CHAPTER- 3

MATERIAL AND METHODS

The soil samples from four different gardens were taken for the study of soil fungi and the physico-chemical properties of soils. The samples were collected from the *Citrus* and Grape gardens. Four samples were designated as A, B, C and D. The soil A was collected from *Citrus* garden having red soil. Sample B was collected from *Citrus* garden having black soil. Sample C was collected from the Grape garden having red soil and soil D was collected from Grape garden having black soil.

The organic manure was supplied to both the fields of *Citrus*. The Grape gardens were also manured and watered once a week.

Collection of soil samples: -

All the soil samples were collected from different gardens of Grape and Citrus. The samples were collected on 5th day of every month. The samples were collected from November 2007 to October 2008. The soil was taken from the depth of 6 inches near the roots of plants. The upper layer of the soil was scrapped with the help of sterile trovel .

Before the collection of soil samples, the trovel and hand was washed with the help of 70 % alcohol. The samples were collected in polythene bags. The samples for the study of soil mycoflora and physico-chemical properties were collected in separate polythene bags. All the samples were brought to the laboratory for further study. Soil moisture and P^{H} was determined on the same day of collection. 1gm air dried soil sample was taken for the study of physico-chemical properties.

Isolation of the mycoflora from the soil samples: -

The fungi from soil were studied by dilution plate method. (Trivedy, R.K;Goel, P.K; and Trisal, C.L.,1987).

The soil to be used for isolation of fungi was silted through a sieve having 2 mm pores. 1gm of soil was taken for dilution. The dilutions were made as 1:100, 1:1000 and 1:10000. For the serial dilutions, sterile distilled water was used. For the preparation of 1:100 dilution, 1gm of soil was mixed in 100 ml sterile distilled water in a conical flask and shaked thoroughly for sometime. To prepare 1:1000 dilution, a sterile test tube was taken and 1 ml of soil suspension from 1:100 dilution was pipetted out and mixed in the test tube containing 9 ml sterile distilled water. For the preparation of 1:10000 dilutions,

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1 ml of soil suspension from 1:1000 dilution was added to another test tube containing 9 ml of sterile distilled water.

For the isolation of fungal flora two types of media were prepared, Potato Dextrose Agar medium and Czapek -Dox Agar medium. Both the media were poured in the sterile petriplates. Before pouring the media in plates the soil suspensions from each dilutions were inoculated in the sterile petriplates. Then the cooled but melted media waspoured in plates and rotated in circular motion. These petriplates then kept in the incubator for 5 to 6 days at $25^0 - 29.5^0C$. The plates were observed regularly.

Methods of preparation of culture media: -

The fungi from soil were obtained on two different culture media these are Potato Dextrose Agar and Czapek-Dox Agar medium.

1) Potato Dextrose Agar (PDA)

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

Peeled and sliced potatoes were boiled in 1000 ml of distilled water for about one hour. Then this was filtered through a muscline cloth. The final volume was made 1000 ml by distilled water. Then Agar agar and Dextrose were added to the filtrate. After cooling of liquid medium, streptomycin was added and then thi medium was poured in petriplates.

2) Czapek- Dox Agar

$Na\ No_3$	- 3 gm.
K_2HPO_4	- 1 gm.
$MgSO_4 .7\ H_2O$	- 0.5 gm.
KCL	- 0.5 gm.
$FeSO_4. 7\ H_2O$	- 0.01 gm.
Sucrose	- 30 gm.

Agar agar	- 15 gm.
Distilled water	- 1000 ml.

All these ingredients were dissolved in 1000 ml of distilled water. This medium was autoclaved. In this medium streptomycin was added before solidifying. Then this medium was poured in the petriplates.

Preparation of slides:

The plates were observed for fungal growth after 6 to 7 days of inoculation. Then the slides were prepared. A small portion of the fungal colony was taken at the centre of the slide. Then cotton blue stain was added to it. Lactophenol was used as a mounting medium. Then slides were sealed with the help of D. P. X. for semi- permanent preparation.

Identification of fungi: -

Various characters of fungi were studied by observing the slides under microscope. Various measurements were taken with the help of micrometry technique. Then these fungi were identified with the help of relevant standard keys. Various species of fungi were identified from "Mucorales of India" by R.N. Tondon (1968),"Hyphomycetes, an account of Indian species, except Cercosporae" by Subramanian, C.V. (1971) and "The Manual of soil fungi" by Gilman (1957), Dematiatous Hyphomycetes by Ellis, M. B. and "More Dematiatious Hyphomycetes" by Ellis, M. B. (1976).

Methods for physiological constituents in soil.

The P^H of the soil was determined with the help of P^H meter. The soil to be taken for measurement of P^H was added in distilled water. P^H meter inserted into the soil solution and P^H of soil was recorded. Copper, Manganese, Zinc, Iron and Magnesium were determined by using the method of Toth S. J., J. L. Prince, A. Wallace and B. Mikkelseen, (1948.) For this, the soil was digested in acids. 1 gm of sieved soil was added to glass beaker containing 10 ml of concentrated HNO_3. The beaker was wrapped with the help of silver wrap and kept for 12 hours. After

12 hours the mixture was kept for condensation. 3 ml of 60% Perchloric acid was added to it. This mixture was condensed up to 3 ml. Then distilled water was added to it and made final volume 100 ml. It was filtered through Whatman filter paper number 4.This solution was used for the estimation of Copper, Manganese, Zinc, Iron and Magnesium by using atomic absorption spectrophotometer.

PHOTOPLATES

Plate- I

Fig. 1. *Citrus* garden in black soil.

Fig. 2. Black soil surrounding the root region of *Citrus* plant.

Plate - II

Fig. 3. *Citrus* garden in red soil.

Fig. 4. Red soil surrounding the root region of *Citrus* plant

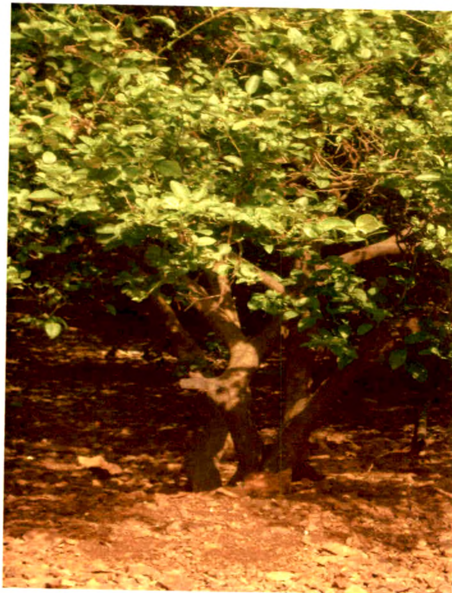

Plate- III

Fig. 5. Grape garden in black soil.

Fig. 6.Black soil surrounding the root region of Grape plant

Plate- IV

Fig. 7. Grape garden in red soil.

Fig.8. Red soil around the root region of Grape plant.

Plate - V

Fig. 9. Petriplates showing fungal colonies isolated from black soil of *Citrus* garden.

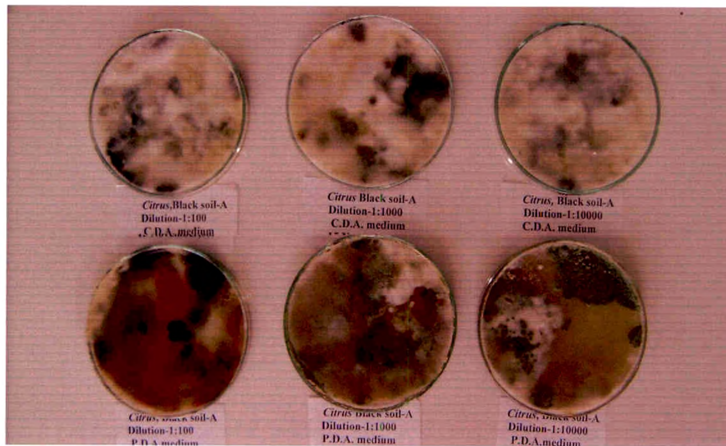

Fig. 10. Petriplates showing fungal colonies isolated from red soil of *Citrus* garden.

Plate- VI

Fig.11. The petriplates showing fungal colonies isolated from Black soil of Grape garden.

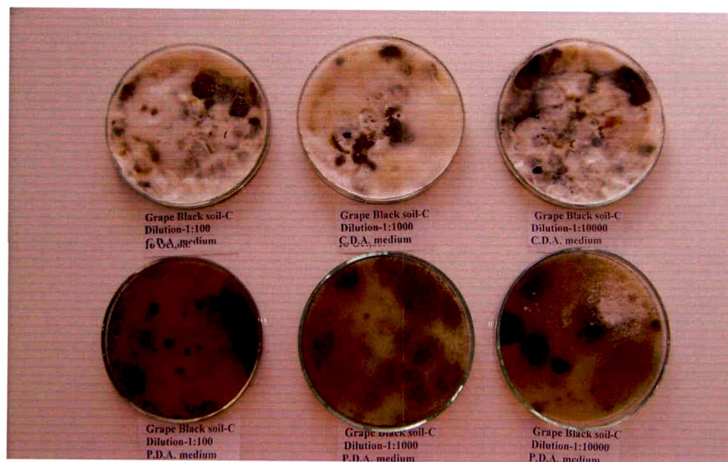

Fig.12. The petriplates showing fungal colonies isolated from red soil of Grape garden.