

Isolation and Identification of Soil-born fungi from Citrus Orchards of ICAR-IARI, New Delhi, India

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Abstract: Most of the soil-born fungi are pathogens and cause diseases to plants. Susceptibility of plants to these soil borne diseases is the most concerning part which present a common threat to agricultural production and productivity. In the present study, the soil samples were collected from different citrus orchards of ICAR-IARI, New Delhi. The isolation of fungal isolates was done on Sabouraud dextrose agar (SDA) media by following serial dilution and spread plate technique at temperature and incubation period of 30°C and 24 hours respectively. Based on the morphological and gram staining, a total of 9 different species of soil-borne fungi were isolated, among which *Rhizopus* and *Mucor* were dominant. Further it was found that 3 different species of *Aspergillus* viz. *A. versicolor*, *A. glaucus* and *A. flavus* were also identified. All three species of *Aspergillus* are considered as pathogens of important crops and *A. flavus* is able to produce potent mycotoxins. It was concluded that the soils of citrus have a good diversity of fungal species and some of the isolates were pathogenic as per the available scientific literature. Hence the research provides the early alarm for the presence of pathogenic fungi that can be detrimental to crops and thus the loss can be prevented by taking early preventive measures.

Keywords: Soil-born fungi, citrus, isolation, serial dilution.

INTRODUCTION

Soil provides home for the growth and development of all living organisms, *i.e.*, plants, animals and microorganisms. Soil has moisture holding capacity and it maintains adequate aerations. Among all microorganisms growing in soil, fungi grows dominantly (Ali-Shtayeh and Jamous, 2000; Rane and Gandhe, 2006). Soil harbors fungi and other macro and microorganisms viz. bacteria, worms, beetles and plants etc. Soil, the mantle of weathered rock contains nutrients and minerals in addition to organic matter (Sylvia *et al.*, 2005). Soil habitats are full of life and the soils which are rich in keratinous material are the most conducive for the occurrence and growth of keratinophilic fungi (Ingham, 2007). Soil habitats can be different and the characteristics of soil affect what lives in it.

Fungi belong to kingdom Myceteeae and are cosmopolitan in distribution. They can

grow in any habitat where life is possible. The microscopic cells of fungi grow usually as long strands or threads called hyphae. The hyphae push their way between soil particles, roots and rocks. The significant characteristics of fungi are that, they are eukaryotic, heterotrophic, lack tissue differentiation and their cell wall is composed of chitin or other polysaccharides and they propagate by spores sexually and/ or asexually (Benson, 2002). Fungi play important services in the fields of water dynamics, nutrient cycling, and disease suppression and they also play important role as decomposers along with bacteria in the soil food web. Decomposers, mutualists and pathogens are the three general categories of soil fungi created on the bases of how they get energy. Decomposers help in breaking down of dead organic matter, mutualistic fungi colonize the plant roots and provide nutrients

to plant in exchange for shelter and carbon (an energy source). Pathogens are responsible for diseases or death of living organism on which they colonize and feed (Tugel *et al.*, 2000; Brady and Weil, 2002).

Citrus orchards of ICAR-IARI represent a rich diversity of fungal flora. The present study was an attempt to know about diversity of mycoflora found here.

MATERIAL AND METHODS

The soil samples were collected from different citrus blocks of ICAR-IARI. Three samples were collected vertically from each block, from the surface of soil and at various depths viz, 0-5cm, 05- 10cm and 10-15cm. These samples were mixed to make a composite sample. This composite sample was then used for the isolation of fungi. Soil samples were collected by using the method of Brown (1958).

In the laboratory the samples were kept in oven for 24 hours to get dry samples. Then the dried soil was grinded and sieved to remove large debris and stones to obtain soil samples with small particles. The soil was then processed, first serial dilution was done by adding one gram of each soil sample to 9 ml of sterile distilled water in an autoclaved test tube. The test tube was shaken well and a serial dilution up to 10^{-3} was made by following the same method. One ml of 10^{-3} dilution was poured in each petri dish containing prepared SDA media. Pouring was done with the help of micropipette and each sample made by three replication plates and then incubated at 32°C for 15 days.

After 15 days good colonies of fungi grew in petri plates. These fungi were then mounted on a clean glass slide, stained with methylene-cotton blue to observe and detect fungal structures (Basu, 1980), before observing under microscope the slide was covered with a cover slip and then examined under microscope and identified on the basis of their colony morphology and spore characteristics. (Ronhede *et al.*, 2005 and Rajankar *et al.*, 2007).

RESULTS AND DISCUSSION

The main aim of current research was to isolate and identify the soil born fungi from different

citrus orchards of ICAR-IARI. In this research the soil samples from different areas were collected and followed by serial dilution. Finally, pour on the SDA media. Then after 15 days the isolated colonies of fungus were identified.

During the course of study 9 different species of fungi belonging to 4 different families were documented which are pathogenic in nature and cause diseases to agricultural crops (Table 1). The families which were isolated are *Nectriaceae*, *Saccharomycetaceae*, *Mucoraceae* and *Trichocomaceae* among these *Mucoraceae* was dominant and the species belonging to these families are *Fusarium oxysporum*, *Candida albicans*, *Rhizopus sp.*, *Mucor sp.*, *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus versicolor* and *Aspergillus niger*, respectively

The Physicochemical analysis of soil showed that pH of soil of different site ranging from 6.4 to 7.2. The diversity of fungi in any soil depends on various factors such as pH, organic content, moisture and texture of soil (Neha *et al.*, 2016).

Table 1: Fungal species isolated from the soil collected from citrus orchards of ICAR-IARI

Name of the fungus	Family	Colour of colony	Number of colonies
<i>Rhizopus</i> sp.	<i>Mucoraceae</i>	White	19
<i>Mucor</i> sp.	<i>Mucoraceae</i>	Black	17
<i>Penicillium</i>	<i>Trichocomaceae</i>	Blue	1
<i>Candida albicans</i>	<i>Saccharomycetaceae</i>	White	11
<i>Fusarium oxysporum</i>	<i>Nectriaceae</i>	White	4
<i>Aspergillus flavus</i>	<i>Trichocomaceae</i>	Green	3
<i>A. glaucus</i>	<i>Trichocomaceae</i>	Green	5
<i>A. versicolor</i>	<i>Trichocomaceae</i>	White yellow	7
<i>A. niger</i>	<i>Trichocomaceae</i>	Brown	2

Many incubated petri plates were filled with different types of fungi in each single petri plate (Fig. 1). These different fungi differ in morphological view and also each fungal colony had different colour. Species like *Aspergillus*, *Penicillium*, *Mucor* etc. were growing in common petri plate which is clearly seen in figure 1.

The soil of different orchards varies accordingly in colour, texture, drainage, moisture content, pH, organic matter and micro or macro nutrients, these all parameter of soil affect the

diversity of mycoflora (Guleri *et al.*, 2012). The nutrients of the soil are affected by soil pH due to reactions with soil particles and other nutrients (Wright *et al.*, 2009). Generally the number of species of fungi decrease with the increase of

depth of soil as the necessary organic matter present on the surface of soil required for fungal growth (Guleri *et al.*, 2016). The dominance as per colony count and species count are shown (Fig. 2).

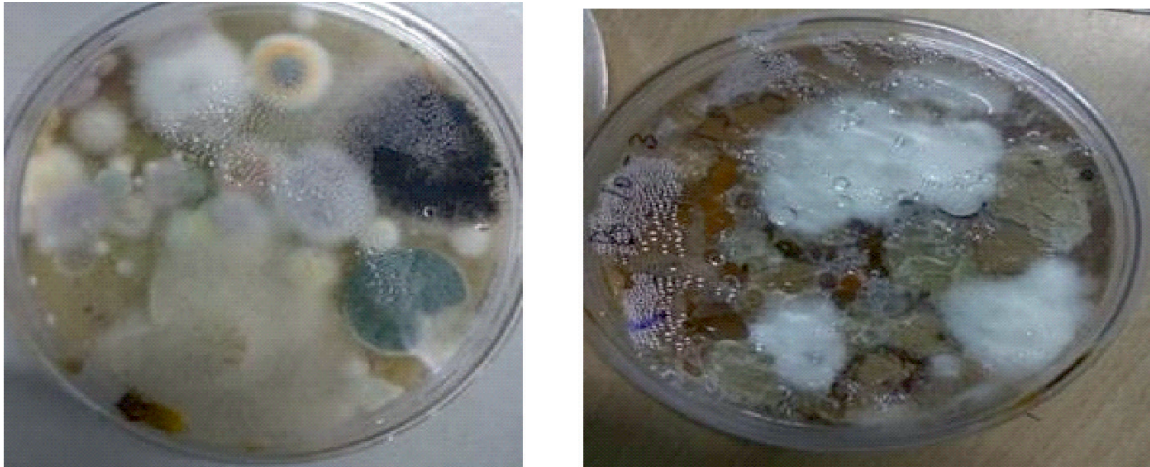


Fig. 1: The morphological view of incubated petri plates showing fungal growth

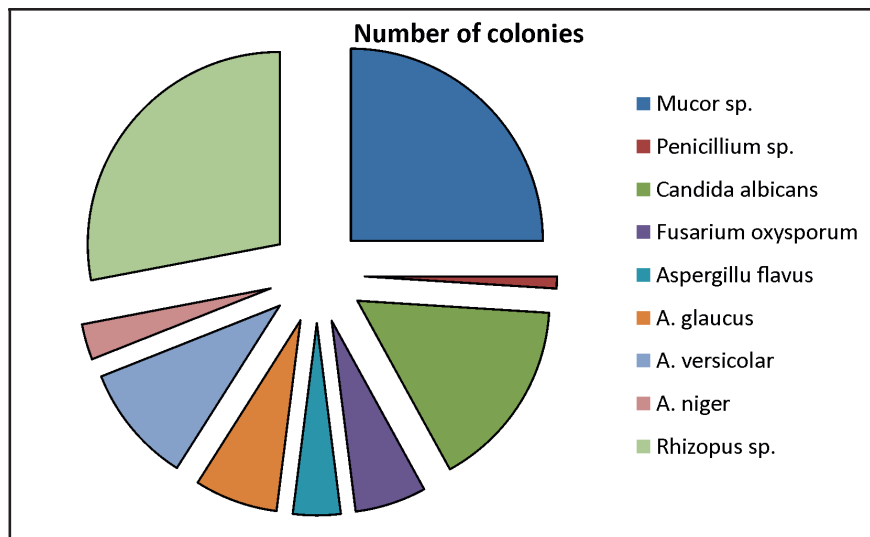
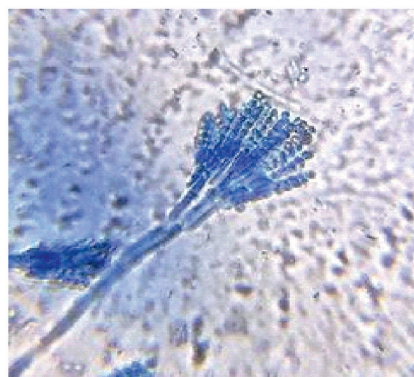


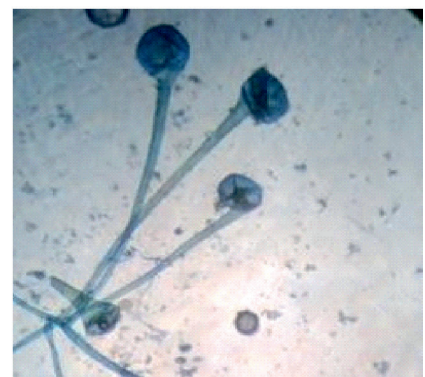
Fig. 2: Dominant species as per colony count



Aspergillus flavus



Penicillium spp.



Mucor spp.

Fig. 3: Microscopic pictures

CONCLUSION

The agricultural sector is of vital importance for the region. In earlier studies, diversity of fungal species was found to have positive relation with the depth and organics of the soil samples (Guleri *et al.*, 2016). During this study, 9 different species of fungi belonging to 4 different families isolated were isolated. The dominating family was *Trichocomaceae* but as per colony count *Mucor sp.* was dominant. The study was concluded that the soils of citrus orchards have a good diversity of fungal species and some of the species are pathogenic as per the available scientific literature. The research provides an early alarm for the presence of pathogenic fungi that can be detrimental to citriculture. Thus, the loss can be prevented by taking early preventive measures.

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