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Quantitative and qualitative enumeration of soil mycoflora in brinjal crop fields

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ABSTRACT

A total of 36 species of fungi belonging to 15 genera were isolated from brinjal crop fields of Kodad. The present study results clearly revealed that the rhizosphere and non-rhizosphere soil showed variation in diversity of mycoflora. *Aspergillus* was observed to be an important component of the present rhizosphere and non-rhizosphere soil fungal flora constituting 70.54% and 58.96%, respectively, and *Aspergillus niger* was observed to be predominant species in the rhizosphere and non-rhizosphere soils.

KEY WORDS: Microflora, non-rhizosphere, rhizosphere

INTRODUCTION

Soil is a complex ecosystem. Many biological processes take place in the soil. Soil is a medium with solids, liquids, and gases in which minerals and organic particles form differently sized aggregates that delimit pores. Soil organic matter plays an important role in determining the fertility and productivity of soils (Tisdall and Oades, 1982; Feller and Beare, 1997). This organization creates microenvironments that are suited for microbial activities to the various extents (Chotte *et al.*, 1997). Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soil. Natural and anthropogenic disturbances can alter the species composition or may have a negative effect on species diversity of decomposer fungi. These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization and may result in disturbances.

Fungi are fundamental for soil ecosystem functioning (Warcup, 1951). Along with bacteria, actinomycetes, and algae, fungi are primary decomposers, agents of biogeochemical transformations and recyclers of stored energy and nutrients of the organic matter already degraded by invertebrates and other microbes for plant growth. The organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. The members and kinds of microorganisms present in the soil depend on many environmental factors such as amount and type of nutrients, moisture, degree of aeration, pH, and temperature (Gaddeyya *et al.*, 2012). Rhizosphere is a site of complex interactions between plants and microorganisms, where environmental factors such as soil physicochemical parameters as well as fertilizers or cultivation practices may have a large effect on microbial communities (Hindumathi and Reddy, 2011). The conservation of diversity of microflora in agricultural fields becomes very essential for the development of sustainable agriculture. This is why the study of interactions in the rhizosphere is a topic of current concern. Despite the importance of rhizosphere in agriculture and forestry, little work has been done regarding the distribution, diversity, and presence of microfungal flora in the soil associated with the brinjal, the vegetable crop in Telangana. The present work is aimed for quantitative and qualitative enumeration of the fungi from rhizosphere and non-rhizosphere soils of brinjal fields collected during different time intervals and to assess the percentage contribution and diversity of different fungal species.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from the rhizosphere and non-rhizosphere regions from agricultural brinjal fields at

three intervals (pre-flowering, flowering, and harvesting period) of the life cycle. For rhizosphere soil sampling, the selected plants were carefully dugout with roots; excess soil was gently shaken off and discarded, leaving only the soil closely adhered to the root system. The roots were cut into pieces and placed in screw cap bottles containing sterile water for soil dilution. The bottles were shaken to remove closely adhering rhizosphere soil.

For the non-rhizosphere soil, samples were collected randomly from different places of experimental brinjal crop at a depth within 10 cm using a metal spatula and pooled together to get a composite sample. The rhizosphere and non-rhizosphere soil samples collected were stored at 4°C for further analysis.

Soil Physicochemical Properties

Soil pH was read using electronic digital pH meter. The moisture content of the soil sample was determined by oven-dried basis by drying 10 g of soil in a hot air oven at 105°C for 24 h, and the dry weight was taken. Soil organic carbon was estimated by a colorimetric method (Anderson and Ingram, 1993).

Dilution Plate Method

Dilution agar plate technique (Waksman, 1922) was used to isolate the fungi from soil samples. 1 g of soil sample was diluted in 10 ml of sterilized distilled water to make microbial suspension of 10^{-1} concentration. 1 ml of this suspension was added to 9 ml of sterilized distilled water to give 10^{-2} concentration. Similarly, serial dilutions were made to give concentrations up to 10^{-6}. Triplicates of each dilution of 10^{-4}, 10^{-5}, and 10^{-6} were used to isolate fungi. 1 ml of each of the dilution was poured and spread on Petri plates containing sterilized potato dextrose agar (PDA) medium and CZA medium. One percent streptomycin solution was added to the medium before pouring into Petri plates for preventing bacterial growth. The plates were incubated in an inverted position at room temperature (26 ± 2°C) for 3-5 days. Fungal colonies growing on the PDA and CZA plates were numbered. Each isolate of fungal species was sub-cultured by transferring onto fresh PDA slants.

The colonies growing on PDA and CZA plates with different morphology were counted separately. A portion from the growing edge of the colony was picked up with the help of a needle and mounted on a clean slide with lactophenol cotton blue stain. The specimen was spread carefully to avoid overcrowding of the fungal mycelium on the slide. The slide was gently heated over a flame of spirit lamp so as to facilitate the staining and remove air bubbles if any. The excess stain was removed with the help of tissue paper, and then, the cover glass was sealed with DPX. The slide was observed under the compound microscope. Photomicrographs of the individual fungal species were also taken.

Identification

Colony color and morphology were noted beside hyphal structure, spore size, shapes, and spore-bearing structures. They were identified using standard manuals (Gilman, 1957; Nagamani *et al.*, 2006).

Isolation and Enumeration of Fungi

Serial dilution plate method (Johnson and Curl, 1972) was followed for the isolation of rhizosphere and non-rhizosphere fungi using PDA and CZA media. Colony-forming units (CFUs) of fungi were estimated by counting the number of fungal colonies. The CFU per gram soil was calculated on the dry weight basis:

$$\text{CFU} / \text{g}^{-1}\text{of soil} = \frac{\text{Number of colonies per plate} \times \text{Dilution factor}}{\text{Dry weight of the soil taken}}$$

The percentage occurrence of each fungal species was calculated using the following formula:

$$\text{Percentage occurrence of fungal species} = \frac{\%\ \text{Occurrence of individual species}}{\%\ \text{Occurrence of total species}} \times 100$$

RESULTS AND DISCUSSION

The data on rhizosphere and non-rhizosphere soil analysis of physicochemical characteristics presented in Table 1 show that the soil pH was neutral (7.1) to moderately alkaline (7.8). The soil moisture was relatively abundant in all soil samples ranging from 26.51% to 30.92%. The soils were optimum in organic carbon ranging from medium (0.5% to 0.75%) to high level (>7.5%). The soil organic carbon ranged from 0.80% to 0.95% in the rhizosphere soil and 0.62-0.79% in the non-rhizosphere soil. Increase in soil organic carbon in the rhizosphere is affected by rhizodeposition, by which carbon enters the soil systems, forms symbiotic associations with fungi which facilitates the flow of carbon to and through this symbiotic interface, resulting in increased carbon content in the root region compared to the bulk soil (Leake *et al.*, 2004).

In the present study, fungal CFU/g^{-1} soil exhibited variations throughout the sampling period in both rhizosphere as well as non-rhizosphere soils. Fungal CFU/g^{-1} soil ranged from 1.18×10^5 to 1.90×10^5 g^{-1} dry soils in rhizosphere soil and 0.75×10^5 to 1.24×10^5 g^{-1} dry soils in the non-rhizosphere soil. Highest fungal CFU/g^{-1} soil was observed at the harvesting stage of plant life cycle in rhizosphere (1.90×10^5) and at flowering stage in non-rhizosphere (1.24×10^5) soil in CZA medium (Table 2). It is evident from the data that there was an increase in CFU/g^{-1} soil with increase in age of the plant growth period showing an association of highest propagule number during the time of flowering. The population of fungal flora showed lower CFU/g^{-1} soil number in the non-rhizosphere than in corresponding rhizosphere soils and are affected by plant development. These results are in correlation with the earlier findings (Hindumathi and Reddy, 2011). It is evident from this study that fungal CFU/g^{-1} soil is higher in the rhizosphere soil than that of the non-rhizosphere soil may be due to the different types of substances known as exudates released from the roots such as carbohydrates (sugars and oligosaccharides), organic acids, vitamins, nucleotides, flavonoids, enzymes, hormones, and volatile compounds (Prescott *et al.*, 1999) that may have stimulated the microbial activities in the root region as compared to the non-rhizosphere soil. The root exudates stimulate the biological and physical interactions between roots and soil microorganisms which modify the biochemical and physical properties of the rhizosphere soil and contribute to root growth and plant survival, resulting in a dense and active microbial population in the root region (Khonglah *et al.*, 2015).

Table 1: Physicochemical properties of rhizosphere and non-rhizosphere soils of brinjal

Soil properties	Sampling period		
	PF	F	H
pH			
R	7.40	7.30	7.60
NR	7.80	7.20	7.50
MC			
R	30.40	30.92	29.48
NR	26.51	28.60	26.90
OC			
R	0.80	0.95	0.82
NR	0.75	0.79	0.62

R: rhizosphere soil, NR: Non-rhizosphere soil, MC: Moisture content (%), OC: Organic carbon (%), PF: Pre-flowering, F: Flowering, H: Harvesting, organic carbon (low<0.05%; medium 0.5-0.75%; high>0.75%)

Table 2: Quantitative enumeration of fungi from non-rhizosphere and rhizosphere soils of brinjal

Period of growth in first column	Soil			
	Rhizosphere CFU/g^{-1} soil		Non-rhizosphere CFU/g^{-1} soil	
	PDA	CZA	PDA	CZA
Period of growth				
Before flowering	1.59×10^5	1.18×10^5	0.75×10^5	0.82×10^5
Flowering	1.78×10^5	1.90×10^5	1.10×10^5	1.24×10^5
Harvesting	1.74×10^5	1.75×10^5	1.14×10^5	1.12×10^5

The present study revealed the occurrence of 36 fungal species in the brinjal vegetable fields representing 15 genera. Among the 15 genera recorded, the genus *Aspergillus* was predominant represented by 14 species, followed by *Fusarium* (4 species), *Trichoderma, Penicillium,* and *Cladospora* by 2 species each. The other genera were represented by one species each (Table 1). The species of *Aspergillus* isolated were *Aspergillus fischeri, Aspergillus flavipes, Aspergillus flavus, Aspergillus fumigatus, Aspergillus funiculosus, Aspergillus humicola, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus sulphureus, Aspergillus sydowii, Aspergillus tamarii, Aspergillus terreus,* and *Aspergillus versicolor*; the species of *Fusarium* were *Fusarium oxysporum, Fusarium poae, Fusarium solani,* and *Fusarium dimerum*; the species of *Cladophora* were *Cladosporium cladosporioides* and *Chaetomium herbarum*; the species of *Penicillium* were *Penicillium islandicum* and *Penicillium aurantiogriseum*; *Trichoderma* spp. were *Trichoderma harzianum* and *Trichoderma viride;* and remaining were *Alternaria* sp., *Macrophomina phaseolina, Monodictys fluctuate, Myrothecium roridum, Phoma glomerata, Rhizoctonia solani, Rhizopus stolonifer, Colletotrichum,* and *Chaetomium* represented by one species each. White sterile and black sterile mycelia were also observed (Figures 1a-h and 2a-h).

Quantitatively species of the genus *Aspergillus* were predominant both in the rhizosphere and non-rhizosphere soils. *A. fischeri, A. flavus, A. funiculosus, A. humicola, Aspergillus nidulans, A. niger, A. ochraceous, Aspergillus sulphureus, A. sydowii, Aspergillus tamarii, A. terreus,* and *A. versicolor* were confined to rhizosphere. *A. flavipes* and *A. fumigatus* were observed in the non-rhizosphere soils. *Alternaria*, *A. humicola, A. ochraceous, A. sydowii, A. tamarii, Chaetomium* sp., *F. dimerum, F. solani,* and *Monodictus* sp. were not observed in non-rhizosphere soil but were found in rhizosphere. The present study results revealed the occurrence of saprophytes (*Aspergillus* and *Penicillium*), plant pathogens (*Fusarium* and *Colletotrichum*), and biocontrol agents (*Trichoderma* and *Cladosporium*) in the soil. *F. oxysporum, F. poae,* and *F. solani* were isolated from the soils of infected fields. The population density of these species was observed to be greater in rhizosphere than non-rhizosphere soils (Tables 3-5).

Aspergillus is observed to be an important component of the present rhizosphere and non-rhizosphere soil fungal flora constituting 70.54% and 58.96%, respectively.

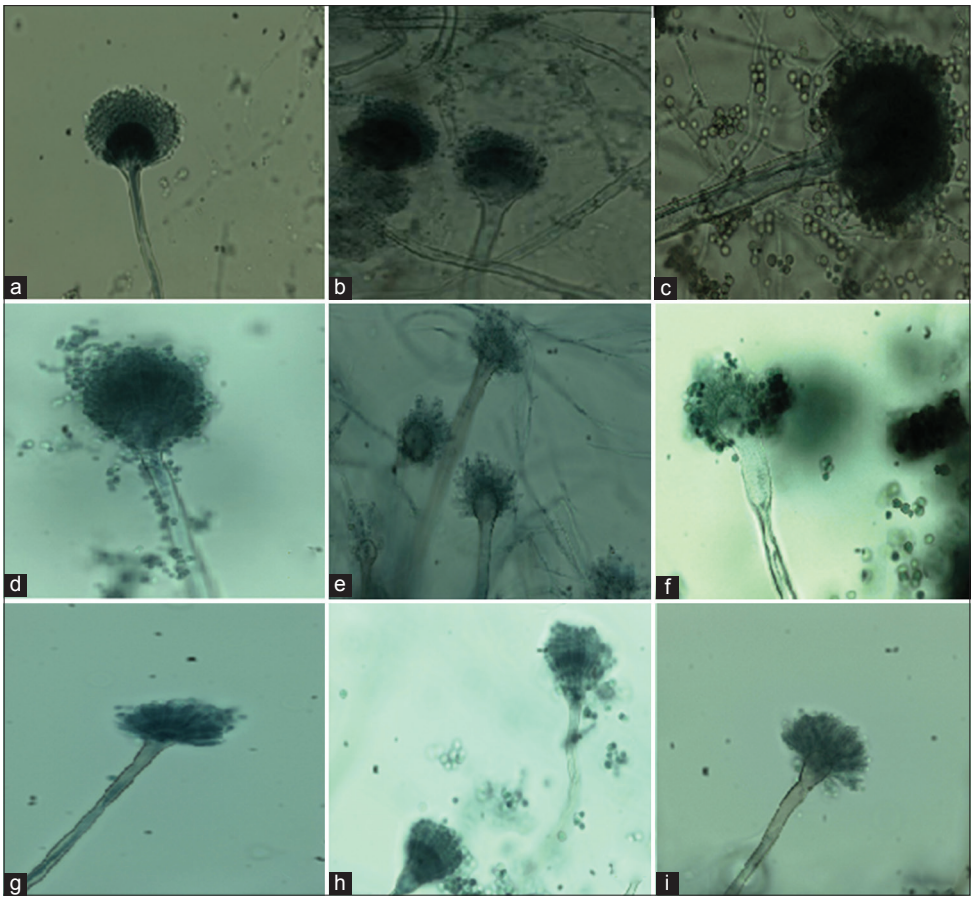

Figure 1: Photomicrographs of mycoflora isolated from rhizosphere and non-rhizosphere. (a) *Aspergillus flavus*, (b) *Aspergillus terreus*, (c) *Aspergillus niger,* (d) *Aspergillus unguis*, (e) *Aspergillus sulphureus*, (f) *Aspergillus* sp., (g) *Aspergillus humicola*, (h) *Aspergillus ochraceus*, (i) *Aspergillus fischeri*

Table 3: Percentage occurrence of mycoflora from rhizosphere soils of brinjal

Mycoflora	Before flowering		Flowering		Harvesting		Total (%)
	PDA	CZA	PDA	CZA	PDA	CZA	
Alternaria alternata	0	0	2	2	3	0	7 (0.84)
Aspergillus fischeri	1	2	2	3	0	3	11 (1.33)
Aspergillus flavus	17	6	4	6	12	13	58 (7.03)
Aspergillus funiculosus	5	4	2	3	4	0	18 (2.18)
Aspergillus humicola	0	0	2	5	2	0	9 (1.09)
Aspergillus nidulans	0	0	13	3	8	9	33 (4)
Aspergillus niger	37	31	28	20	22	22	160 (19.39)
Aspergillus ochraceous	0	0	1	10	5	6	22 (2.66)
Aspergillus sulphureus	0	0	2	3	3	4	12 (1.45)
Aspergillus sydowii	0	0	2	2	3	3	10 (1.21)
Aspergillus tamarii	0	0	1	2	2	1	6 (0.72)
Aspergillus terreus	29	18	15	18	17	19	116 (14.06)
Aspergillus versicolor	0	0	1	0	0	2	3 (0.36)
Chaetomium herbarum	2	0	2	3	0	2	9 (1.09)
Cladosporium cladosporioides	0	0	4	0	5	2	11 (1.33)
Cladospora herbarum	0	5	1	5	3	2	16 (1.93)
Colletotrichum	4	2	3	0	0	2	11 (1.33)
Curvularia clavata	1	0	7	8	5	3	24 (2.9)
Fusarium dimerum	0	2	1	2	0	2	7 (0.84)
Fusarium oxysporum	4	3	9	8	8	10	42 (5.09)
Fusarium poae	2	0	0	2	3	5	12 (1.45)
Fusarium solani	0	0	2	3	0	2	7 (0.84)
Macrophomina phaseolina	2	1	2	3	5	2	15 (1.81)

(Contd…)

Table 3: *(Continued)*

Mycoflora	Before flowering		Flowering		Harvesting		Total (%)
	PDA	CZA	PDA	CZA	PDA	CZA	
Monodictys fluctuate	0	0	2	8	3	5	18 (2.18)
Myrothecium roridum	3	5	7	6	2	2	25 (3.03)
Penicillium aurantiogriseum	3	0	3	3	4	5	18 (2.18)
Penicillium islandicum	1	4	2	6	5	8	26 (3.22)
Rhizoctonia solani	2	1	7	3	2	5	20 (2.42)
Rhizopus stolonifer	0	0	3	0	3	2	8 (0.96)
Trichoderma harzianum	3	0	3	2	2	3	13 (1.57)
Trichoderma viride	9	1	4	4	4	3	25 (3.03)
Black mycelium sterile	1	4	5	7	3	2	22 (2.66)
White mycelium sterile	5	8	4	6	5	3	31 (3.75)
Total	131	97	146	156	143	152	825 (100)

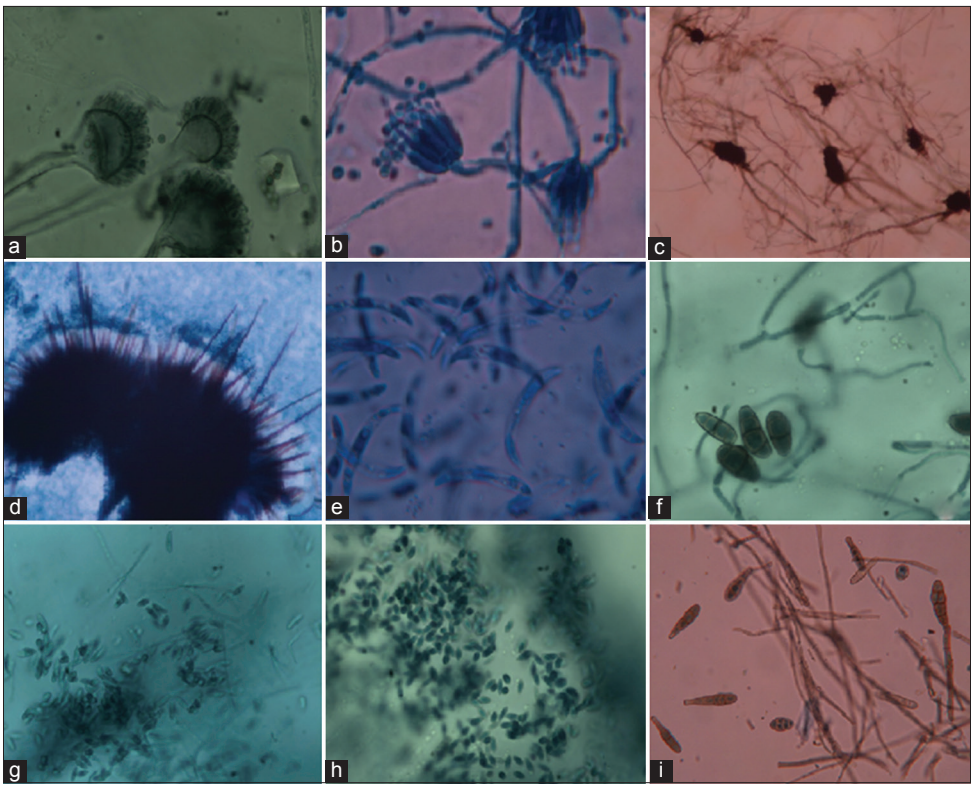

Figure 2: Photomicrographs of mycoflora isolated from rhizosphere and non-rhizosphere soils of brinjal. (a) *Aspergillus fumigatus*, (b) *Penicillium* sp., (c) *Macrophomina phaseolina*, (d) *Colletotrichum falcatum*, (e) *Colletotrichum flacatum* conidia, (f) *Curvularia lunata*, (g) *Cladosporium cladosporioides*, (h) *Myrothecium* sp., (i) *Alternaria alternata*

Among *Aspergillus* species, *A. niger* was the predominant occupant with percentage frequency of occurrence constituting 19.39% and 15.12% in rhizosphere and non-rhizosphere soils, respectively. Our observations are in agreement with those of Deshmukh *et al.* (2013) who reported the dominance of *Aspergillus*. In the rhizosphere, *Fusarium* populations were increased in number and occurrence, when compared to non-rhizosphere soils as plant growth was associated with root exudates with increasing plant age similar to earlier reports (Watanabe*et al.*, 1974).

Fungal activities increased with plant age. In early plant growth stage (before flowering), the number of fungal CFU/g^{-1} 0.75 × 10^5 increased progressively with the growth period. When near maturity, the CFU/g^{-1} remained constant in rhizosphere attributed to the competitiveness between mycoflora. These observations are in agreement with those of Hindumathi and Reddy (2011).

In the present study, there was the occurrence of *Trichoderma* species, which would be a scope in using these fungi as a native biocontrol agent to suppress the plant pathogenic organisms (Ramaraju *et al.*, 2016).

Table 4: Percentage occurrence of mycoflora in from non-rhizosphere soils of brinjal field

Mycoflora	Before flowering		Flowering		Harvesting		Total (%)
	PDA	CZA	PDA	CZA	PDA	CZA	
Aspergillus fischeri	3	5	2	0	3	0	13 (2.55)
Aspergillus flavipes	0	2	3	2	2	0	9 (1.76)
Aspergillus flavus	8	9	15	11	12	13	68 (13.35)
Aspergillus fumigatus	6	5	3	5	9	3	31 (6.09)
Aspergillus nidulans	5	3	4	5	6	7	30 (5.89)
Aspergillus niger	7	8	13	16	15	18	77 (15.12)
Aspergillus sulphureus	2	0	3	4	3	4	16 (3.14)
Aspergillus terreus	8	9	12	6	6	8	49 (9.62)
Aspergillus versicolor	3	0	2	1	4	3	13 (2.55)
Cladosporium cladosporioides	2	0	1	2	0	2	7 (1.38)
Cladosporium herbarum	2	1	0	2	1	2	8 (1.57)
Colletotrichum falcatum	0	2	0	0	2	0	4 (0.78)
Curvularia lunata	0	4	2	3	3	0	12 (2.35)
Fusarium oxysporum	2	3	4	6	5	4	24 (4.71)
Fusarium poae	1	4	2	4	1	3	15 (2.94)
Macrophomina phaseolina	2	2	3	5	2	3	17 (3.33)
Myrothecium roridum	0	2	2	0	3	0	7 (1.37)
Penicillium aurantiogriseum	2	0	3	4	1	2	12 (2.35)
Penicillium islandicum	2	3	3	3	2	4	17 (3.33)
Phoma glomerata	0	0	3	4	0	1	8 (1.57)
Phoma glomerata	2	0	2	2	4	2	12 (2.35)
Rhizoctonia solani	0	0	2	2	2	3	9 (1.76)
Rhizopus stolonifer	0	0	0	2	0	1	3 (0.59)
Trichoderma harzianum	0	2	2	3	2	1	10 (1.96)
Trichoderma viride	2	0	0	2	1	2	7 (1.37)
Black mycelium sterile	3	2	2	4	2	3	16 (3.14)
White mycelium sterile	0	2	3	4	3	3	15 (2.94)
Total	62	68	91	102	94	92	509 (100)

Table 5: Abundance of mycoflora in rhizosphere and non-rhizosphere soils of brinjal

Mycoflora	Rhizosphere	Non-rhizosphere
Alternaria alternata	+	-
Aspergillus fischeri	+	+
Aspergillus flavus	++	+++
Aspergillus flavipes	-	+
Aspergillus funiculosus	+	+
Aspergillus fumigatus	+	+
Aspergillus humicola	+	-
Aspergillus nidulans	+	+
Aspergillus niger	+++	+++
Aspergillus ochraceous	+	-
Aspergillus sulphureus	+	+
Aspergillus sydowii	+	-
Aspergillus tamarii	+	-
Aspergillus terreus	+++	++
Aspergillus versicolor	+	+
Chaetomium globosum	+	-
Cladosporium cladosporioides	+	+
Cladosporium herbarum	+	+
Colletotrichum falcatum	+	+
Curvularia clavata	+	+
Fusarium dimerum	+	-
Fusarium oxysporum	++	+
Fusarium poae	+	+
Fusarium solani	+	-
Macrophomina phaseolina	+	+
Monodictys fluctuate	+	-
Myrothecium roridum	+	+
Penicillium aurantiogriseum	+	+
Penicillium islandicum	+	+
Phoma glomerata	-	+
Rhizoctonia solani	+	+
Rhizopus stolonifer	+	+
Trichoderma harzianum	+	+
Trichoderma viride	+	+
Sterile black mycelium	+	+
Sterile white mycelium	+	+

Detected isolates: +++: High (11 and above), ++: Medium (6-10), +: Low (1-5)

CONCLUSION

The fungal numbers and species varied in relation to plant growth stage as well as rhizosphere and non-rhizosphere soils. This type of studies help in better understanding of the conditions favorable for the occurrence of soilborne fungal pathogens and enable us to take possible precautions to prevent the establishment and control of fungal pathogens, thereby reducing loss. Lewis and Papavizas (1985) mentioned that *Trichoderma* in natural soil requires substrates as a source of nutrients to enhance growth, survival, and competitiveness. The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture.

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