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Isolation and characterization of novel rhizospeheric soil fungi associated with important vegetable (Egg Plant) *Solanum melongena* L. from Kashmir valley

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Abstract
Rhizosphere is store house of microbiota which influences the growth of plant directly or indirectly. The study was carried out to isolate and identify the various rhizospeheric fungi associated with important vegetable *Solanum melongena* L. The metabolic processes like accumulation of various primary and secondary metabolites governed by mycoflora. The most fungal species isolated and characterized from *Solanum melongena* L. include *Rhizopus stolonifer*, *Pencillium notatum*, *Fusarium oxysporum*, *Aspergillus niger* and *Trichoderrma* sp. The relative association frequency of these fungi with the concerned plants differs from fungi to fungi and also with the plant species. Among all the isolated fungi species *Pencillium notatum* was found to possess highest association frequency and relative abundance plant species.

Keywords: Rhizosphere; *Solanum melongena*; mycoflora; *Rhizopus stolonifer*, *Pencillium notatum*, *Fusarium oxysporum*, Aspergillus *niger* and *Trichoderrma* sp

Introduction
The uppermost layer of earth's crust and biologically active medium is known as soil, serving as a reservoir of essential nutrients and water. Soil being a diverse range of available niches, habitats, sustains most of the earth's genetic diversity. It is a complex environment in which the biological activity is governed mostly by the microorganisms. The beneficial effects of soil microorganisms are enormous especially playing an important role in maintaining the growth and development of the plant.

Rhizosphere microflora increases the productivity of crops and is known as the economic, organic and sustainable inputs (Smith and Read, 1997). Rhizosphere (Hiltner, 1904) [5] is the zone of soil immediately adheres the plant roots together with root surfaces and evolved to adapt to their surrounding environment by optimizing their functional architecture to use resources in heterogeneous soils. The co-evolution of rhizosphere and plant roots play an important role in soil physical, chemical and biological processes that sustain biodiversity, provide soil carbon sequestration, and cycle nutrients in natural and agricultural systems. Root exudates affect the biomass and activity of soil microorganisms (Raaijmakers *et al.*, 2009) [9]. Particular soil harbors particular indigenous microorganisms that influence the plant root activity on rhizosphere microbial community (Singh *et al*., 2007). Plants maintains the indigenous microbial populations in soil, each plant species is thought to select specific microbial populations for which root exudates are a driving force. The composition of root exudates varies from plant to plant and affects the abundance of microorganisms in the vicinity of the root. Rhizospheric soil with root exudates of plants harbors a rich microbial population, and also consists of free oxygen, specific ions, water, mucilage, enzymes and carbon containing primary and secondary metabolites. Rhizosphere microbial community affected by plant growth as root exudates change during the plant's life cycle and seasonal environment responses.

The important microbiota (fungi) typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). Fungi is ubiquitous in nature and play very important roles in regulating the activity of most ecosystems like biogeochemical cycles, organic matter decomposition, plant growth and disease development and control (Raaijmakers *et al.*, 2009) [9]. Rhizosphere fungi are closely linked to plant health and growth, owing to their roles in antagonizing pathogens, decomposing plant residues and providing nutrients. It is estimated that there are 1.5 million fungal species on earth, of which 75,000 have been described up to now (Hawksworth, 1991; Hawksworth *et al.*, 1995, Hawksworth, *et al*., 1997) [2, 3, 4]. There are various categories of fungi like saprophytes, mycorrhizal and pathogenic, the saprophytic fungi are present in large proportion in soil among fungal species and are the major decomposers in most terrestrial and some aquatic ecosystems and play a critical role in biogeochemical cycles (Gadd, 2007) [4] and in many food webs. Mycorrhizal associations enhance the uptake of inorganic nutrients such as nitrate and phosphates from soils (Lindahl, *et al.*, 2007) [6]. Mycorrhizal colonized roots and most of the actively absorbing rootlets are extending to the surrounding soil for nutrient uptake. There is a critical link between plants and rhizospeheric soil. There is fungal abundance about 10-20 times more in the zone of rhizosphere than in the rhizoplane soil (Lynch, 1990; Morgan *et al*., 2005). Rhizosphere fungal isolates belonging to *Penicillium pinophilum, Aspergillus niger* and *A. fumigatus* identified from different plants species, can effectively solubilize tricalcium phosphate or rock phosphate (Wahid and Mehana, 2000). Not only medicinal and aromatic plants but vegetables have great fungal diversity. Vegetables like *Allium cepa, Hibiscus esculentus, Trigonella foenum-graecum, Lycopersicum esculentum, Daucus carota* are rich in rhizospheric fungal

diversity (Mehrota and Kakkar (1972) [7]. *Aspergillus* and *Rhizopus* is abundant in the rhizosphere of *Saccharum officinarum*. Fungal microbiota have an close association with medicinal and aromatic plants viz. *Ocimum sanctum* and *Centella asiatica* (Sagar and Kumari, 2009) [10]. The rhizosphere soils of *Phellodendron amurense* showed three different groups of Arbuscular Mychorhizal fungi namely *Glomus*, *Hyponectria* and *Scutellospora* (Cai *et al*., 2009) [1], followed by zygomycotina, ascomycotina, oomycotina and coelomycetes.

Materials and Methods

Collection of rhizospeheric soil samples

Soil samples were collected from rhizospheric portion of *Solanum melongena* L. from different places of Kashmir. Soil samples were collected by digging out a small amount of soil from the depth of 10 cm. to 15 cm. by using a small sterilized hand auger or any other suitable instrument and wearing a hand glove. Soil samples were collected and packed in a small sterilized zipped polythene bags and brought to the laboratory. Each sample bag was labeled appropriately by indicating the geo coordinates, date and place of collection. Soil samples were stored in a refrigerator for further analysis and stud

Washing of glassware

All the glassware (test tubes, beakers, measuring cylinders etc.) were cleaned properly. After washing with labolene, the glassware were thoroughly washed with tap water and allowed to air dry.

Sterilization of media and glassware

After washing the glassware, sterilization was done by physical methods via dry heat (Flaming, Hot air oven), Wet heat or Sterilization by chemical methods

Preparation of media

The media used for the isolation and culturing of soil fungi in the present study was

- Potato dextrose agar (PDA)
- Richard's medium

Isolation of fungal flora

Isolation of the fungi associated with rhizosphere of *Solanum melongena* L. was done by dilution method (Dickson and Pugh, 1965). In this method 1g of soil was taken from the soil sample. This 1g of soil sample was dissolved in 10 ml of distilled water in sterilized test tubes to get 10^{-1} dilution. From this 1 ml of soil suspension was taken and added to 9 ml of distilled water to get 10^{-2}. This was repeated until a final dilution of 10^{-6} was obtained. Inoculation was done in laminar air chamber 10 ml of agar medium was transferred to each petri plate. 1 ml of soil suspension was inoculated in each petriplate. Plates were rotated gently to get uniform distribution of soil suspension into the medium. Antibiotics were also added to the petriplates to prevent bacterial contaminations. Then the plates were incubated 25±2° C for 5 – 7 days. These petriplates were then observed for fungal growth and the numbers of fungal colonies were recorded.

Sub-culturing of fungi

Individual colonies from mixed or mother culture were sub-cultured on fresh PDA plates for obtaining pure culture of each isolate. The pure culture was isolated for further identification through lactophenol cotton blue staining technique.

Staining technique for fungi

Inoculating needles were flamed over burner. Then using the needle, a small portion of the growth on the culture plate was transferred into a drop of lactophenol in cotton blue on the slide.

Identification of fungi

Rhizosphere mycoflora were identified based on colony and microscopic characters. The colony growth which includes length and width of colony, the presence and absence of aerial mycelium were some macro-morphological characters evaluated. The specimen was observed under the microscope for identification. Identification was done by monographs and relevant literature.

Frequency of fungi

The frequency of fungi isolated from soil was calculated by the formula.

$$\text{Frequency} = \frac{\text{Number of plates containing a particular fungus}}{\text{Total number of plates poured}} \times 100$$

Relative abundance

The relative abundance of isolated fungi was calculated by the following formula.

$$\text{Relative abundance} = \frac{\text{Total number of colonies of a fungus}}{\text{Total number of colonies of all the fungi}} \times 100$$

Raising and maintenance of pure culture

The pure culture of fungi encountered during isolation from the petri plates was maintained on PDA medium. Isolation was done for the separation of a strain from a mixed population, in order to identify the fungi of interest. Spores or mycelia or agar block containing these fungal structures were aseptically transferred into plates with solidified agar media. Then the plates were incubated for 5 - 7 days. These petriplates were then observed for fungal growth.

Results

The present study was undertaken to study the rhizospheric fungi associated with *Solanum melongena* L. in different localities of Kashmir. The soil fungi identified on the basis of cultural and microscopic characters were: *Rhizopus stolonifer*, *Penicillium notatum*, *Aspergillus niger*, *Trichoderma* sp., *Fusarium oxysporum* and *Mucor* sp.

Characteristics of rhizospheric fungi associated with *Solanum melongena* L.

Rhizopus stolonifer Lind.

Cultural characteristics

Colonies of *Rhizopus stolonifer* grow very rapidly on PDA. The mycelium appears as a cottony growth. The colour of colony is white with black raised heads (Plate 1, Fig. A).

Microscopic characteristics

The mycelium is coenocytic and profusely branched having three types of hyphae: sporangiophores, stolons and rhizoids

(Plate 1, Fig. B).

***Penicillium notatum* Thom.**
Cultural characteristics
The colonies of *Penicillium notatum* are bluish green with velvety texture. The colonies grow as small as concentric circles (Plate 2, Fig. C).

Microscopic characteristics
Mycelium is highly branched, multinucleate and septate. Conidia are borne on long, erect, and branched conidiophores. Each branch of conidiophores ends in bottle shaped sterigmata bearing a group of conidia. The conidia are ovoid in shape and are blue in colour (Plate 2, Fig. D).

***Mucor* sp.**
Cultural characteristics
Mucor is a fast-growing fungus on PDA. The colonies of *Mucor* appears in the form of white cottony growth within 3 days (Plate 3, Fig.E).

Microscopic characteristics
Mycelium is differentiated into aerial and prostrate hyphae. The aerial branches of mycelium bear sporangia at their tips are known as sporangiophores. Sporangium is present at the tip of sporangiophore. (Plate 3, Fig.F).

***Aspergillus niger* Van Tieghem.**
Cultural characteristics:
The colony of *Aspergillus niger* grow very rapidly on PDA medium. They are black in colour, irregular in outline and mostly consist of a dense tuft of erect conidiophores (Plate 4, Fig. G).

Microscopic characteristics
Mycelium is well developed, septate. Conidia are produced on conidiophores. The conidiophores are unbranched, aseptate, smooth walled and are becoming darker at the apex and terminating in a globose vesicle. The finger like projections called sterigmata are present over the entire surface of vesicle. The conidia are globose, present in chains over the sterigmata (Plate 4, Fig. H).

***Fusarium oxypsporum* Link.**
Cultural Characteristics:
Fusarium oxysporum is a rapidly growing fungus on PDA. Pink coloured colonies were obtained (Plate 6, Fig. I).

Microscopic characteristics
Species of *Fusarium* typically produce both macro- and micro conidia from slender phialides. Macro conidia are hyaline, two to several-celled, fusiform to sickle shaped, mostly with an elongated apical cell and pedicillate basal cell. Micro conidia are one to two-celled, hyaline, smaller than macro conidia, pyriform, fusiform to ovoid, straight or curved. Chalamydospores may be present or absent (Plate 6, Fig. J).

***Trichoderma* sp**
Cultural characteristic
Trichoderma is a rapidly growing fungus on PDA. It matures in 3-5 days. Growth begins as white tufts which then turns green (Plate 5, Fig.K).

Microscopic characteristics:
Hyphae are septate and hyaline. Conidiophores are branched. Phialides extend from the conidiophores (Plate 5, Fig. L).

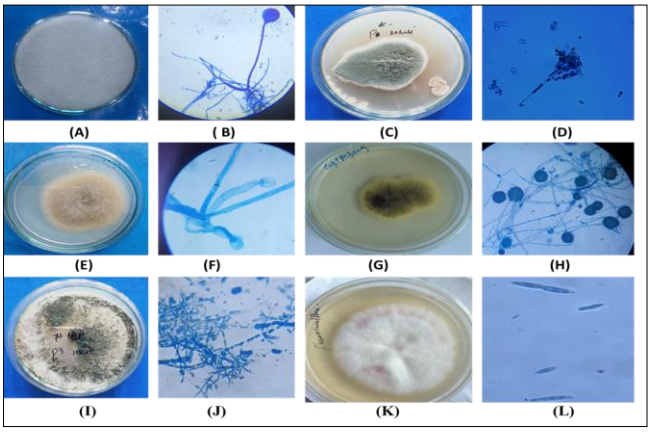

Fig 1: Plate1 (A) Culture of *Rhizopus stolonifer* (B) Mycelium of *R. stolonifera*

Plate 2 (C) Culture of *Pencillium* sp. (D) Mycelium of *Pencillium* sp.
Plate 3 (E) Culture of *Mucor* sp. (F) Mycelium of *Mucor* sp.
Plate 4 (G) Culture of *Aspergillus niger* (H) Mycelium of *Asperigilius niger*
Plate 5 (I) Culture of *Fusarium oxysporum* (J) Mycelium of *Fusarium oxysporum*
Plate 6 (K) Culture of *Trichoderma* sp. (L) Mycelium of *Trichoderma* sp.

Table 1: Rhizospeheric soil fungi associated with *Solanum melongena* L.

Soil fungi	Rhizospeheric fungal population No. of Plates					
	1	2	3	4	5	6
Penicillium notatum	-	-	+	+	+	+
Rhizopus stolonifer	+	-	+	+	-	-
Mucor sp.	+	+	-	+	-	-
Aspergillus sp.	-	+	+	-	+	-
Fusarium oxysporum	-	-	+	+	-	-
Trichoderma sp.	-	-	-	-	+	+

+ Presence, - Absence

Frequency of rhizospeheric fungi associated with *Solanum melongena* L.
The fungi isolated from the rhizospeheric soil of plants of brinjal (*Solanum melongena L.*) were *Aspergillus niger* Tiegh., *Penicillium notatum* Thom., *Mucor* sp., *Trichoderma* sp., *Fusarium oxypsporum* Link., and *Rhizopus stolonifera* Lind, as shown in Table 1. The highest frequency of fungi were of *Penicillium notatum* (66.67%), followed by *Rhizopus stolonifer* (50%), *Mucor* sp. (50%), *Aspergillus* sp. (50%), *Fusarium oxysporum* (33.33%) and *Trichoderma* sp. (33.33%) as shown in Table 2.

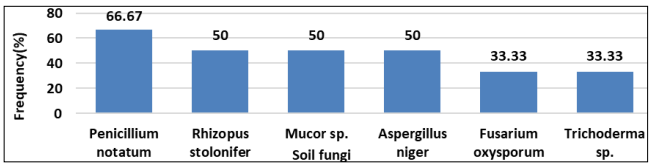

Fig 2: Frequency of soil fungi associated with *Solanum melongena* L.

Relative abundance of soil fungi associated with *Solanum melongena* L. The highest relative abundance was found in *Penicillium notatum* (48.78%), followed by *Rhizopus stolonifer* (14.63%), *Mucor* sp. (12.19%), *Aspergillus* sp. (9.76%), *Fusarium oxypsporum* (7.32%) and *Trichoderma* sp. (7.32%) as shown in Table 3

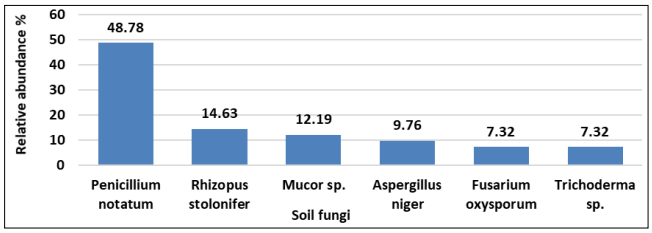

Fig 3: Relative abundance of soil fungi associated with *Solanum melongena* L.

Conclusion and Discussion

This study revealed that the rhizospeheric fungi associated with Solanum *melongena* L. in different localities of Kashmir valley were *Penicillium notatum*, *Rhizopus stolonifer*, *Mucor* spp., *Aspergillus niger*, *Fusarium oxysporum* and *Trichoderma* spp.

The quality of plants is largely influenced by both abiotic and biotic factors of the rhizosphere. There was found a significant variation in the frequency and relative abundance of fungi associated with *Solanum melongena* L. The highest frequency was found for *Penicillium notatum*. Relative abundance of fungi isolated from rhizosphere soil also shows significant variation. Similar studies were carried out Motta *et al*., (2003) [8] and isolated 49 species of filamentous fungi from the rhizospheric soil of *Helianthus annus*. *Penicillium* and *Aspergillus* were the genera that presented highest number of species.

Conflict of interest

The authors declared that there is no conflict of interest.

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