MYCOSTASIS IN SEMI-ARID SOIL OF WEST PAKISTAN

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Eleven species of fungi were tested by agar disk technique for sensitivity to mycostasis of six different soils. The capability of spore germination of all fungi except a species of *Alternaria* was reduced to a varying degree due to mycostasis. All the six soils were mycostatic, the University of Karachi campus soil being the least. Autoclaving of the soils depleted the inhibitory factor. Autoclaved soil, when kept exposed to the atmosphere for 30 days allowing contamination by aerial microflora, regained mycostasis indicating probable microbial origin of the inhibitory factors. The upper layer of the soil, the site for intense microbial activity, was more mycostatic than the soil at lower depth. The soil containing moisture of 90% water holding capacity greatly diminished mycostasis but did not eliminate it.

Introduction

The occurrence of substances inhibitory to the growth and survival of soil fungi and bacteria has been reported from different parts of the world particularly from the temperate regions.[1,3,4,11] In tropical humid soil complete inhibition of germination of spores of fungi belonging to different taxonomic groups was noted.[9] Similar inhibitory effect against *Fusarium oxysporium* f. *cubense* was found in humid tropical soil of Honduras.[17] The phenomenon has been termed as fungistasis in earlier literature. Here the term mycostasis is being used following the suggestion of Dobbs, Hinson and Bywater.[5]

The cause and nature of mycostasis is not understood. Lockwood[13] suggested that diffusible toxic substances produced by *Streptomyces* sp. might be a cause of soil mycostasis. He obtained lysis of fungal mycelia in contact with unsterilized soil.[14] Lingappa and Lockwood[12] being unable to detect any fungitoxin in the soil postulated that the antibiotics produced by the microorganisms on the agar film used in the experiment was the cause of mycostasis.

The present work was undertaken to determine the presence of mycostasis in the tropical semi-arid region of West Pakistan where the annual rainfall is *ca.* 20 cm and the temperature ranges between 10°C and 43°C. The investigation was made to elucidate some aspects of the nature of mycostasis.

Materials and Methods

Organisms.—The following fungi were used. *Alternaria* sp.,* *Aspergillus flavus* Link, *A. fumigatus* Fres, *A. niveus* Bloch, *A. rugulosus* Thom & Raper, *A. ochraceus* Wilhelm, *A. tamarii* Kita, *A. ustus* (Bainier) Thom & Church, *A. terreus* Thom, *A. variecolor* (Bark & Br.) Thom & Raper, *Cunninghamella echinulata* Thaxter and *Monilia* sp. These were isolated from Karachi University Campus soil except *Cunninghamella echinulata* which was isolated from decaying sugarcane leaf. These were maintained on Czapek's agar medium.

Six different soils were tested for the presence of mycostasis using eleven species of fungi. For the experiments with the autoclaved soils the samples after moistening with distilled water were autoclaved at a pressure of 20 lb/in^2 for ½ hr.

Soils.—The soil samples were collected from the upper 15 cm layer unless otherwise mentioned. The pH and the maximum water holding capacity of the soil samples were determined by standard technique.[16] The soil samples employed were:

(A) Natural soils not fit for cultivation.

1. Karachi University Campus—fine and mixed with pieces of rocks, scrubby grasses and other herbs of low density and frequency, pH 7.8, water holding capacity (WHC) 11.6%.

2. Sea shore—fine sand, swampy, high salt content, no vegetation, pH 7.8, WHC 15.8%.

3. Hillock—rocky mixed with loamy fine sand, calcareous, pH 8.1, WHC 18.3%. The soil was pulverized before use.

4. Canal bed—samples from the bed of a flowing canal of sweet water, heavy growth of algae on the bed, fine sand, pH 8.4, WHC 16.5%.

(B) Cultivated. These were from agricultural belt adjacent to Karachi metropolis. The area has been under intensive cultivation for over 50 years.

1. Lucerne corn plot—loamy fine sand, standing crop of lucerne (*Medicago sativa*) and corn

*Gilman's nomenclature 7 has been followed.

(*Zea mays*) two-month old at the time of sampling, pH 8.9, WHC 15.5%.

2. Papaya-grass plot—loamy fine sand, standing crop of papaya (*Carica papaya*) two-year old at the time of sampling with ground cover of dog grass (*Cynodon dactylon*), pH 8.8, WHC 15.8%.

Jackson's [9] agar disk technique was used for assessing soil mycostasis. Air dried and sieved (2 mm sieve) soil samples (50 g each) were placed in petri dish and brought to 60% water holding capacity by adding distilled water. Four 10 mm square pieces of filter paper were placed equidistant from each other on the surface of the soil. An agar disk (2% distilled water agar) of 10 mm diameter and 1.5 mm thickness cut out by sterilized cork borer was placed on each of the filter paper squares and allowed to stand for 4 hr before seeding the agar disk surface with spore suspension. In subsequent experiments filter paper squares were replaced by tissue paper of the same size which allowed better diffusion of the mycostatic factor into the agar disks. The spore suspension was prepared in double distilled water and each agar disk was inoculated with a drop of this suspension. Spore suspension was obtained from actively growing fungal colonies on Czapek's agar medium. Control plates were similarly prepared with filter paper in place of soil. After inoculation the petri dishes were incubated at room temperature (28–32°C) for 14–16 hr which was found to be sufficient to give germination in control plates. At the end of this period the disks were covered with a drop of lactophenol to stop further growth of germ tubes. The disks were removed to the microslides and counts of germinated and ungerminated spores were taken in four fields per disk. Duplicate soil plates for each soil type were made and duplicate agar disks for each organism on each soil plate were employed, thus giving four disks for each organism and soil type. The data are based on about 800 spore counts in each case.

Results

Widespread Mycostasis and Effect of Autoclaving.—The data presented in Fig. 1 indicate that the germination of spores of all fungi except *Alternaria* sp. was inhibited. This inhibition to spore germination varied in different soils. The sea shore soil (No. 2) and the hillock soil (No. 3) severely inhibited the spore germination of most fungi. The lucerne-corn soil (No. 5) was more inhibitory than the other cultivated soil from papaya-grass plot (No. 6). The campus soil (No. 1) was least mycostatic. The per cent germination of all fungi in autoclaved soils was much higher than in respective unautoclaved soils indicating that most of mycostatic factor was thermolabile.

The different species of fungi varied in their sensitivity towards mycostasis. *Aspergillus flavus* was the most sensitive followed by *A. Ochraceous* and *A. niveus*.

Regeneration of Mycostasis Destroyed by Autoclaving.—One part of each of the six soil types was moistened (60% WHC), autoclaved and kept in 11-cm petri dishes with the lid open for 30 days in the laboratory to allow natural contamination prior to use. The soil samples were maintained approximately at 60% WHC by adding tap water from time to time. The other part of the same soil was autoclaved just before use. The pressure and time of autoclaving was the same as has been mentioned elsewhere. *Aspergillus flavus* was used as test fungus. The data (Table 1) are expressed in percentage of filter paper control where germination was 86%. The spore germination on soils autoclaved 30 days before experiment was greately inhibited and approached the data obtained on unsterilized soil in previous experiments. The inhibitory factor was partially but not completely removed by autoclaving as the per cent germination in freshly autoclaved soil was lower than in filter paper control.

Vertical Distribution of Soil Mycostasis.—The soil samples from the University Campus were collected from the surface (0 cm) and at depths of 10, 20, 30, 40, 50 cm. A trench of about 60 cm depth was dug. The soil samples were obtained from the respective depths mentioned above by pushing spatuala starting at lowest depth first. These were air dried, sieved and brought to 60% WHC by adding distilled water. *Aspergillus flavus* was used as test fungus. The germination of fungal spores was nil on surface soil with a gradual increase with depth (Fig. 2). The inhibitory factor was still present at a level of 50 cm depth where the germination did not rise above 40%. The effect of mycostasis was highest in the surface soil—the seat of maximum metabolic activities of microorganisms.

Decrease of Mycostasis with Increase of Soil Moisture Content.—The air-dried and sieved soil samples were moistened with distilled water so as to bring to 60, 70, 80 and 90% WHC. The soils used were from the Campus, sea shore and lucerne-corn plot. *Aspergillus flavus* and *Cunninghamella echinulata* were used as test fungi. The results are shown in the Table 2.

The inhibition of spore germination on campus and lucerne-corn soils was highest at a moisture

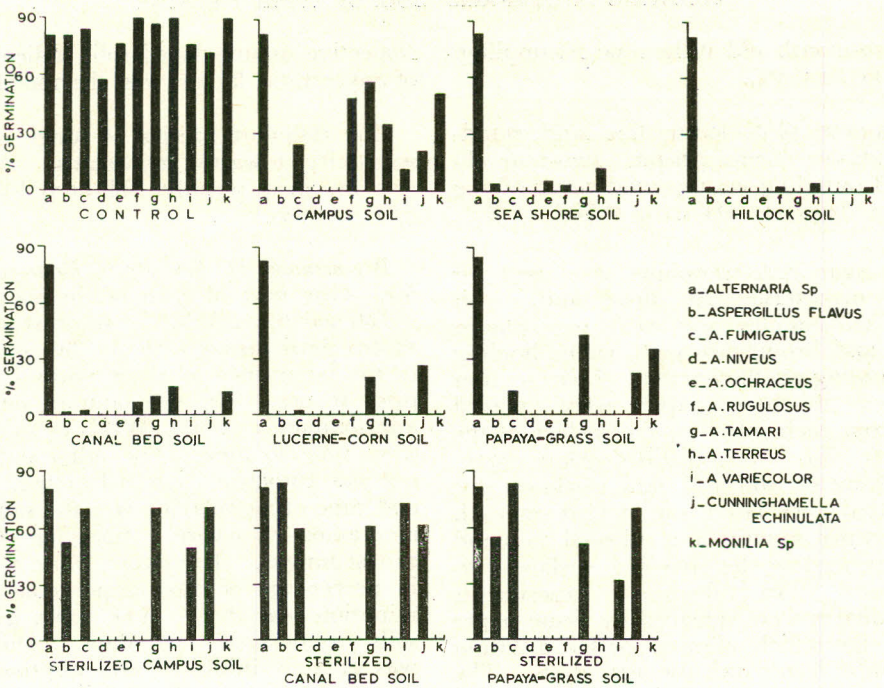

Fig. 1.—Percentage germination of spores of different speices of fungi on filter paper control, unsterilized and sterilized soils.

TABLE 1.—GERMINATION OF SPORES OF *Aspergillus flavus* ON SIX DIFFERENT SOILS FRESHLY AUTOCLAVED AND EXPOSED TO ATMOSPHERE FOR 30 DAYS.

Soil types	Percentage germination of spores on soils autoclaved	
	Fresh	30-day old
Campus ..	49	0
Sea shore ..	48	1
Hillock ..	59	2
Canal bed ..	69	0
Lucerne-corn ..	54	0
Papaya-grass ..	54	3

content of 60% WHC than at any other. The per cent germination of the spores of both the fungi gradually increased with the increase of moisture content of the soil. The inhibition existed even at soil moisture content of 90% WHC. The effect of mycostasis in sea shore soil remained unchanged with the increase of soil moisture content. The nature of the mycostasis in the former two soils and in the sea shore one was probably not the same.

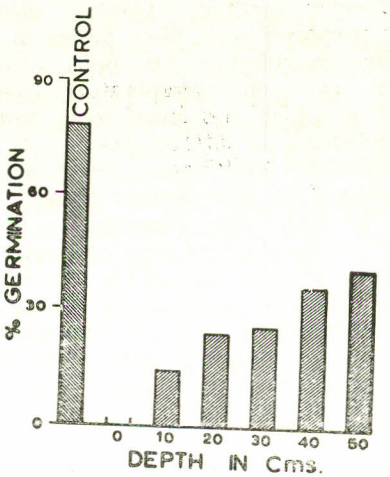

Fig. 2.—Percentage germination of spores of *A. flavus* on filter paper control and on soils of various depths.

Discussion

Except one species of *Alternaria* all fungal species tested were susceptible to mycostasis to varying degrees. The spore germination of *Alternaria* sp. remained unchanged. Similar variations in sensitivity of fungal species to mycostasis was also noted by other workers.[3,9,15] Chinn[3] noted that

TTBLE 2.—PERCENTAGE GERMINATION OF SPORES OF *A. flavus* AND *C. echinulata* ON SOILS MOISTENED TO 60,70,80 AND 90% OF WHC.

Organisms	Soil types	Percentage germination of spores on control and on soils moistened to				
		Control	60	70	80	90 (WHC)
A. flavus	Campus	75	3	11	9	33
	Sea shore	75	0	0	0	0
	Lucerne-Corn	75	6	12	18	28
C. echinulata	Campus	90	22	30	30	65
	Sea shore	90	0	0	0	0
	Lucerne-Corn	90	20	27	32	63

the spores of *Penicillium notatum* and *Stachybotrys atra* germinated in contact with soil but those of *Cladosporium* sp., *Aspergillus* sp., and *Fusarium culmorum* did not germinate in that condition. Jackson,[9] giving a list of fungi as "affected" and "unaffected" to mycostasis, mentioned that the spores of *Helminthosporium ravenelli* were stimulated to germinate in presence of soil. Thus the sensitivity to mycostasis is not related to any taxonomic group.

All the six soils investigated displayed some degree of mycostasis. Since these soils were obtained from different localities varying in physical and chemical composition, it might be said that other soils from the semi-arid zone would have shown this property if tested. This substantiates the statement of Dobbs and Hinson[4] who first mentioned about "widespread fungistasis in soil."

The different soils differed in their degree of mycostasis. The soils from hillock and sea shore were highly inhibitory to germination of fungal spores whereas the Campus soil was least mycostatic. None of these three soils carries any vegetation. The hillock soil is highly calcarious and the sea shore soil is saline and water-logged favouring the anaerobic microbes. The microbial population is presumably low in these soils as compared to the cultivated soils. Although speculative it is not unlikely that the high salt content in the hillock and sea shore soils largely contributes to the inhibitory factor in these soils. Dobbs[6] also suggested the possibility of inorganic origin of mycostasis. There is no clear evidence uptil now as to the origin of the mycostasis. It is argued that the antibiotics may accumulate in soil to produce an overall mycostatic activity.[2,10] The low mycostatic effect in the Campus soil could be due to low bioactivity. This activity in the two cultivated soils was higher than that in the campus soil. The inhibitory factor in the cultivated soils may have been due to micro-organisms as well as to higher plants. Mallik[15] showed that the mycostatic activity of a soil was influenced by its crop history.

The mycostatic factor was greatly reduced by autoclaving. Similar results were obtained by Jackson.[9] The mycostasis depleted by autoclaving was regenerated after some time when the soil was allowed to be contaminated with micro-organisms from air and water. This is in agreement with the findings of Jackson[10] who obtained restoration of mycostasis in autoclaved soil after inoculating it with unsterilized soil. Dobbs, Hinson and Bywater [5] found a return of the inhibitory factor in the soil once destroyed by chromic acid treatment by mixing the treated soil with untreated one; although they failed to get the similar result in an earlier experiment. However, they concluded that the widespread inhibition was of biological origin. Griffin[8] noted a regeneration of mycostasis in autoclaved soil after it was reinoculated with a variety of microorganisms. He suggested that soil mycostasis resulted from the accumulation of metabolites of the normal saprophytic activities of soil microflora.

The mycostasis was more intense at the surface soil than at lower depths. Jackson [9] reported mycostasis as low as at 90 cm deep in Nigerian soil. Dobbs and Hinson [4] found inhibition from surface and sub-surface soils. Stover[17] noted a direct correlation between the number of soil microflora and the fungistasis. Microbial population and their activities are maximum at the surface soil than further below. The accumulation of microbial metabolites in surface soil is responsible for higher mycostatic activity.

The maximum inhibition of spore germination was obtained at soil moisture content equivalent to 60% WHC. The mycostasis gradually decreased with the increase of soil moisture content but was still present at 90% WHC. This indicates that in nature some spores may be able to germinate at favourable soil moisture content. But in semi-arid region the soil moisture content seldom becomes favourable when the toxic factor is diminished sufficiently to allow the germination of fungal spores.

Inhibitory substances are present in soils of geographically separated areas and have more or less the same effect on germination of soil inhabiting fungi. The origin and cause of mycostasis is not yet clearly established. The evidence provided here supports the theory of biological origin of this toxicity but the possibility of inorganic salts contributing to mycostasis cannot be completely ruled out.

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