INTRODUCTION

Techniques for the study of fungi that occur mostly in the form of mycelia on root surfaces of young plants are very few and are beset with many limitations. All the techniques tested hitherto and proved valuable have each relative advantages but do not give full scope for the development and spore production of all the fungi that occur. For a fuller evaluation of the fungi of the rhizosphere, it is found necessary to minimise the effects of masking of certain fungi by fast growing and heavily sporulating forms. The differential dispersion of the fungal spores and mycelial bits in the water medium used for dilutions and the very small sample drawn thereof for plating generally restrict the number of species that appear on the agar plates. With a view to overcome the major limitations, an agar slide technique has been devised and tested in this laboratory. The aim was to provide a minimum-nutrient agar medium on a slide for initiation of growth of the fungi present on the root surface and inducing of spore formation by them, for ready and rapid scrutiny under a light microscope. Results of a preliminary study of the fungi occurring on the root surface of Crotalaria juncea L. seedlings under infection with Dolichos Enation Mosaic Virus (DEMV) by this method are reported here.

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MATERIALS AND METHODS

Seedlings of *C. juncea* L. were raised, four in each earthenware pot containing 500 g of sieved garden soil; the moisture was maintained at 40% level. Roots of seedlings were sampled daily from the day after sowing to study its surface mycoflora. One half of the seedlings were inoculated with DEMV (sap inoculum) on the 8th day after sowing, roots were sampled from 9th day up to the 17th day when all the infected seedlings withered completely.

Petri dishes containing one or two glass slides placed over S shaped rods were sterilized in a hot air oven. A few drops of Martin's agar with Rose Bengal and streptomycin were spread along the centre of the glass slide, leaving a margin of ca 3/4 cm on all sides, with a sterile glass rod. A small volume of sterile water was added to each petri dish to keep the chamber moist. Roots were sampled about two hours before plating and were kept on dry filter paper inside sterilized petri dishes after removing the excess soil particles from its surface by gently tapping the roots. Before plating the roots were again gently tapped to remove the remaining small lumps of soil from their surface. The roots were then cut into approximately 2½ inch bits and each bit was placed in the centre of the agar smear on the glass slide, carefully pressing to ensure contact of the full length of the root with the agar medium. These plates (plate 1, Fig. 1) were incubated at 25°C for 30 to 36 hours. The slides were taken out the following day, air dried, fixed, stained and examined. Fungal mycelia were observed to grow radially from the surface through the agar medium on to the glass surface. As, by this method, it is possible to observe the pattern of attachment of spores to sporophores, identification is facilitated.

RESULTS AND DISCUSSION

The data are presented for root lengths 0–2½ inch, 2½–5, 5 to 7½ inch and 7½ inch and above from the base of the stem in Tables 1 to 4.

Several genera of fungi not met in dilution plates in the extensive series of sampling of the roots of the same plant, both healthy and DEMV infected, were observed on the slides by this technique, in addition to the fungi which appeared in the soil-dilution plates. These were Acremonium, Cephalotrichum, Cunninghamella, Drechslera, Fusarium, Memnoniella, Pullularia and some phycomycetes (Plate 1, Figs. 3, 5 and Plate 2, Figs. 7, 8 and 9).

Till the third day only a few fungi namely Aspergilli, Mucor and Rhizopus that are quick growing were observed (Table 1). From the 4th day to 10th day 'stable' forms (see Parkinson) began to appear.