A COMPARATIVE STUDY OF CULTURE MEDIA AND SOIL DILUTIONS FOR ISOLATING FUNGI FROM CITRUS SOIL

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A comparative study of five culture media and five soil dilutions was made for the slection of a suitable medium and determination of an appropriate soil dilution for isolating fungi from citrus soil nurseries. Higher number of fungal colonies were obtained on peptone dextrose agar and Jensen's medium than on Oxgall agar, Waksman's medium and potato dextrose agar. The number of colonies on peptone dextrose agar and Jensen's medium were about the same, but species of *Chaetomium*, *Rhizoctonia* and *Trichothecium* which were isolated on peptone dextrose agar were not isolated on Jensen's medium.

On the five culture media, almost similar fungi were recorded in soil dilutions 1: 100; 1: 1,000 and 1: 10,000, but soil dilutions 1: 100,000 and 1: 1,000,000 did not permit the isolation of all the fungi. The number of colonies of fungi isolated decreased with the dilution of the soil sample. However, dilution 1: 10,000 permitted isolation of all the fungi and gave colonies which could easily be counted and isolated.

INTRODUCTION

For the isolation of fungi infesting soils, the selection of a suitable medium and determination of an appropriate dilution of soil solution is essential. Such a medium should be adequate to provide quantitative estimation of the soil fungi and should permit the isolation of the various species of fungi infesting the soil. The appropriate dilution of the soil should be such that the quantitative estimation of separate colonies of different fungi is possible.

The present investigation embraced a comparative study of the five commonly used culture media and five soil dilutions of a sample from a citrus nursery to determine the most suitable medium and appropriate soil dilution for the isolation of fungi. This paper presents the results of this comparative study.

REVIEW OF LITERATURE

Tyner (1944) suggested the use of boric acid in culture media, whereas Smith and Dawson (1944) suggested the addition of rose bengal in glucose nitrate soil extract agar and peptone dextose agar. Littman (1947)

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developed peptone dextrose Oxgall crystal violet streptomycin agar for the isolation of soil fungi and inhibition of bacterial growth. Warcup (1950) found Czapek—Dox+0.5 per cent yeast extract agar, acidified with phosphoric acid to pH 4.0, a satisfactory medium for the growth and sporulation of many soil fungi. Martin (1950) used potato dextrose agar, peptone dextrose agar and glucose nitrate soil extract agar with the addition of sulphuric acid (pH 4.0), rose bengal, streptomycin and crystal violet in various combinations. He found that crystal violet and streptomycin in combination gave best result in peptone dextrose agar, but crystal violet caused some deformation of fungi. Peptone dextrose agar with rose bengal (1:30,000) and streptomycin (30 µg/ml) was reported to be the best medium for the isolation of soil fungi. However, Anwar (1949) used 1: 1000 soil dilution for isolation of soil fungi on Waksman's synthetic medium.

MATERIALS AND METHODS

Five soil dilutions (1; 100; 1: 1,000; 1: 10,000; 100,000; 1: 1,000,000) of a soil sample collected from Lyallpur citrus nursery were plated on five culture media including Oxgall agar, potato dextrose agar, peptone dextrose agar, Jensen's medium and Waksman's medium. This sample consisting of about 250 gm of soil was taken from a depth of 4 to 12 inches, after removing 4 inches of the surface soil. The soil sample was dried in shade, pulverized in a sterilized mortar, sieved through 9 mesh sieve into a glass container.

Modified Johnson's soil dilution and plate count technique (Johnson *et al.*, 1959) was used for isolating the fungi infesting the soil. Five gm. of the soil from the original sample (on a dry soil basis) was made up to 50 ml. in graduated sterile cylinder with sterilized distilled water. This suspension was shaken thoroughly in a 250 ml. sterile conical flask and was used for making the five soil dilutions. One ml. of the final dilution in each case was transferred to a sterile petri dish and was mixed with 20 ml. of the medium. The composition of the media used for isolation of soil fungi is as follows:

Oxgall agar; Agar, 20 gm; Peptone (mycological), 10 gm; Dextrose, 10 gm; Oxgall, 15 gm; Rose bengal (1: 30,000); Streptomycin (30 µg/ml); Distilled water to make one litre.

Potato dextrose agar; Agar, 20 gm; Potato starch, 20 gm; Dextrose, 20 gm; Rose bengal (1: 30,000); Streptomycin (30 µg/ml); Distilled water to make one litre.

Peptone dextrose agar; Agar, 20 gm; Potassium dihydrogen phosphate, 1 gm; Magnesium sulphate, 0.5 gm; Peptone (mycological), 5 gm; Rose bengal (1: 30,000); Streptomycin (30 µg/ml); Distilled water to make one litre.

Jensen's medium: Agar, 25 gm; Glucose, 20 gm; Asparagin, 2 gm; Monopotassium phosphate, 1 gm; Magnesium sulphate, 0.5 gm; Rose bengal (1: 30,000); Streptomycin (30 µg/ ml); Distilled water to make one litre.

Waksman's medium: Agar, 20 gm; Peptone (mycological), 5 gm; glucose 10 gm; Monopotassium phosphate, 1 gm; Magnesium sulphate, 0.5 gm; Rose bengal (1: 30,000); Streptomycin (30 µg/ ml); Distilled water to make one litre.

EXPERIMENTAL RESULTS

The average number of colonies per dish in each soil dilution for the five media used (Table 1) showed that higher number of colonies were obtained on peptone dextrose agar and Jensen's medium than on Oxgall agar, Waksman's medium and potato dextrose agar. Soil dilutions 1: 100 and 1: 1,000 gave the highest number of colonies on all culture media used, but soil dilution 1: 10,000 gave the number of colonies which could be easily counted and isolated. The fungi isolated from the soil sample included species of *Aspergillus*, *Fusarium*, *Penicillium*, *Chaetomium*, *Alternaria*, *Curvularia*, *Neocosmospora*, *Stachybotrys*, *Mucor*, *Thielavia*, *Rhizopus*, *Sclerotium*, *Pullularia*, *Rhizoctonia* and *Trichothecium*.

TABLE 1.—*Number of colonies of fungi obtained from five dilutions of Lyallpur soil samples on five culture media.*

Soil dilution	Average number of colonies per petri dish on				
	Oxgall agar	Potato dextrose agar	Peptone dextrose agar	Jensen's medium	Waksman's medium
1: 100	293.3	198.0	599.3	607.7	269.3
1: 1,000	38.3	29.0	118.7	121.3	34.7
1: 10,000	8.3	6.0	26.0	26.7	8.0
1: 100,000	1.7	1.0	5.7	6.0	1.7
1: 1,000,000	0.0	0.3	1.0	1.3	0.0

The average prevalence of different fungi isolated from five dilutions of the soil sample on five culture media (Table 2) showed that species of *Aspergillus* predominated the isolates and constituted 90.6 per cent of them. Species of *Fusarium* accounted for 3.4 per cent of the isolates. Species of *Penicillium*, *Chaetomium*, *Alternaria*, *Curvularia*, *Neocosmospora*, *Stachybotrys*, *Mucor*, *Thielavia*, *Rhizopus*, *Sclerotium*, *Pullularia*, *Rhizoctonia* and *Trichothecium* in combination comprised only 6 per cent of the isolates.

The differential effect of culture media resulted in the differences in the relative proportion of fungi isolated on five culture media and the fungi that were isolated (Table 2). Species of *Aspergillus* predominated the isolates on all the culture media, but the relative proportion was different; 296 colonies on Oxgall agar, 200 colonies on potato dextrose agar, 695 colonies on peptone dextrose agar, 710 colonies on Jensen's medium and 277 colonies on Waksman's medium. *Fusarium* species was the third most prevalent on potato dextrose agar and the second most prevalent on the other culture media. *Penicillium* species was the second most prevalent on potato dextrose agar. The prevalence of *Fusarium* species was 18 colonies on Oxgall agar, 11 colonies

TABLE 2.—*Nature and number of colonies of fungi isolated on five culture media.*

Species of fungi isolated	Number of colonies on					Average prevalence (per cent)
	Oxgall agar	Potato dextrose agar	Peptone dextrose agar	Jensen's medium	Waksman's medium	
Aspergillus ..	295.7	200.3	694.7	710.3	277.0	90.62
Fusarium ..	18.3	10.7	19.3	17.0	15.0	3.37
Penicillium ..	4.0	11.7	6.3	4.0	0.0	1.08
Chaetomium ..	4.3	0.0	8.7	0.0	5.3	0.76
Alternaria ..	4.3	0.0	6.0	6.0	0.0	0.68
Curvularia ..	0.0	0.0	3.3	6.3	4.3	0.58
Neocosmospora ..	0.0	0.0	4.3	5.7	3.3	0.56
Stachybotrys ..	0.0	4.7	1.0	2.3	4.3	0.51
Mucor ..	6.7	4.7	0.0	0.0	0.0	0.47
Thielavia ..	4.3	1.3	2.3	3.0	0.0	0.46
Rhizopus ..	0.0	0.0	0.0	4.7	3.7	0.35
Sclerotium ..	1.7	0.0	1.7	3.7	0.0	0.29
Pullularia ..	2.3	1.0	0.0	0.0	0.0	0.13
Rhizoctonia ..	0.0	0.0	2.0	0.0	0.0	0.08
Trichothecium ..	0.0	0.0	1.0	0.0	0.0	0.04
Total ..	341.0	234.4	705.6	763.0	131.6	

on potato dextrose agar, 19 colonies on peptone dextrose agar, 17 colonies on Jensen's medium and 16 colonies on Waksman's medium. The prevalence of *Penicillium*, *Chaetomium*, *Alternaria*, *Curvularia*, *Neocosmospora*, *Stachybotrys*, *Mucor*, *Thielavia*, *Rhizopus*, *Sclerotium*, *Pullularia*, *Rhizoctonia* and *Trichothecium* in combination was 28 colonies on Oxgall agar, 22 colonies on potato dextrose agar, 37 colonies on peptone dextrose agar, 36 colonies on Jensen's medium and 21 colonies on Waksman's medium.

Species of *Curvularia*, *Neocosmospora*, *Rhizoctonia*, *Rhizopus*, *Stachybotrys* and *Trichothecium* were not isolated in all dilutions on Oxgall agar. Likewise, species of *Alternaria*, *Chaetomium*, *Curvularia*, *Neocosmospora*, *Rhizoctonia*, *Rhizopus*, *Sclerotium* and *Trichothecium* were not isolated in all dilutions on peptone dextrose agar. Similarly, species of *Chaetomium*, *Mucor*, *Pullularia*, *Rhizoctonia* and *Trichotheclum* were not isolated in all dilutions on Jensen's medium, whereas species of *Alternaria*, *Mucor*, *Pullularia*, *Penicillium*, *Rhizoctonia*, *Sclerotium*, *Thielavia* and *Trichothecium* were not isolated in all dilutions on Waksman's medium.

The relative proportion of fungi isolated from different dilutions of soil samples on the five culture media taken together is summarised in Table 3. The relative proportion of different fungi isolated was different. In dilution 1: 100, *Aspergillus* species constituted 1854 colonies as compared to 113 colonies of the remaining fourteen fungi isolated (species of *Fusarium*, *Penicillium*, *Chaetomium*, *Alternaria*, *Curvularia*, *Neocosmospora*, *Stachybotrys*, *Mucor*, *Thielavia*, *Rhizopus*, *Sclerotium*, *Pullularia*, *Rhizoctonia*, and *Trichothecium*). Similarly, in the dilution 1: 1,000 *Aspergillus* species comprised 274 colonies as compared to 68 colonies of the remaining fourteen fungi isolated. In dilution 1: 10,000, *Aspergillus* species formed 41 colonies as compared to 34 colonies of the remaining fungi isolated. Whereas, in dilution 1: 1,000,000, *Aspergillus* species constituted 7 colonies as compared to 9 colonies of the remaining fungi isolated, in soil dilution 1: 1,000,000, *Aspergillus* species constituted 2 colonies as compared to 1 colony of the remaining fungi. Thus the relative proportion of different fungi was also different. *Aspergillus* species predominated the isolates from all dilutions but relative proportion generally decreased with the increase in soil dilution up to 1: 100,000. On the other hand, the relative proportion of *Fusarium* species generally increased with an increase in soil dilution. The relative proportion of the other fungi increased with an increase in soil dilution up to 1: 100,000.

All the fungi were isolated in soil dilution 1: 100 and 1: 1,000. However, *Pullularia* sp. was not isolated in soil dilution 1: 10,000. Species of *Chaetomium*,

Pullularia and *Trichothecium* were not isolated in soil dilution 1: 1000,000. On the other hand, only four fungi comprising species of *Aspergillus, Fusarium, Stachybotrys* and *Alternaria* were isolated in dilution 1: 1,000,000.

TABLE 3.—*Nature and number of fungi isolated on five soil dilutions.*

Species of fungi isolated	Number of colones on soil dilution					Average prevalence (percent)
	1: 100	1: 1,000	1: 10,000	1: 100,000	1: 1,000,000	
Aspergillus	1854.3	273.7	41.0	7.3	1.7	90.62
Fusarium	44.7	21.0	13.0	2.0	0.3	3.37
Penicillium	13.0	8.7	3.3	1.0	0.0	1.08
Chaetomium	8.0	6.7	3.0	0.7	0.0	0.76
Alternaria	6.3	5.3	3.7	0.7	0.3	0.68
Curvularia	5.3	5.7	2.0	1.0	0.0	0.58
Neocosmospora	6.0	4.7	3.2	0.3	0.0	0.56
Stachybotrys	8.0	3.7	0.3	0.0	0.3	0.51
Mucor	7.3	3.0	1.0	0.0	0.0	0.47
Thielavia	5.3	3.0	1.7	1.0	0.0	0.46
Rhizopus	3.0	3.0	1.0	1.3	0.0	0.35
Sclerotium	3.0	2.0	1.7	0.3	0.0	0.29
Pullularia	2.7	0.7	0.0	0.0	0.0	0.13
Rhizoctonia	0.3	0.7	0.7	0.3	0.0	0.08
Trichothecium	0.3	0.3	0.3	0.0	0.0	0.04
Total	1967.5	342.2	75.0	15.9	2.6	

The comparative study of culture media and soil dilution for one soil sample showed that peptone dextrose agar and Jensen's medium gave higher number of colonies in all soil dilutions than Oxgall agar, potato dextrose agar

and Waksman's medium. The number of colonies of different fungi isolated on peptone dextrose agar and Jensen's medium were about the same. However, three species of fungi including *Chaetomium*, *Rhizoctonia*, and *Trichothecium* which were isolated on peptone dextrose agar, were not isolated on Jensen's medium. On all the media used for isolation, almost similar fungi were recorded in soil dilution 1: 10,000 as in soil dilution 1: 100 and 1: 1,000, but soil dilution 1: 10,000 gave the number of colonies which could easily be counted and isolated.

DISCUSSION

As expected, the culture media used for isolation and the dilutions of soil solution had marked influence on the number of colonies obtained, the fungi that could be isolated from the soil sample and their relative prevalence.

Higher number of colonies of fungi were obtained in all soil dilutions on peptone dextrose agar and Jensen's medium than on Oxgall agar, Waksman's medium and potato dextrose agar. Soil dilutions 1: 100 and 1: 1,000 gave the highest number of colonies on all culture media used, but soil dilutions 1:10,000 gave the number of colonies which could be easily counted and isolated.

Species of *Curvularia*, *Neocosmospora*, *Rhizoctonia*, *Rhizopus*, *Stachybotrys* and *Trichothecium* were not isolated in all dilutions on Oxgall agar. Likewise, species of *Alternaria*, *Chaetomium*, *Curvularia*, *Neocosmospora*, *Rhizoctonia*, *Rhizopus*, *Sclerotium* and *Trichothecium* were not isolated in all dilutions on peptone dextrose agar. Similarly, species of *Chaetomium*, *Mucor*, *Pullularia*, *Rhizoctonia* and *Trichothecium* were not isolated in all dilutions on Jensen's medium, whereas species of *Alternaria*, *Mucor*, *Pullularia*, *Penicillium*, *Rhizoctonia*, *Sclerotium*, *Thielavia*, and *Trichothecium* were isolated in all dilutions on Waksman's medium.

Species of *Aspergillus* predominated the isolates on all culture media but the relative proportion was different on different culture media. There were as many as 710 colonies on Jensen's medium as against 200,277,296, and 695 colonies on potato dextrose agar, Waksman's medium, Oxgall agar, and peptone dextrose agar respectively. In dilutions 1:100, *Aspergillus* species constituted 1854 colonies as compared to 113 colonies of the remaining fourteen fungi isolated. Similarly, *Aspergillus* species comprised 274, 41,7 and 2 colonies as compared to 68, 34, 9 and 1 colonies of the remaining fourteen fungi in soil dilutions 1: 1,000; 1: 10,000, 1: 1000,000 and 1: 1,000,000 respectively.

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