SOIL MYCOFLORA AS PRODUCER OF VOLATILE FUNGISTATIC FACTORS*

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Ten soil samples collected from Madhya Pradesh and South India were investigated for their fungistatic properties. Spore germination of *Alternaria alternata* (Fr.) Keissler, *Curvularia geniculata* (Tracy and Earle) Boedijn, *Drechslera rostrata* (Drechsler) Richardson and Fraser and *Pestalotia* sp. was effectively checked over these soils when tested for fungistasis by cellphane agar-disc and U-tube techniques.

INTRODUCTION

The role of volatile inhibitors in the ecology and morphogenesis of fungi is now fairly well documented (Hora and Baker 1970; Hutchinson 1971, 1973; Fries, 1973; Robinson *et al.* 1968, 1969).

Balis and Kouyeas (1968) and Hora and Baker (1970, 1972 *a, b*) have shown that volatile inhibitors play a major role in soil fungistasis. Balis and Kouyeas observed that soil blocks incubated in close containers over silver nitrate and mercuric perchlorate solutions lost their fungistatic property against the conidia of *Arthrobotrys oligospora* Corda. Hora and Baker (1970) presented evidence for a *p*H-dependent volatile fungistatic factor in various soil types. In their latter reports (1972 *a, b*) these workers have pointed out that soil actinomycetes were amongst the more active members of soil microflora responsible for volatile inhibitors of spore germination. Present paper describes the role of soil fungi in the elaboration of volatile inhibitors and their implications in soil fungistatis.

MATERIALS AND METHODS

Soil samples were collected from Madhya Pradesh and Tamil Nadu (Table I). Spore germination of *Alternaria alternata, Curvularia geniculata, Dechslera rostrata,* and *Pestalotia* sp. was evaluated by cellophane agar-disc (Schuepp and Green 1964) and U-tube (Hora and Baker 1970) techniques. Spore suspension of test fungi from an actively growing culture (6–8 day old) was placed on pre-activated agar discs which had earlier been left in the atmosphere of unsterilized soil for 24 hr. The volatile nature of the fungistatic factors was confirmed by removing the agar discs to an ordinary moist chamber for another 24 hr; stimulation of spore germination, if any, was recorded.

In the other technique, 30 g unsterilized soil was placed in each sterile Petri plate and a U-tube was placed over it. Sterile glass slide containing agar discs (2 mm

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thick) was placed on the U-tube and the whole assembly was incubated at 28°C for desired length of period. Spore suspension was placed on the discs after suitable interval and germination counts for at least 200 spores were made after a further incubation of the discs for 24 hr.

Soil fungi and bacteria were isolated and quantitated by using soil plate (Warcup, 1950) and soil dilution (Waksman 1927) methods. Czapek's Dox and potato dextrose agar media were employed for stock cultures and propagation of the test fungi.

Results and Discussion

The fungistatic level of different soil types varied within wide limits (Table I, column 'A'). This variation was traceable both, amongst soil types and in the germination behaviour of the four test fungi. Least germination of test spores was recorded on a forest soil. Black cotton with lime and white and brown sands supported good germination of test spores. The sensitivity of test fungi declined in the order, *Curvularia* → *Pestalotia* → *Alternaria* → *Drechslera* The removal of these discs to a moist chamber resulted in increased germination percentage of the spores (Table I, column 'B'). In some cases this increase paralleled that observed on unactivated agar discs (control). The increase in spore germination apparently resulted from the diffusion of volatiles from agar discs outwardly whereby fungistatic action of the inhibitors was gradually released. These results are in conformity with those of Hora and Baker (1970).

Table I

Production of volatile inhibitors of spore germination by soils of Madhya Pradesh and Tamil Nadu

Soil Type	pH	Germination of test spores (%)							
		A. alternata		*C. geniculata*		*D. rostrata*		*Pestalotia*	
		a	b	a	b	a	b	a	b
Black cotton	8.5	75	90	20	75	50	95	0	95
Red morum	9.4	40	90	0	75	15	95	40	95
Black cotton with lime	8.2	70	90	35	85	45	95	30	95
Forest soil	9.0	10	90	0	35	0	95	0	95
Natural soil	8.0	15	90	0	50	20	95	0	95
Mandpam sand	5.0	45	90	20	65	15	95	25	95
Yellow sand (Mahabalipuram)	4.5	75	90	15	55	45	95	20	95
Black sand I (Mahabalipuram)	4.3	40	85	0	45	20	55	25	90
Black sand II (Mahabalipuram)	4.3	20	90	0	50	25	95	15	95
Vivekanandpuram sand	4.5	55	90	20	90	40	95	20	95

a — Germination of test spores on agar discs exposed to the unsterilized soil for 24 hr, using a cellophane agar-disc technique.

b — Stimulation of spore germination recorded when agar discs were removed to an ordinary moist chamber. This allowed diffusion of volatiles outwardly.

TABLE II

*Composition of the mycoflora isolated from various soils**

Soil mycoflora	Soil Type									
	Black cotton	Red morum	Black cotton with lime	Forest soil	Natural soil	Mandpam sand	Yellow sand	Black sand I	Black sand II	Vivekanandapuram sand
Asspergillus flavus	+	+	+	+	+	+	—	—	—	—
A. fumigatus	+	+	+	+	+	+	+	+	—	—
A. nidulans	+	—	—	—	+	—	—	+	—	—
A. niger I	+	+	+	+	+	—	+	+	+	—
A. niger II	—	—	—	—	—	—	+	+	—	—
A. terreus	+	—	—	+	+	+	—	—	—	—
Aspergillus sp.	—	—	—	—	—	—	—	—	—	+
Fusarium culmorum	—	—	—	—	+	+	+	—	—	—
Hormodendrum sp.	—	—	—	—	+	—	+	+	—	—
Mycelia sterilia	+	+	+	+	+	+	+	+	—	—
Penicillium chrysogenum	—	—	—	—	—	—	+	+	—	—
P. nigricans	—	+	+	—	+	—	+	—	—	—
Rhizopus sp.	+	+	—	—	+	—	—	+	—	—
Sclerotium sp.	+	—	—	—	—	—	—	—	—	—
Syncephalastrum sp.	—	+	—	—	+	—	—	—	—	—
Trichoderma sp.	—	—	—	—	—	+	+	+	—	—
Wardomyces sp.	—	—	—	—	—	+	—	—	—	—

— = absent, + = present

*Isolations were made by soil plate method (Warcup 1950).

In order to assess the contribution of mycoflora in the production of volatiles, isolations from soils were made. A total of seventeen fungi were recorded (Table II). *Aspergillus fumigatus* appeared to be the most frequent fungus, followed by Mycelia sterilia, *A. niger,* and *A. flavus.* These four fungi were consistently recovered from all the ten soils. *Penicillium chrysogenum, Wardomyces* sp., and *Trichoderma* sp. were present in soils of south only. Both, gram + and — bacteria were also isolated but they are known not to contribute significantly in the production of fungistatic factors (Singhai 1973). The quantitative distribution of fungi showed little relation to the level of spore inhibition recorded in Table I.

Three of the above ten soils, viz., Madras sand (black sand), natural soil, and forest soil were further screened for the production of volatiles by U-tube technique. Germination of test spores was studied as a function of the incubation period of unsterilized soil (Figs. 1 to 4). Spores of *D. rostrata* exhibited maximum sensitivity to the volatile inhibitors (Fig. 3); agar discs incubated for only 2 days showed inhibition of test spores from 35 to 50 per cent. At this stage of incubation, other test spores were able to germinate quite well (80–90 per cent). Germination of all the four test spores, however, declined with an increase in incubation period from 2 to 10 days. Only a small percentage (10–35) of *Alternaria, Drechslera,* and *Pestalotia* spores were able to germinate on 10-day exposed agar discs. Natural and forest soils were slightly better producers of volatiles than the sample of Madras sand. Since no inhibitory activity was detectable in agar discs suspended over sterilized soils, it was evident that soil microbes were responsible for the production of volatile inhibitors.

The relative efficacy of the members of soil mycoflora in elaboration of volatile inhibitors was also studied. Inoculum of each of the eight test fungus was individually mixed in 30 g sterilized natural soil and the cultures were incubated at 28°C for desired length of period. Germination of test spores on pre-activated agar discs was evaluated with the U-tube technique. Test fungi included four species each of *Aspergillus* and *Penicillium.*

All eight fungi were able to inhibit germination of test spores at one time or the other (Fig. 5). *Aspergillus fumigatus* brought about maximum inhibition. Incubation of this fungus for 4 days was sufficient to bring down spore germination of *D. rostrata, Pestalotia* sp., and *C. geniculata* by 40, 45, and 50 per cent respectively. Further increase in incubation to 10 days resulted in a complete check of spore germination of the first two test fungi. *Aspergillus terreus* exhibited a similar pattern of spore germination; spores of *A. alternata* and *C. geniculata* were affected to a comparatively lesser degree. The resistance of test spores to the inhibitors produced by some fungi and not the others, would suggest that the substances secreted by these soil fungi are very likely, different chemically.

Amongst Penicillia, *P. jenseni* brought about considerable inhibition of spore germination (Fig. 5). The incubation of this fungus in soil for 4 days allowed only 50 to 55 per cent spores of *C. geniculata* and *Pestalotia* sp. to germinate; spores of *A. alternata* and *D. rostrata* showed 90 and 70 per cent germination at this stage respectively. Spore germination of *D. rostrata* however, dropped to 15 per cent after 10 days. *Penicillium notatum* and *P. nigricans* exhibited a similar inhibitory pattern. *Penicillium chrysogenum,* a known producer of non-volatile antibiotics, appeared to be least effective in elaborating their volatile counter part.

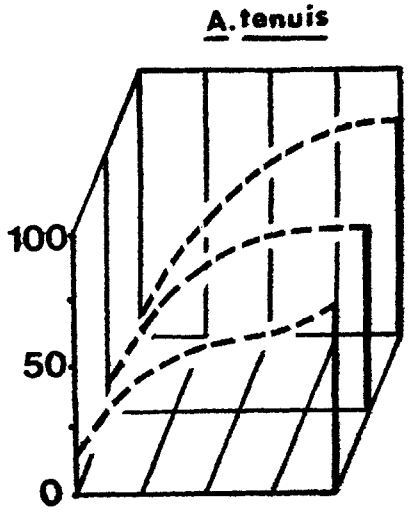

Fig. 1

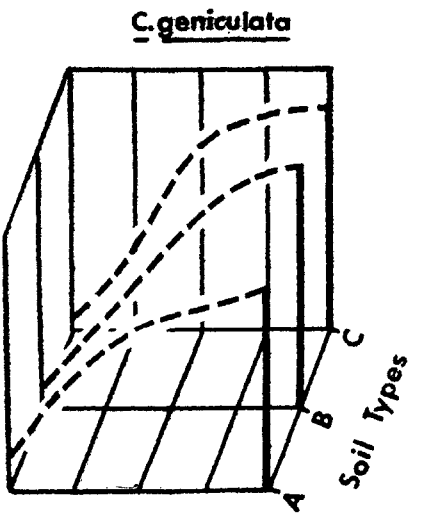

Fig. 2

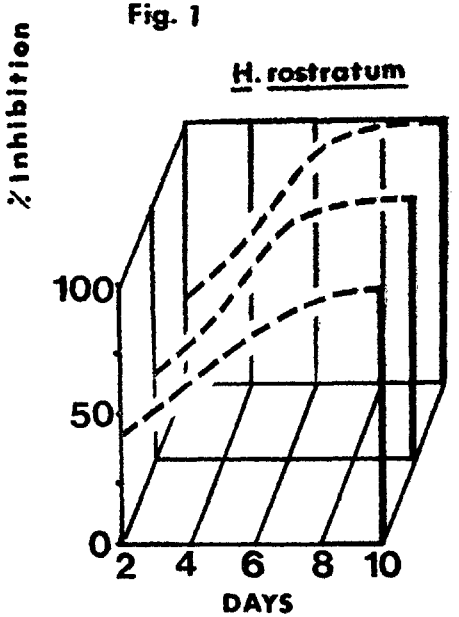

Fig. 3

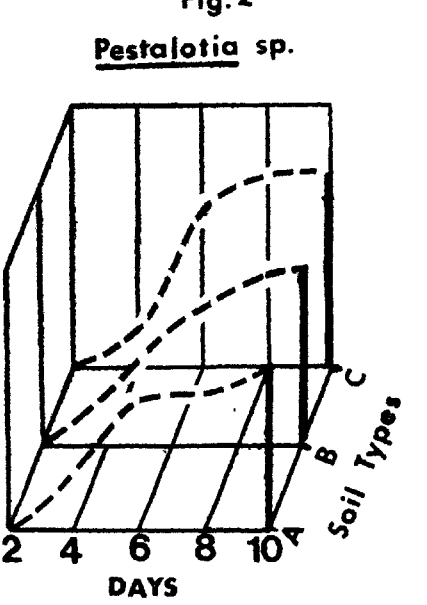

Fig. 4

Figs. 1–4. Germination of spores of test fungi on agar discs exposed in the atmosphere of unsterilized soils and sands for various length of period. A, Madras sand (black sand); B, Natural soil (unamended garden soil); C, Forest soil. The germination values have been expressed in terms of per cent inhibition. A U-tube technique was employed for evaluation of volatiles.

Note : On this and the figure following *A. tenuis* should read *A. alternata* and *H. rostratum* as *D. rostrata*

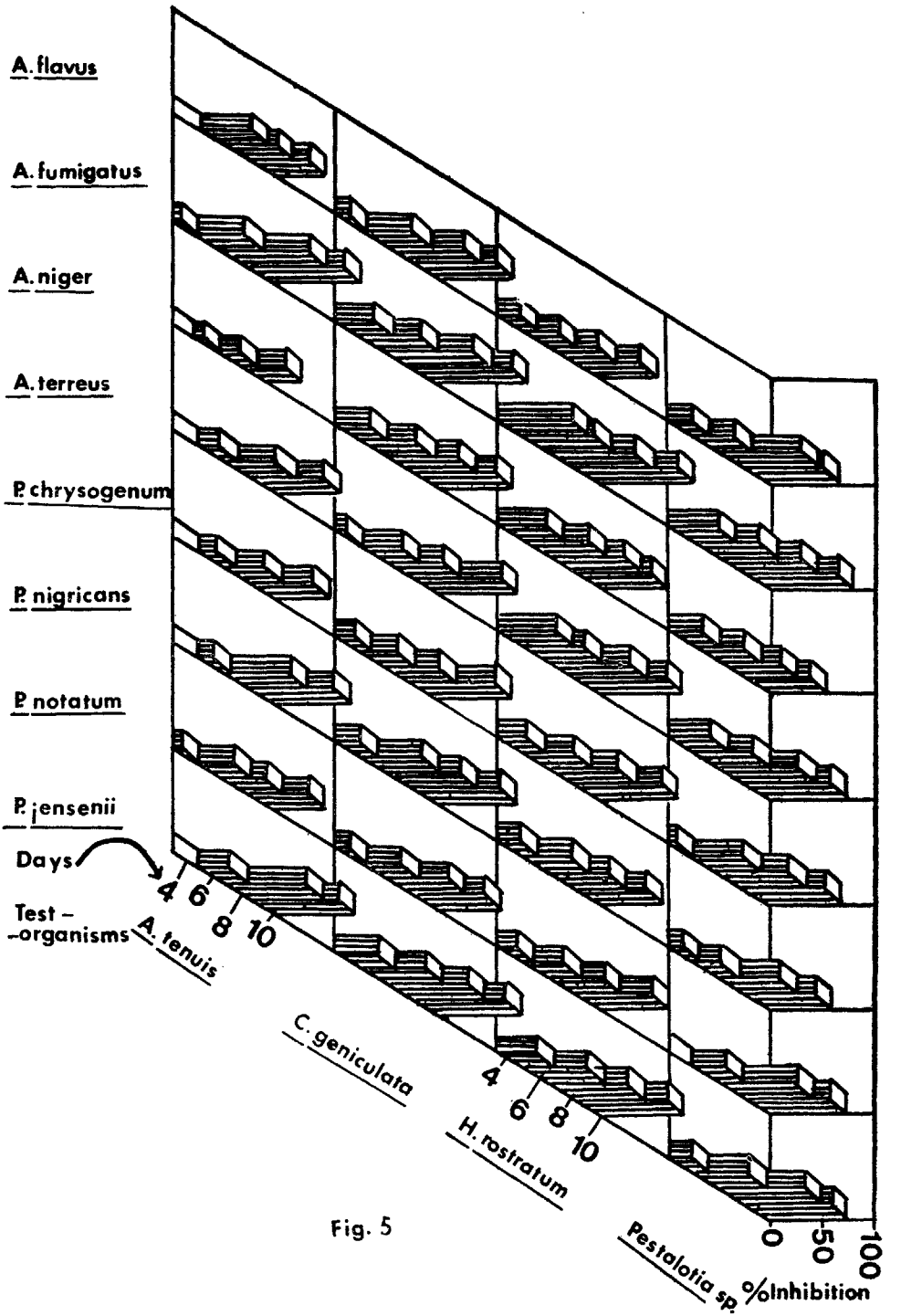

Fig. 5

Fig. 5. *In vivo* production of volatile fungistatic factor(s) by soil mycoflora in relation to the length of incubation. Inhibition of spore germination was assessed by a U-tube technique in sterilized natural soil re-infested by an inoculum of Aspergilli and Penicillia.

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