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Research Article

Prevalence of *Penicillium* species in Rhizosphere soils of selected economically important trees in district Punjab, Pakistan.

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Abstract

Penicillium species are common soil inhabitants exhibiting a range of beneficial and harmful effects. The composition of *Penicillium* spp from rhizosphere soils of different plants (*Citrus limonia, Dalbergia sisso, Eucalyptus globulus, Psidium guajava*) collected from ten districts (Hafizabad, Kasur, Lahore, Nankana sahib, Narowal, Okara, Sahiwal, Sahiwal, Sialkot, Sheikhpura and Syedwala) of Punjab province of Pakistan was determined using the soil dilution plate method. Five composite samples from each locality of rhizosphere soil samples collected from May to September 2009 were plated onto malt extract agar medium and fungal colonies were identified based on morphological characteristics. A total of nine species of *Penicillium* were recovered from 50 composite soil samples. The results indicate that eight of these species belonged to the subgenus *Furcatum* and only one species belonged to subgenus *Penicillium*. The species isolated were *Penicillium atrovenetum, P. canescens, P. italicum, P. janthinellum, P. melinii, P. oxalicum, P. simplicissimum, P. velutinum* and *P.waskmanii*. Overall frequency %age showed that *Penicillium oxalicum* was the most frequent species with frequency %age 30.8 followed by *P. canescens* (18.96%) and *P. italicum* (17.5%). Whereas *P. melinii* and *P. simplicissimum* showed 11.99% and 10.16% respectively. On the other hand *Penicillium atrovenetum, P. janthinellum, P.velutinum* and *P. waskmanii* exhibited the least frequency %age, 4.09, 3.6, 2.03 and 0.82 respectively in the rhizospheric soil samples, amongst the genus *Penicillium.*

Keywords: Rhizosphere, *Penicillium, Furcatum,* frequency % age

Introduction

Soil is a complex and dynamic environment in which the biological activity is mostly governed by microorganisms (Bridge & Spooner, 2001). It is also an area of intense microbial activity and consequently of interest because of its effect on the host (Singh, 1980). The interactions among microorganisms and the plant roots are essential for the nutrition, growth and productivity of the plant. On the other hand, the microorganisms also benefit from their interactions with the plant.

Fungi are important in an enormous variety of ways e.g. food spoilage, plant, animal and human diseases, as biocontrol agents of plant diseases, production of antibiotics, in food processing, as recyclers, in mycorrhizal associations and as plant growth stimulators etc. Fungi play a role in just about every part of our lives. Of about 1.5 million expected fungal species only 70,000 (5%) have been fully described, others are yet to be discovered (Alexopoulos *et al*., 1996).

Penicillium species play an important role in agriculture. They recycle important nutrients e.g. *Penicillium bilaii* is the phosphate solublising fungus (Grant *et al*., 2002) and used to increase the yield of crops. *Penicillium* species also produce Arsenicals (Trimethyl arsine), which are used in agriculture as pesticides and defoliants (Manwar *et al.,* 2000). In addition to the beneficial role *Penicillium* also has

harmful effects for plants and animals. *Penicillium* is the major cause of degradation of agricultural products during pre harvesting and post harvesting stages, thus *Penicillium* cause substantial economic losses due to spoilage. *Penicillium* also cause Blue-eye mold in corn. *Penicillium* causes rots in citrus fruits.

Keeping in view the versatility and importance of the genus *Penicillium*, the projected study aims to isolate and record the *Penicillium* species from different areas of Punjab. Approximately 200 species of *Penicillium* from worldwide have been isolated and recorded whereas in Pakistan only 50 species (Ahmed *et al*., 1997) have been reported so far however there is no proper record of isolates. The main goal of this research is to isolate maximum species of *Penicillium* from Punjab and develop appropriate record and catalogues for data on the basis of morphological characteristics for their future use in various fields such as teaching, industry, and research work.This study was aimed to isolate the *Penicillium* species from the rhizosphere of different plants, identify and preserve them for further studies.

Study of Penicillia will substantially enhance our knowledge about this economically important genus. The main objectives are:

1. To evaluate the prevalence of *Penicillium* species in rhizosphere soils of selected trees.
2. Identification and long term preservation of *Penicillium* species.

Materials and Methods

Sample collection

Test samples were collected from ten districts of Punjab province of Pakistan, viz, Hafizabad, Kasur, Lahore, Nankana sahib, Narowal, Okara, Sahiwal, Sahiwal, Sialkot, Sheikhpura and Syedwala during May to September 2011. Fifty composite rhizoshere soil samples were collected from root vicinity different plants, *Citrus limonia*, *Dalbergia sisso*, *Eucalyptus globulus*, *Psidium guajava*. Rhizosphere soil samples were collected to a depth of 10 cm with a sterile auger and bulked to a composite sample. Soil samples were stored in sterilized polyethylene bags in a refrigerator at 4°C until further use.

Isolation of fungi

The fungi were isolated using the serial dilution technique (Mehan *et al*., 1991) and soil plate method (Warcup 1950). Soil samples were thoroughly mixed and 25 g of each sample was suspended in 225 ml of sterilized distilled water (SDW) (1:10) and 10 ml of this suspension were added to 990 ml of SDW (1:1000). These suspensions were stirred for 20 min before making 7-fold falling dilutions then; 1 ml of the desired suspensions (10^{-4}) was plated in triplicate in Petri dishes containing 2% Malt extract Agar composed of: Malt extract 20g; agar 15g in 1 L of distilled H_2O (pH 7.2). The suspension was evenly spread on the surface of medium by a sterile glass rod while rotating the Petri dish. Soil plates were prepared in triplicate by uniformly distributing the 0.5 g soil directly by sprinkle method on the surface of 2% MEA medium. The plates were incubated at 26$\pm$2°C for six days before counting growing fungal colonies. Individual fungal colonies were further isolated by sub-culturing onto new MEA plates.

Identification of fungi and long-term preservation

The isolated fungi were identified to the genus and species level on the basis of macro (Colony size, colony shape, colony color, colony reverse, texture and exudates) and micro (Conidiophores, metulae, phialides and conidia) morphological characteristics using the most updated keys for identifications (Pitt 1979; Domsch *et al*., 1980). Identified *Penicillium* species were preserved by direct lyophilization of the fungal mat grown in 2% Malt Extract (ME) broth for 7-10 days. Each fungal mat was washed three times with distilled sterile water and dried in paper towel. Fungal mat was transferred in sterile falcon tubes and tubes were covered with nylon mesh (200µm). Falcon tubes were placed in the vacuum bottles and the samples were lyophilized in a freeze dryer (TFD5505, Ilshin, Korea) under vacuum at ≤ 50 °C for 6-8 hours. Samples were removed from vacuum bottles when pressure reached at 25 bars and lyophilized samples were kept at 4 ^{o}C and room temperature.

Results

A total of nine *Penicillium* species were isolated from 50 rhizosphere soil samples of different types of plants**,** growing in ten districts of Punjab. Eight

species belong to subgenus *Furcatum and one species* to subgenus *Penicillium*. Out of eight species, six species belong to section *Divaricatum* and two species belong to *Furcatum*.

Following six species of *Penicillium* are new records from Pakistan; *Penicillium atrovenetum, P. canescens, P. melinii, P. simplicissimum, P.velutinum* and *P. waskmanii*. Although previous records of *Penicillium italicum, P. janthinellum* and *P. oxalicum* exist from Pakistan, these are new isolates from plant rhizosphere.

Overall frequency %age showed that *Penicillium oxalicum* was the most frequent species with frequency %age 30.8 followed by *P. canescens* (18.96%) and *P. italicum* (17.5%). Whereas *P. melinii* and *P. simplicissimum* showed 11.99% and 10.16% respectively. On the other hand *Penicillium atrovenetum, P. janthinellum, P.velutinum* and *P. waskmanii* exhibited the least frequency %age, 4.09, 3.6, 2.03 and 0.82 respectively in the rhizospheric soil samples, amongst the genus *Penicillium* (Figure 1a,1b).

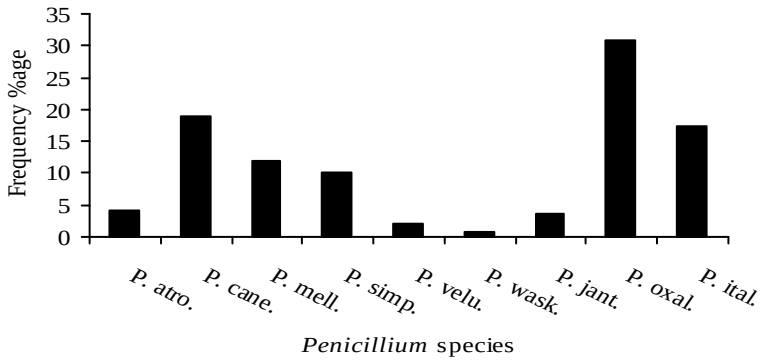

Fig. 1a: Overall frequency %age of different *Penicillium* species amongst the genus *Penicillium*.

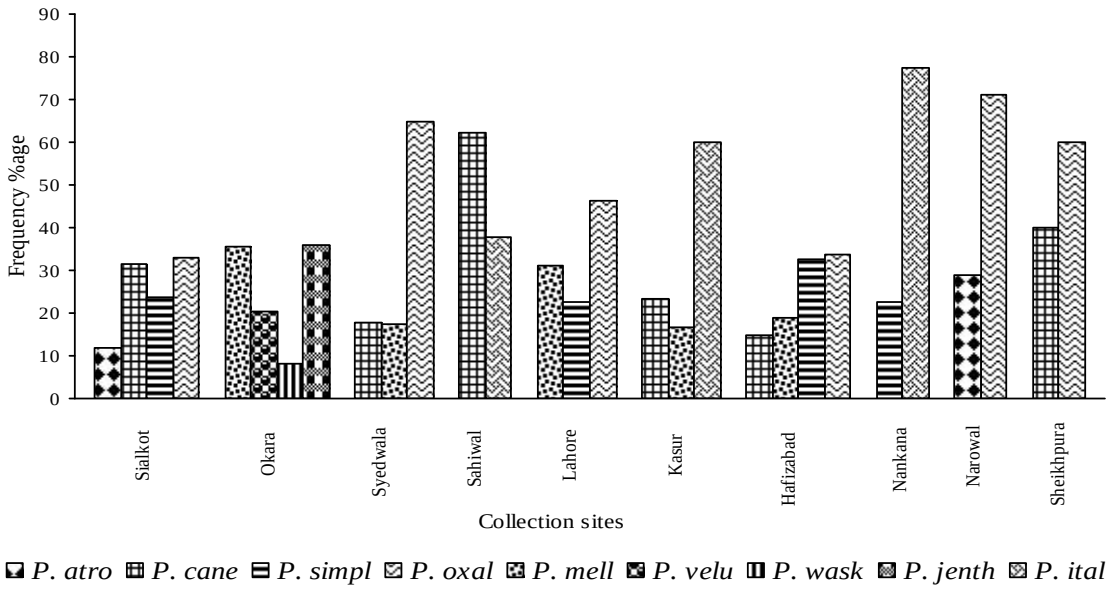

Fig.1b: Frequency %age of *Penicillium* species from different localities of Punjab.

Discussion

Results indicate that the *Penicillium* species belonging to subgenus *Furcatum* are the most frequently present in rhizosphere. In rhizospheric soil of grassland *Furcatum* also reported as dominant subgenus (Menna *et al.*, 2007). The populations of *Penicillium* and *Trichoderma* species were also found to dominate the rhizosphere of established tea bushes in various tea growing locations in India (Pandey *et al.*, 2001). This study presents the extent of *Penicillium* species diversity in rhizosphere of some economically important plants. Distribution patterns of microorganisms provide important clues about the underlying mechanisms about structure ecological communities and preservation priorities (Green and Bohannan, 2006).

The prevalence of *Aspergillus* and *Penicillium* occurs probably because these genera have a high number of species and are capable of surviving in dry environments (Dix and Webster 1995). *Aspergillus* and *Penicillium* have been reported as the dominant genus in the microfungi of the rhizosphere of *Zygophyllum qatarense,* native plants of Negev, Israel, melon plants and *Lycopersicon esculentum* (Mandeel 2002; Grishkan *et al*., 2006; Flavia *et al*., 2010).

In the present study *P. janthinellum* and *P. melinii* belonging to subgenus *Furcatum* were isolated from rhizosphere of *Citrus limonia*. *Penicillium thomii, P. spinulosum, P. janthinellum, Penicillium* sp., *P. melinii,* along with other fungal species, has also been reported as the most occurring fungi of forest rhizosphere (De Bellis *et al*., 2007). *Penicillium canescens* and *P. simplicissimum* also has been isolated along with other *Penicillium* species from the rhizosphere of *Eucalyptus, Dalbergia sissoo* and *Psidium guajava* respectively, in our study. Menna *et al.,* (2007) had also reported *P. canescens, P. simplicissimum* along with other *Penicillium* species from rhizosphere.

According to our results *Penicillium atrovenetum, P. canescens, P. melinii, P. simplicissimum, P. velutinum* and *P. waskmanii* are the newly reported isolates from the rhizospheric fungal flora of Pakistan (Ahmed *et al*., 1997). *Penicillium* species like *P. atrovenetum, P. canescens, P. melinii, P. simplicissimum, P. velutinum, P. waskmanii, P. italicum, P. janthinellum* and *P. oxalicum* are economically important. These species can be used according to their potential in biocontrol, industrial biotechnology and bioremediation. The distribution of some *Penicillium* isolates at all sites rhizospheric soil in our study underlined their cosmopolitan nature. This is the first report on the rhizosphere soil-associated *Penicillium* species, and hopefully it will provide a basis for new and innovative concepts in the industrial biotechnology, bioremediation and biological control of fungal pathogens.

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