relation to fungi (M u k e r j i, 1966).

This paper deals with the studies on the relative pH tolerance and growth-optima pH of some Aspergilli obtained from 'U s a r' soils and their counterparts from fertile soils.

Materials and Methods

Aspergilli form the dominant fungal flora of 'U s a r' soils and consequently, species of this genus were selected for the present investigations. Pure cultures of the following strains obtained from soils of dif-

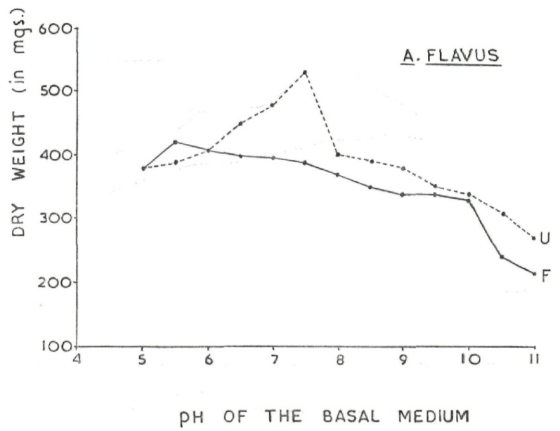


ferent pH values (noted against each) were utilised: *A. aeneus* (pH 9); *A. striatus* (pH 10); *A. flavus, A. sulphureus* and *A. sydowii* (pH 8, 9, 10, 11); *A. versicolor* and *A. carneus* (pH 8, 9 & 10). Corresponding strains of each of these species isolated from different fertile soils (pH 6.2—7.3) were utilised for comparative study.

Growth optima were determined by dry-weight method using Czapek-Dox liquid medium whose pH-values, ranging between 5—11, were adjusted at intervals of 0.5 and the final pH of the medium was recorded after autoclaving. For each treatment, 50 ml. of the medium was dispensed in 250 ml. Erlenmeyer flasks. After inoculations by dropping 0.5 ml. of spore-suspension of each strain, the flasks were incubated at 30 ± 1° C. generally for 10 days (7 days for the first three species) and dry weight of the mycelial mat along with the final pH of the basal medium, after extraction, was recorded in each case. While observations were made for all the strains mentioned earlier, the data presented in the table pertains to only one strain of each of the species and are the averages of at least three sets of experiment.

Observations

Two main observations are evident from the tabulated data and the graphs viz., (i) that, 'U s a r' strains can tolerate higher pH values and, (ii) their optimum growth is generally at a relatively higher pH when compared with their fertile counterparts. The corresponding pH values for the growth optima of 'U s a r' and fertile strains of *Aspergillus* spp. have been found to be as under:

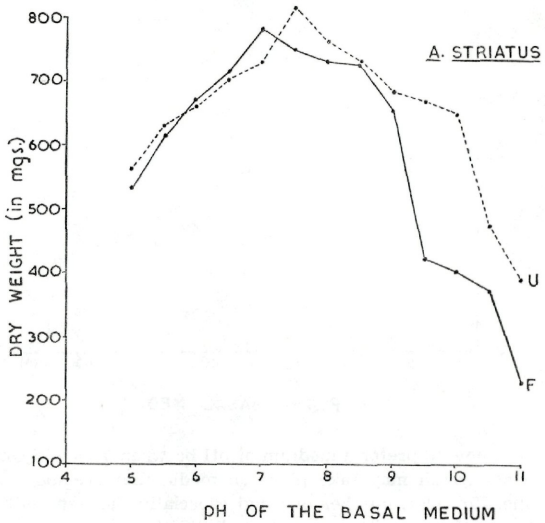


6.0/5.0 for *A. aeneus;* 6.5/5.5 for *A. versicolor* and *A. carneus;* 6.0/5.0 for *A. sulphureus;* 7.0/6.5 for *A. sydowii;* 7.5/5.5 for *A. flavus* and 7.5/7.0 for *A. striatus* (Graphs).

A comparative study of the growth — optimum pH values, obtained for the various 'U s a r' strains of each species, reveals that strains from

optimum growth in comparison to those that have been obtained from the lower pH soils. The respective pH optimum for *A. sulphureus* and *A. sydowii* strains isolated from 9, 10 & 11 — pH soils are 6.5 and 7.0 as against 6.0 and 6.5 for those from lower pH soils. Similar differential values have been obtained for the various strains of *A. flavus* also e. g., pH 11 strain has its growth optimum at pH 7.5; pH 10 strain at 7.0 and the lower ones at 5.5.

The final pH of the basal medium, after extraction of the mycelial mat, was also recorded in every case. A definite shift in the initial value was observed nearly in all cases after the fourth day of the growth. The general trend of results is that the lower pH values drift to become higher and the higher ones become lower. The shift on either side, of the intermediate values (pH 7.0—8.0) is relatively small. As far as this property is concerned, the 'U s a r' and fertile strains exhibit similar trends. The lower pH values of the medium especially in case of *A. sydowii, A. carneus* and *A. versicolor* drift to become neutral while, the higher pH values, on the other hand, lower down



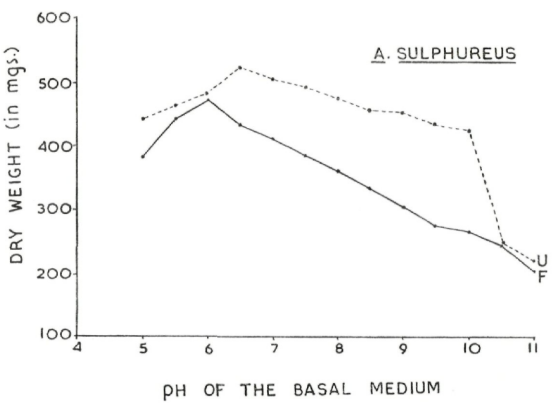
only upto 8.5—9.0 except in case of *A. flavus* in which they dropped considerably viz., upto 4.5—5.7. Strains of *A. aeneus* and *A. striatus* however, behaved differently and the initial pH values ranging between 5—11 either rose up or dropped down to the values between pH 8.5—9.5.

In earlier studies with *Aspergillus fumigatus* var. *acolumnaris* it was observed that the initial pH 7 of the basal medium dropped down to 3.4.

Discussion

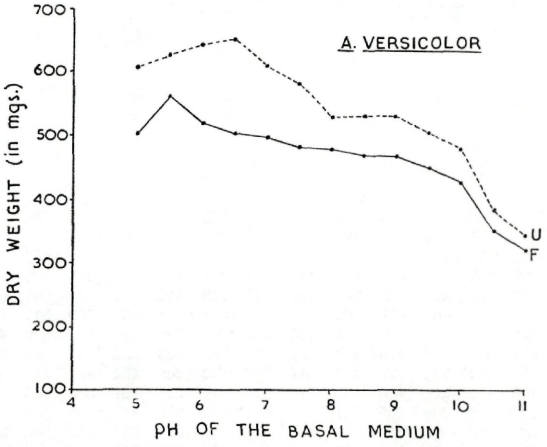
Soil provides a complex habitat supporting an abundant and extremely diverse population of microorganisms with a number of biotic and abiotic influencing either singly or in combination the existence of such microbes. Adaptations in an organism including the physiological changes, appearing as a result of its interaction with the environment particularly in relation to higher plants, has been known for long time. However, comparatively very little work on this aspect has been done in relation to microorganisms native to alkaline soils.

Of the various abiotic factors influencing the behaviour of an organism in the soil, pH is known to have marked effect on its growth, sporulation and other cultural characteristics sometimes, even inducing a certain degree of specialization due to its prolonged impact (Taber, 1960; Sen, 1963; Mukerji, 1966). Most fungi, for their satisfactory



growth, are know to prefer a medium of pH between 5—6 and complete inhibition of growth may take place on media that are too acidic or too alkaline. However, number of fungi especially the Aspergilli, have been found to favourably grow on media with a wide range of pH 2 to 12 (Johnson, 1923; Thom & Steinberg, 1939; Steinberg & Thom, 1940 a&b; Wolf et al, 1950; Agarwal, 1958; Brewer, 1960 and Agnihotri, 1964) while *Helicostylum piriforme* has been shown to exhibit two pH growth-optima (Mehrotra & Mehrotra, 1962). The present studies, with seven species

of *Aspergillus* (which is the most commonly occurring form in the 'U s a r' soils), reveal that the 'U s a r' strains exhibit an increased pH-tolerance than the corresponding fertile soil strains. It was also observed that the strains isolated from higher pH soils generally, possess a higher pH growth-optima as against those obtained from soils of lower pH values. This difference in the pH-tolerance of 'U s a r' strains is indicative of their ecological specialization (adaptation) to higher alkaline habitats. Although enough data is not available on such studies with regard to fungi, T a b e r (1960) isolated 107 acid-sensitive strains of Actinomycetes from 9 alkaline and 1 acidic Canadian soils that were able to grow at pH 6.2—8.5. S e n (1963) isolated 2 strains of a *Bacillus* from the alkaline soils near Delhi, both showing maximum growth at pH 9,0. Results supporting some such observations were obtained earlier in this laboratory by M u k e r j i (1966) with respect to 2 fungi isolated from alkaline and fertile soils. In the present study also, the 'U s a r' (alkaline) soil strains have been found to possess a higher growth and increased pH-tolerance than their fertile counterparts.



The fungi are known to alter the pH of the basal media on which they grow. Several workers have reported rise and fall of the initial pH values for fungi (E d w a r d & D u r b i n, 1959; B r e w e r, 1960; A g n i h o t r i, 1962 & 1963 and M e h r o t r a & A g n i h o t r i, 1963). The results obtained here with regard to 7 species of *Aspergillus*, have also recorded a definite shift in the initial pH values of the medium that were adjusted between 5—11. So far as this property is concerned, the behaviour of both the 'U s a r' and fertile strains is

nearly the same. While, the pH values of 7.0 and its neighbourhood do not generally record a significant shift on either side, the lower pH values in at least 5 species viz., *A. aeneus, A. carneus, A. sydowii, A. striatus* and *A. versicolor* rise upto 7.0—9.2. Similar trend of results has been shown by A g a r w a l (1958) and H a s i j a (1965) for *Curvularia penniseti* and *Colletotrichum inamdarii* respectively. In contrast to this, the drop of the higher pH values is relatively less except in *A. flavus* where the initial pH 10—11 drops down to 4.0—5.7. Results of similar nature have also been obtained with *Aspergillus fumigatus* var. *acolumnaris* in which the initial pH 7.0 of the medium lowered to become 3.4 after 10 days of its growth. This property of both the Aspergilli may be of some significance in utilising microorganisms for reclamation of 'U s a r' soils.

T a b l e — Showing the relative growth as exhibited by dry weight measurements of 'U s a r' and fertile strains of seven Aspergilli.

	Average dry weights of mycelial mat in mgs.													
	A. aenus		*A. carneus*		*A. flavus*		*A. striatus*		*A. sulphureus*		*A. sydowi*		*A. versicolor*	
Initial pH	F	U	F	U	F	U	F	U	F	U	F	U	F	U
5.0	692	542	428	459	380	380	538	562	380	444	433	445	501	603
5.0	542	587	441	496	422	384	612	631	437	463	437	463	557	624
6.0	428	674	434	508	412	412	671	663	471	482	464	498	521	644
6.5	366	517	430	531	401	447	714	701	435	521	517	506	506	650
7.0	351	475	416	525	398	478	783	728	412	504	504	557	498	608
7.5	324	451	406	481	392	528	752	815	382	493	500	547	481	577
8.0	291	402	398	480	371	400	732	757	365	471	455	509	484	532
8.5	215	343	398	476	352	393	727	736	328	452	448	480	476	532
9.0	191	253	382	427	342	384	654	688	302	452	418	442	471	528
9.5	169	205	364	413	342	357	421	671	270	431	390	425	452	506
10.0	152	192	342	305	331	344	404	652	260	422	300	397	428	477
10.5	152	188	278	348	238	309	376	472	235	242	228	348	352	380
11.0	150	182	252	302	218	270	228	386	200	218	208	310	328	340

F = Fertile soil strain
U = 'U s a r' soil strain

Summary

The paper deals with the studies on the relative pH-tolerance and growth-optima pH of seven species of *Aspergillus* occurring commonly in Indian 'U s a r' (alkaline) soils. The 'U s a r' soil strains show rela-

tively higher pH-tolerance and their optimum growth is also generally at a higher pH as compared to their fertile counterparts. This behaviour of 'Usar' strains is indicative of an ecological adaptation in response to the conditions prevailing in these highly alkaline soils. A definite shift in the initial pH of the basal medium has also been recorded nearly in all the cases.

Acknowledgements

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Explanation of Figures

Showing the effect of pH on the growth of 'Usar' and fertile soil strains of certain Aspergilli.

U = 'Usar' soil strain
F = Fertile soil strain