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Preliminary studies on phylloplane fungi associated with *Syzygium cumini* and *Ocimum sanctum*

***Uzma Bashir and Sobia Mushtaq**

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
**Corresponding author's email: uzmamppl@yahoo.com, uzma.iags@pu.edu.pk*

Abstract

Phylloplane is natural habitat of microbial community on leaf surface which comprises of both saprophytes and pathogens. The present study was designed to investigate fungal abundance on leaves of two medicinal plants viz., *Syzygium cumini* (L.) Skeels and *Ocimum sanctum* L. Direct inoculation with and without surface sterilization of leaf and the leaf suspension method were used for the assessment of associated phylloplane fungal flora. A total of five fungal species belonging to three different genera namely *Alternaria, Aspergillus* and *Fusarium* were isolated. Fungal abundance was significantly higher on *O. sanctum* than on *S. cumini*.

Key words: *Ocimum sanctum*, phylloplane fungi, species richness, *Syzygium cumini.*

Introduction

Phyllopalne is natural habitat of a large number of various microorganisms on the surface of the leaf. These microorganisms includes a variety of epiphytic and endophytic that colonizes the surface andinternal tissues of the plants, respectively (Inacio *et al.*, 2002; Lindow and Brandl, 2003; Yadav *et al.*, 2005; Stapleton and Simmons, 2006). However, quality and quantity of the microorganisms on the leaf surface differ with age of the plant, leaf area, morphology, and atmospheric factors such as temperature and humidity. Leaf surface topography and nutrients present on the leaf surface are generally recognized as important regulators of phyllosphere microbial communities. Little research has been done at the whole community level (Hirano and Upper, 2000; Yadav *et al.*, 2005; Yadav *et al.*, 2008). Amongst the different microorganisms, two groups of phylloplane fungi i.e. residents and casuals are generally present on leaves surface. Residents can multiply on the surface of healthy leaves without noticeably affecting the host whereas casuals land on the leaf surface but cannot grow (Prabakaran *et al*., 2011). The phylloplane flora is also reported to decompose plant material while act as allergic air borne spores (Lindow and Brandl, 2003; Osono, 2006).

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value (Nostro *et al*., 2000). *S. cumini* and *O. sanctum* are the important medicinal plants that are traditionally used for treatment of several aliments (Shyamala and Vasantha, 2010). Both medicinal plants have complex chemical composition. Some commonly recognized biologically active phytochemical constituents like eugenol, urosolic acid, alkaloids, flavonoids; tannins and carbohydrates have been reported in *O. sanctum* (Kumar *et al*., 2013). On the other hand, vitamin C, gallic acid, tannins, anthocyanins, cyanidin, petunidin, malvidin, glucoside and other components were documented in *S. cumini* (Gowri and Vasantha, 2010). The present study was, therefore, planned to investigate the phylloplane fungi associated with *S. cumini* and *O. sanctum* using different methods.

Materials and Methods

Collection of Plant Samples

Leaves of *O. sanctum* and *S. cumini* were collected from mature plants growing in University of the Punjab, Lahore, Pakistan and put in plastic bags, immediately brought to the laboratory and stored at 4 °C until isolation procedure was accomplished.

Surface-sterilization of leaf

Leaves were washed with sterilized water to remove dust, cut into discs of 0.5 cm^2 and sterilized with 0.1% mercuric chloride for one minute and washed three to four times with sterilized distilled water to remove chemicals from the surface. The washed discs of leaves were transferred to pre-sterile Petri plates containing 2% malt extract agar (MEA) medium. The plates were incubated at 25±2 °C for 7 days.

Without surface-sterilization of leaf

Leaves were washed with sterilized water to remove dust and debris and cut into 0.5 cm^2 discs,

these leaf discs were transferred to sterile Petri plates containing 2% MEA followed by incubation at 25±2 °C for 7 days.

Leaf suspension method

Leaves were washed with sterile distilled water and were cut into 0.5 cm diameter disks. The leaf discs were transferred to a conical flask with 50 mL sterile water and mechanically shaken in a reciprocating shaker at 180 rpm for 20 minutes. One milliliter of washed aliquots was transferred to each sterile Petri plate containing growth medium (2% MEA) and plates were incubated at 25±2 °C for the development of fungal colonies.

Results and Discussion

A total of five fungal species belonging to three different genera namely *Alternaria*, *Aspergillus* and *Fusarium* were isolated. The isolated fungal species include *Alternaria alternata*, *Aspergillus fumigatus*, *A. flavus*, *A. niger*, and *Fusarium* sp. The higher number of fungal spp. was found on *O. sanctum* than on *S. cumini* (Fig.1 and 2).

On *O. sanctum* leaf, three species of *Aspergillus*, one each of *Alternaria* and *Fusarium* were isolated using three different methods. *A. fumigatus* was the most abundant in all the three methods of isolation and *A. alternata* was only observed in sterilized leaf samples (Fig. 1).

Three *Aspergillus* spp. viz. *A. niger*, *A. flavus* and *A. fumigatus* were isolated from sterilized leaf pieces of *S. cumini.* Maximum number of colonies was recorded for *A. niger* followed by *A. flavus* and *A. fumigatus*. In unsterilized leaf pieces of *S. cumini*, *Fusarium* sp. was also isolated along with three species of *Aspergillus*. In leaf suspension methods, type of fungi was same as was recorded in previous two methods (Fig 2).

Currently reported species of fungi are regarded as commonly occurring primary saprobes on attached leaf surfaces of wide variety of plants throughout the world (Pandey, 1990; Andrews, 1996; Osono, 2006). These sparobic fungi can withstand adverse conditions such as desiccation, UV radiation and microbial lysis by producing thick walled pigmented multicellular spores and microsclerotia (Sadaka and Ponge, 2003). These fungi are normally encountered as epiphytes, but some can also occur as endophytes (Petrini, 1991). The maximum occurrence of *Aspergillus* followed by *Fusarium* were in well agreement with those found by Srivastava and Chandra (1985). According to them, these are the most frequent members of the mycobiota of some medicinal plants like coriander, cumin, fennel and fenugreek

S. cumini facilitates less growth of fungi than *O. sanctum*. It is attributed to waxy leaf surface of *S. cumini* which possibly not entertain prevalence of fungal spores. By contrast, roughness in the leaf surface of *O. sanctum* likely to allow more fungi to prevail. The abundance of *A. flavus* on leaves of *S. cummini* and *A. fumigatus* on leaves of *O. sanctum* might be due to the compatibility of plant and fungus. Surface sterilized method has a slight edge over the other two methods might be due to washing of unwanted spores during sterilization thus provide better insight of associated mycoflora.

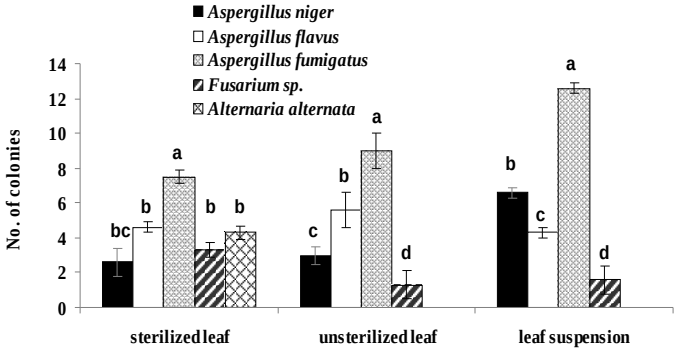

Fig. 1: Phylloplane fungi from *Oscimum sanctum*.

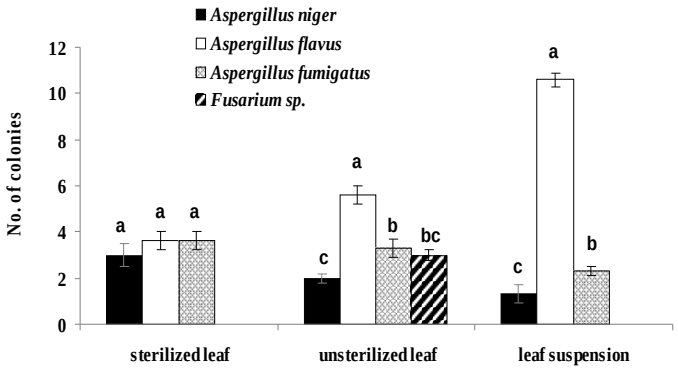

Fig. 2: Phylloplane fungi from *Syzygium cummini*.

Present study revealed that despite the variation in physical, chemical and phenological properties in the two leaf types, the fungal species isolated were more or less, similar and common. Further investigations on the endophytic and epiphytic fungal species compositions associated with the same host leaves at other sites or during different seasons and increased sampling efforts could yield more fungal taxa and could further clarify the effect of host leaf on the fungal populations.

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