EVALUATION OF MYCOBACILLIN AND VERSICOLIN AS AGRICULTURAL FUNGICIDES

II. STABILITY IN SOIL

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The effect of paddy soils on mycobacillin and versicolin was investigated. Soil inactivated mycobacillin as determined by spectral analysis and microbiological assay. Soil can inactivate mycobacillin only at or above the threshold concentration (125~130 μg per 10 mg of soil), the excess being unreacted. No new peak appears in the ultraviolet spectrum (240~300 nm) while mycobacillin is inactivated. Soil is without any effect on versicolin.

The fate of herbicides, fungicides and insecticides in soil has been studied[1~4] systematically. Recently, new techniques were developed to study the degradation pattern of several herbicides under different conditions[5~6]. We isolated two antifungal antibiotics, *viz.* mycobacillin[7~10] and versicolin[11~12] and preliminary observations on these antibiotics as agricultural fungicides were reported recently[13].

The present work deals with the effect of soil on these antibiotics, as studied by ultraviolet absorption analysis and microbiological assay.

Materials and Methods

All studies were made in the laboratory. The properties of the soil samples under study are summarized in Table 1. The samples were collected from the paddy fields of 24-Parganas (Baruipur, Sonarpur, Diamond Harbour), West Bengal, India, dried in air, powdered and cleaned before use.

Mycobacillin was isolated from the culture filtrate of *Bacillus subtilis* B_3 according to the method of MAJUMDER and BOSE[14]. Versicolin was isolated from the culture filtrate of *Aspergillus versicolor* N_5 by a method of NANDI and BOSE[15].

For assay of antibiotics adsorbed by soil, mycobacillin (50~500 μg in 0.1 N $NaHCO_3$) or versicolin (10~100 μg in water) was added in various concentrations to 10 mg of soil and the final volume was made up to 1.0 ml with 0.1 N $NaHCO_3$ or water respectively, using stoppered centrifuge tubes. In another set of experiments various amounts of soil (5~50 mg) were added to a fixed amount of mycobacillin (200 μg) or versicolin (50 μg), and the final volume was made up to 1.0 ml with 0.1 N $NaHCO_3$ or water. All the reaction mixtures were then shaken at room temperature (25°C) for 24 hours and centrifuged. The clear supernatants thus prepared were adjusted to pH 6.4 and dried under vacuum and extracted with 5.0 ml of methanol. The methanolic solutions were subjected to UV absorption study, using a Beckman spectrophotometer, model DU-2: 50 μg of mycobacillin and 10 μg of versicolin per ml correspond to 0.32 O.D. at 276 nm or 0.10 O.D. at 220 nm respectively. The antibiotics in these methanolic solutions were also assayed microbiologically whenever possible, using as the test organisms *Aspergillus niger* G_3Br[10] for mycobacillin and *Trichophyton rubrum*[11] for versicolin. Controls with pure antibiotics were run side by side.

To test for antibiotic activity left in the soil after removal of the supernatant by centrifugation, the residue was washed thrice with saline to which was then added 5 ml of medium followed by inoculation of the sensitive organism.

Table 1. Properties of soils used.

Field obtained	Soil type	pH (H_2O)	Total carbon (%)	Total nitrogen (%)	Water sol. salts (ppm)	Depth (cm)
i) Baruipur	Paddy, clay and clay loam	6.9	0.5	0.1	0.045	0~35
ii) Sonarpur	Paddy, clay	7.2	0.7	0.07	0.625	0~35
iii) Diamond Harbour	Paddy, clay and clay loam	7.4	0.4	0.06	0.40	0~35

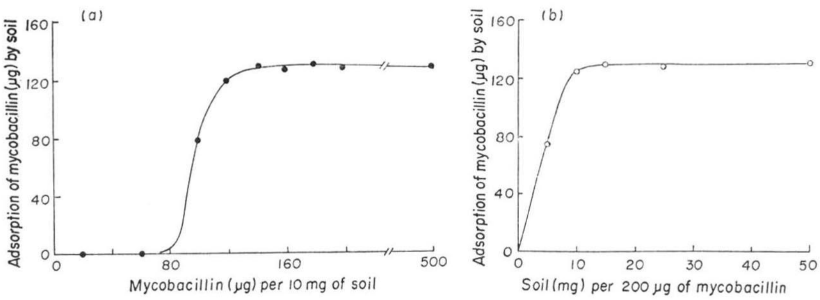
Results

Adsorption of Antibiotics by Soil

Mycobacillin: The adsorption of mycobacillin by 10 mg of soil containing different concentrations of the antibiotic is shown in Fig. 1 (a). It appears that soil does not adsorb any mycobacillin below the concentration 100 μg per 10 mg of soil, the added antibiotics being completely recovered in the supernatant. Once this threshold concentration of mycobacillin is exceeded, soil *e.g.* 10 mg, adsorbed 125 μg of the antibiotic; but it could not adsorb any more mycobacillin even if added in excess of the threshold concentration, that being the adsorption limit for the given quantity of the soil. Reciprocally the adsorption of mycobacillin by various amounts of soil in presence of a fixed concentration of the antibiotic (*e.g.* 200 μg) is shown in Fig. 1 (b). It indicates that 5 mg of soil adsorbed 75 μg of mycobacillin and correspondingly 10 mg of soil under the same conditions adsorbed 125 μg of the antibiotic. However, residual mycobacillin was no more adsorbed even when the amount of soil was increased without the concomitant increase in the added antibiotic as designed in the given experiment. Thus combining these two experiments it is of interest to note that 10 mg of soil can adsorb 125 μg of mycobacillin and no more mycobacillin is adsorbed when either the soil or mycobacillin is increased within the limits of concentration so far studied.

Versicolin: Soil does not adsorb versicolin.

Fig. 1. Adsorption of mycobacillin by soil
(a) Different amounts of mycobacillin added to 10 mg of soil. (b) different amounts of soil to 200 μg of mycobacillin. Mycobacillin adsorbed by the soil was calculated from the spectrophotometrically assayed residual mycobacillin of the supernatant.



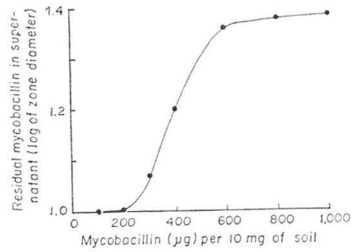
Effect of Soil Treatment on Antifungal Activity as measured by the Cup-plate Method

Mycobacillin: Studies on the antifungal activity of increasing quantities of mycobacillin added to a fixed amount of soil (Fig. 2) indicate that the antifungal activity of the supernatant could not be detected much below (due to the insensitivity of the method) or at the threshold concentration (due to inactivation); above the threshold, the activity increased with the amount of antibiotic added for values between 300 and 400 μg per 10 mg of soil, beyond which the linear relation was not observed.

No antifungal activity was observed in soil residues left after removal of the supernatants.

Versicolin: The antifungal activity of versicolin is unaltered in presence of soil.

Fig. 2. Effect of soil treatment on antifungal activity of mycobacillin
Different amounts of mycobacillin (100～800 μg) added to 10 mg of soil and the activity measured by the cup-plate method.

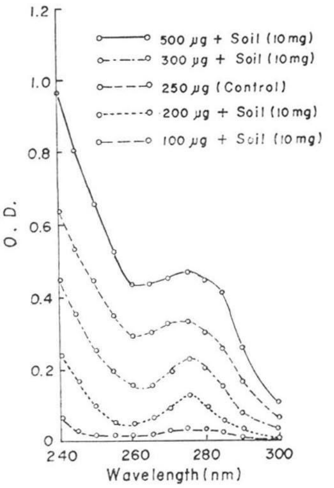


Effect of Soil on the Ultraviolet Spectrum of Mycobacillin and Versicolin

Mycobacillin: The spectral behavior over UV range (240～300 nm) of the supernatants of the various samples containing different concentrations of mycobacillin in presence of a fixed amount of soil (10 mg) is shown in Fig. 3. It appears that at the concentration of 100 μg of the antibiotic per 10 mg of soil, there occurred very negligible spectral absorption of the antibiotic which indicates that it was practically inactivated by the soil. With the increasing concentration of mycobacillin characteristic absorption peak of the antibiotic at 276 nm appeared but no new peak was observed while the antibiotic was being inactivated by soil.

Versicolin: The spectral behavior of versicolin under identical conditions does not exhibit any change.

Fig. 3. Spectral analysis of supernatants from mycobacillin treated soil samples.



Discussion

This work was undertaken to study the influence of soil on mycobacillin and versicolin. The effect of soil was followed both by UV absorption and microbiological assay. It is observed that soil could not adsorb mycobacillin below the threshold concentration. Adsorption occurs only when the threshold concentration (*e.g.* 125～130 μg/10 mg of soil) was reached. Mycobacillin added in excess of the threshold remains unadsorbed, which indicates that the soil has a limiting capacity to inactivate mycobacillin. Reciprocally when different amounts of soil were added to a fixed concentration of mycobacillin, above the threshold concentration (*e.g.* 200 μg/10 mg), adsorption occurred which goes on increasing as long as the antibiotic present is at or above the threshold. With excess of soil, the threshold concentration of mycobacillin could not be attained and adsorption failed to occur. Thus 10 mg of soil adsorbed only 125 μg of mycobacillin, whereas 20 mg of soil could not adsorb more than what is adsorbed by 10 mg, since the existing concentration is 200−125=75 μg/20 mg of soil, which is much below the threshold. The whole phenomenon can be explained on the basis of an interaction between some soil ingredients and mycobacillin leading to the formation of a complex whose dissociation or reassociation is conditioned by the concentrations of the ingredients.

Since the characteristic absorption peak of mycobacillin, which is due to the tyrosine residue[16~17] of the antibiotic, is abolished in the presence of soil, it is presumed that tyrosine may be involved in the mycobacillin inactivation by soil. It is of interest to note that the antifungal activity of mycobacillin is in some way related to its tyrosine residue (unpublished work). However, no new peak appears during this interaction.

Similar studies with versicolin gives entirely different result. Soil has practically no action on versicolin.

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