MATERIALS AND METHODS

IV - MATERIALS AND METHODS

A) Material :-

Four types of soils are selected for this study and were designated as A, B, C and D.

Two types of soils A and B were taken from sugarcane fields. Soil A was deposited clay, brownish in colour, collected from sugarcane field, located near Koyana river and soil B was black cotton, black in colour, collected from sugarcane field, located East side of S.G.M.College, Karad.

Both the soils from sugarcane fields were supplied with organic manures and watered once in a week, except in rainy season. There were some common weeds like Cynodon dactylon, Cyperus rotundus, Parthenium hysterophorus, Digitaria sp. and Euphorbia geniculata.

Soil C and D were taken from Banana fields. Soil C was deposited clay, brownish in colour, collected from banana field, located near Krishna river and Soil D was black cotton, black in colour, collected from banana field, located West side of S.G.M.College, Karad.

Both the soils from Banana field were manured with organic manures and watered ten days interval, except in rainy season. There were some common weeds like Cynodon dactylon, Cyperus rotundus, Digitaria sp. Parthenium sp. Vernonia sp.

B) Collection of soil samples :-

Soil samples were collected on the 5th day of every month from January 1989 to December 1989. Collections were made by taking composite samples upto a depth of 6 inches, after scraping off an inch of surface soil with a sterile trovel. A pit was dug with the trovel which was sterilized with 70% alcohol. The soil was collected in polythene bags and was brought to the laboratory. Soil moisture and soil reaction were determined on the same day and later soil was taken from the same sample for determining the fungal numbers, after removing stones and debris present. Soil for chemical analysis were put in separate bags. They were dried in air and analysed sodium, calcium, manganese, magnesium, iron, copper, zinc, potassium, nitrogen and phosphorus. For determination of water holding capacity oven dried soil was used.

C) Isolation of the fungal flora from the soil samples :-

The soil microfungi were studied by dilution plate method (Waksman, 1922).

Soil to be diluted is silted through a sieve with 2 mm. pores. One gram of the sieved dry soil was mixed with 100 ml. sterile water in conical flask and thoroughly shake for sometime. To obtain 1:1000 dilution with sterile

pipette, pipetted out 1 ml. of soil suspension and added to 9 ml of sterile water in test tube. After thorough shaking added 1 ml. of this dilution to 9 ml. of sterile water in test tube which gives 1:10000 dilution. From these dilutions pipette 1 ml. sample and place in sterilized petriplates. To these plates add approximately 20 ml. of melted but cooled culture media. Then rotated the discs by hand in a broad swirling motion, so as to disperse the samples in the agar uniformly. Incubated the plates for 5-6 days at room temperature (25-29.5°c), before counting for fungal colonies. Examined the plates regularly.

D) Culture media :-

The fungi were isolated on three different media, Potato Dextrose Agar, Czapek-Dox Agar and Peptone Rose-bengal Agar.

1) PDA (Potato Dextrose Agar)

Potato	-	200 gm.
Dextrose	-	20 gm.
Agar	-	15 gm.
Distilled water	-	1000 ml.
Streptomycin	-	30 mg.

Potatoes are peeled and sliced, later boil in a litre of water for one hour. Filtered it through a musclin cloth and make up volume to one litre. Add Agar and Dextrose

in filtrate. The medium is autoclaved. Streptomycin is added to the cooled liquid medium before pouring it in petridishes.

2) Czapek-Dox Agar (Thom and Raper, 1945)

$NaNO_3$	-	3 gm.
K_2HPO_4	-	1 gm.
$MgSO_4.7H_2O$	-	0.5 gm.
KCL	-	o.5 gm.
$FeSO_4.7H_2O$	-	0.01 gm.
Sucrose	-	30 gm.
Agar	-	15 gm.
Distilled water	-	1000 ml.

All ingredients dissolve in 1000 ml. distilled water and medium is autoclaved. Streptomycin is added to the cooled medium before solidifying.

3) Peptone - Dextrose - Rose bengal Agar (Martin 1950)

Dextrose	-	10 gm.
Peptone	-	5 gm.
KH_2PO_4	-	1 gm.
$MgSO_4.7H_2O$	-	0.5 gm.
Streptomycin	-	30 mg.
Agar	-	15 gm.
Rose Bengal	-	0.035 gm.
Distilled water	-	1000 ml.

All materials except Rose bengal and streptomycin are dissolved in water. The mixture is heated slowly while stirring until it starts to boil. It is removed from heat and Rose bengal is added. After autoclaving and before pouring medium in petridishes, add streptomycin in cool liquid medium.

E) Transfering fungi to slant :-

Some fungi are slow growing. After two to three days some fast growing fungi like Mucor, Rhizopus contaminated the slow growing fungi and after five to six days conidial stages of Aspergillus, Trichoderma gives rise to secondary colonies, thus there was contamination. Slow growing fungi were picked up and transferred to the slants. So that pure culture were obtained.

Transfer of the fungi from colonies to the slants were made by using flamed sterilized chrome loop. At the time of the transfer, the test tube containing the slant or organisms was held near the flame. This helps in avoiding the contamination during the transfer.

F) Identification :-

Identification of the fungi was done with the help of relevant standard keys.

G) Preservation :-

For maintaining the pure cultures the cultured tubes were kept in refrigerator. Because of reduced temperature, the drying of the tubes is slowed down and subculturing intervals can be increased four months. This is widely used procedure.

H) Method for counting the number of fungi in the soil :-

It has been pointed out by Waksman (1922) that in the determination of the number of fungi by dilution plate method is so great, as to makes the results absolutely worthless. To reduce the variability of the number of fungi on the plate and thus obtain a slow probable error, low dilutions have to be used so as to have 30 to 100 fungus colonies developing on the plate. This would necessiate a dilution of only 500 to 2000 for an ordinary fertile soil.

Waksman (1922) and others have long made use of the fact that when a culture of a fungus is wanted free from bacteria, raisin agar which is acid in reaction may be used. A medium has, therefore, been devised having a reaction acid enough to prevent the development of actinomycetes and the great majority of bacteria. The following synthetic medium was used.

Agar	-	25 gm.
Glucose	-	10 gm.
KH_2PO_4	-	1 gm.
Peptone	-	5 gm.
$MgSO_4.7H_2O$	-	0.5 gm.
Distilled water	-	1000 ml.

The P^H was adjusted with phosphoric acid to 3.6 and with agar 4.

The soil was diluted in regular manner to only (1:100) to (1:1000). To obtain an accurate count and a low probable error ten plates were prepared for each soil.

The plates were incubated for seventy two hours at 25^oc. The colonies may be counted after 48 hours, then after 72 hours, due to the fact that in some soils, rich in mucorales, the spreading forms will tend to overgrow the plate in 72 hours.

I) Methods for physiological constituents in soil :-

The determination of P^H was done by P^H meter. Sodium, Calcium, Potassium, Iorn, Magnesium, Manganese, Copper and Zinc were estimated by the (1:5 dilution) method, described by Richards L.A. (1954), with the help of PERKIN-ELMER 3030. Atomic Absorption Spectrophotometer. Estimation of total Nitrogen was done by Kjeldahl distillation method, described by Trivedy et al. (1987). And estimation of phosphorus was done by the colorometric method described by Trivedy et al., (1987).

COLOURED PHOTOGRAPHS

PLATE-1

Fig. 1 : Sugarcane field in deposited clay soil.

Fig. 2 : Deposited clay soil surrounding the root region of the sugarcane plants.

PLATE-2

Fig. 3 : Sugarcane field in Black cotton soil.

Fig. 4 : Black cotton soil surrounding the Sugarcane plants.

PLATE-3

Fig. 5 : Banana plantation in deposited clay soil.

Fig. 6 : Deposited clay soil surrounding the root region of the Banana plants.

PLATE-4

Fig. 7 : Banana plantation in Black cotton soil.

Fig. 8 : Black cotton soil surrounding the root region of the Banana plants.

PLATE-5

Fig. 9 : Selected types of soils (A, B, C, and D).

Fig.10 : Different types of fungi from soil grown in a petridish after incubation.

PLATE-6

Fig. 11 : Conical flasks containing cultures of different fungi.

PLATE-1

FIG.1

FIG.2

PLATE-2

FIG.3

FIG.4

PLATE-3

FIG.5

FIG.6

PLATE-4

FIG.7

FIG.8

PLATE-5

FIG.9

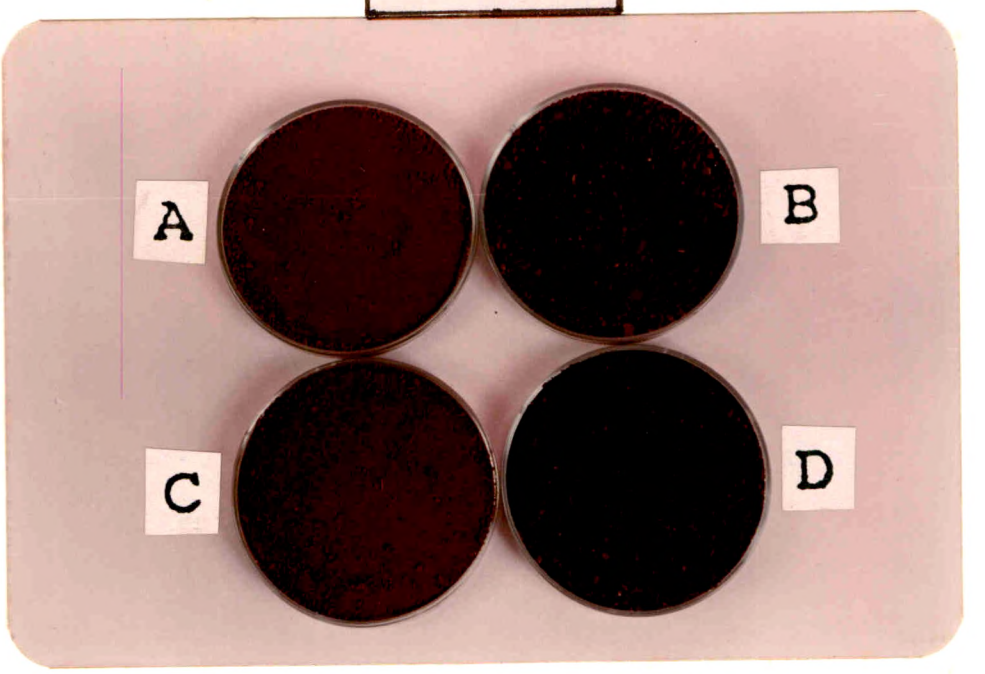

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FIG.10

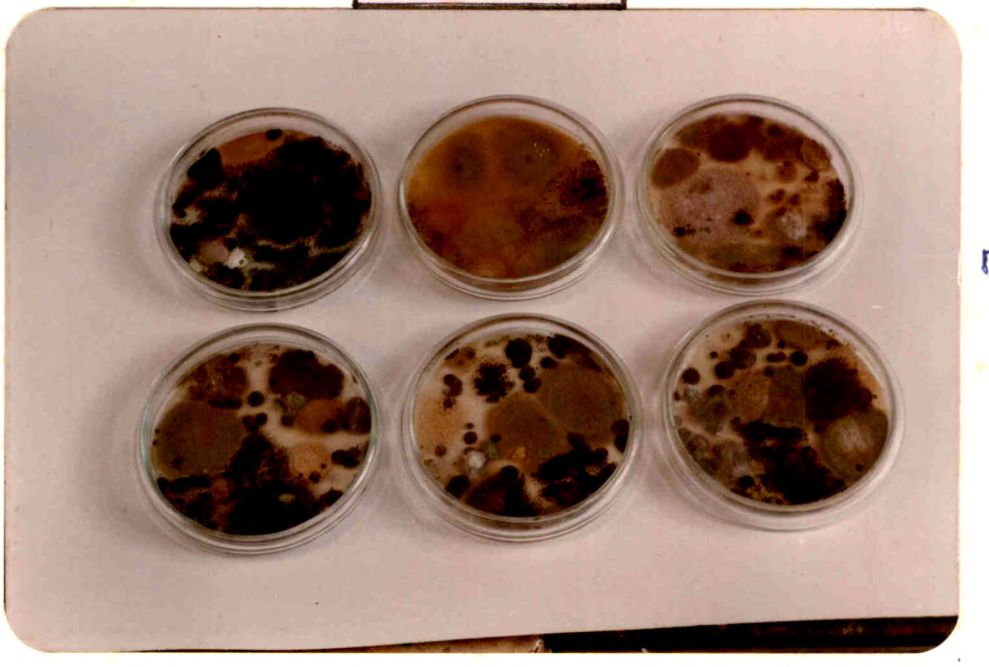

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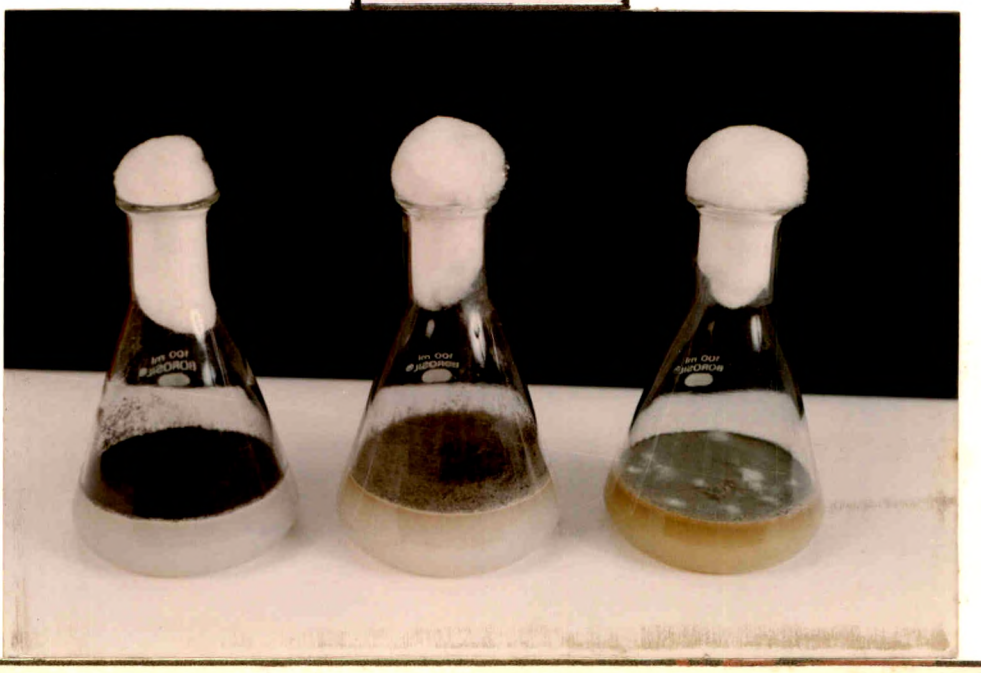
PLATE-6

FIG.11



Aspergillus niger, Rhizopus stolonifer, Penicillium sp.