Material & methods

A. MATERIAL

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

Soil A was taken from the Kavalapur in Sangli district. Soil sample was collected from the Rizosphere of grape field. The grape is perennial plant. The grape field was brought under cultivation about 4 years ago. The soil was manured with organic manures and watered once in week except in rainy season. In hot months no weeds were seen in the field, but in rainy season there were some weeds like, Parthenium sp., Sisamum, Celosia argentia, Argemone mexicana, etc. The colour of soil is reddish.

Soil B was taken from the Shivaji University Campus, Kolhapur where seasonal herbs, covered the entire area. The following were the common plant covers.

Commelina sp., Cyanotis sp., Mollugo pentaphylla, Polygala chinensis, Sisamum laciniatum, Tridax procumbens Linn. Besides there were shrubs like Calotropis gigantia and some grasses. The soil first collected was dry and brown in colour.

Soil C was taken from the Samdoli in Sangli district which is saline soil. There was no any shrubs and herbs in saline soil from where the soil was taken. The colour of soil is black in colour but surface of soil is whitish in colour.

B. COLLECTION OF SOIL SAMPLES

Soil samples were collected from July, 1983 to March, 1984. Collection were made by taking composite sample 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 reactions were determined on the same day and later soil was taken from same sample for determining the fungal numbers. After removing stones and vegetables debris present in soil for chemical analysis were put in separate bags. They were dried in oven and analysed for water holding capacity, total nitrogen, potassium, calcium, chlorides, total soluble salts etc.

C. ISOLATION OF THE FUNGAL FLORA FROM THE SOIL SAMPLES

The soil microfungi were studied by dilution plate method (Waksman, 1922). Soil to be dilute 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 some time. 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 shakeing added 1 ml of this dilution to 9 ml of sterile water in test tube, which gives 1:1000 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 swiring motion so as to disperse the samples in the agar uniformly. Incubuted the plates for 5-6 days at room temperature (25-29.5°C) before counting for fungal colonies. Examined the plate regularly.

D. CULTURAL MEDIA

The fungi were isolated on four different media P.D.A., Soubourd's media, Peptone dextrose rose bengal agar and Waksman media.

1) P.D.A. (Potato-Dextrose Agar)

Potato	200 gm
Dextrose	20 gm
Agar	15 gm
Distil water	1000 ml
Streptomycin	30 mg

Potatoes are peeled and sliced, later boiled in one half liter water for about one hour. Then filtered it through a musclin cloth. Agar and dextrose is melted in another 500 ml water. The potato juice is strained into the melted agar and the volume is adjusted to 1000 ml with water. The medium is autoclaved. Streptomycin is added to the cooled liquid medium before pouring it in petri dishes.

2) Sabouraud's media

Maltose	40 gm
Peptone	10 gm
Agar	15 gm
Distil water	1000 ml
Streptomycin	30 mg

3) Peptone Dextrose Rose Bengal Agar (Martin) 1950

Agar	20.0 gm
KH_2PO_4	1.0 gm
$MgSO_4, 7H_2O$	0.5 gm
Peptone	5.0 gm
Dextrose	10.0 gm
Rose bengal (1%)	3.3 ml
Distil water	1000.0 ml
Streptomycin	30.0 gm

All the material 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 into plates, streptomycin is added to the cooled liquid medium.

E. TRANSFERING FUNGI INTO SLANT

Some fungi are slow growing. After 2,3 days some are fast growing like Mucor, Rhizopus contaminated the slow growing fungi and after 5-6 days conidial stage of Aspergillus, Trichoderma gives rise secondary colonies. Thus there was contamination. Slow growing fungi were picked up and transfered to the slant, so that pure cultures were obtained.

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

F. IDENTIFICATIONS

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

G. PRESERVATION

For maintaining the pure cultures the cultured tube were kept in refrigerator. Because of reduced temperature the drying of the tubes is slowed down and subculturing intervals can be increased 4 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. To reduce the variability of the number of fungi on the plate and thus obtain a low 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 300 to 1500 for an ordinary fertile soils.

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 acidic 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.

Glucose	10	gm
KH_2PO_4	1	gm
Peptone	5	gm
$MgSO_4,7H_2O$	0.5	gm
Distil water	1000	ml

The pH 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 incubuted for seventy two hours at 25°C. The colonies may be counted after 48 hours, then after 72 hours, due to the fact in

some soils, rich in mucorales, the spreading forms will tend to over grow the plates in 72 hours.

I. METHODS FOR PHYSIOLOGICAL CONSTITUENTS IN SOIL :

Chlorides was estimated by the method suggested by Volhard (1956). The determination of pH was done by pH meter. Potassium, sodium and calcium were estimated by the direct intensity method with the help of Flame photometer.