

**Research article**

Study of rhizospheric soil mycoflora of *Cajanus cajan* L. Millisp. in Halol taluka of Gujarat, India

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Abstract: The rhizospheric fungi are predominantly in close association with plants and essentially are important for plant well-being. Characterization of soil mycoflora for a crop will help in the improvement of agricultural techniques. In the present investigation six genus of fungi were isolated from the soil near root region of *Cajanus cajan* grown in agricultural fields of Halol taluka. In order to identify the fungi specific to the rhizospheric region a comparative study was conducted with the non-rhizospheric mycoflora. There was only 22% similarity in mycoflora between the two soil type which was predominantly due to the organic matter decomposers belonging to *Aspergillus* genera. The most common soil fungi identified were organic matter decomposers and a few phytopathogenic fungi such as *Curvularia* and *Fusarium*. The Shannon-Wiener diversity index obtained for the rhizospheric sample is 0.3, indicating high fungal diversity in this soil. Overall, our study revealed that all the mycoflora identified from the *C. cajan* rhizospheric soil from agricultural field in Halol area is rich in fungal diversity and it is different from the non-rhizospheric soil.

Keywords: Pigeon pea - Fungal flora - Rhizosphere - Non-rhizosphere.

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INTRODUCTION

Microorganisms are found in large numbers in soil predominantly consisting of bacteria and fungi. Fungi are one of the key components of the soil microflora. They are present either as mycelia, rhizomorphs or as spores. Mycoflora play a significant role in the decomposition of organic matter and indirectly contribute to soil fertility. Rhizosphere can be defined as a narrow region of the soil that is directly influenced by roots. This region has a very diverse mycoflora which is determined by the type of the plant and its interaction with the microbes. The rhizospheric fungi are involved in numerous biological process like biodegradation, mineralization, symbiotic and non-symbiotic association with plant which improves soil fertility, protects from plant pathogen and enhances plant growth (Kennedy & Smith 1995). A few rhizospheric fungi are in very close association with the root tissues in the form of mycorrhiza where the fungal hyphae grows within the root tissue and around the root system (Phillips & Hayman 1970). This association not only promotes the plant growth but also protects plant from dehydration. Thus, understanding the rhizospheric microflora is significant for conservation of the micro-environment in soil ecosystem for crop improvement. The plant rhizosphere system, in turn, affects biomass and activity of soil microorganism that is generally enhanced due to root exudates. The rhizospheric mycoflora of a plant will vary with the nature of soil and climatic condition (Deshmukh *et al.* 2016). As the root exudates change plant life cycle, interestingly the composition of rhizospheric mycoflora also varies during different developmental stages of the plant (Houlden *et al.* 2008, Deshmukh *et al.* 2016). Therefore, the rhizospheric mycoflora is unique for a plant which is dependent on the property of soil, root exudate and interaction with microbes. Most of the reported studies have focused only on bacterial communities in the rhizosphere. The present research is focused to study the fungal diversity of the rhizospheric region of *Cajanus cajan*. crop plant in agricultural field. Pigeon pea is the sixth important legume crop of the world and it is one of the major crops of Gujarat state in India. Pigeon pea is cultivated in 2.65 lakh hectare area of Gujarat leading to production of 2.94 lakh ton of the pulse annually (<http://agropedia.iitk.ac.in>). A comparison of the

mycoflora of the rhizospheric soil and non-rhizospheric soil has been analyzed in order to identify the fungi specific to *C. cajan* in Halol region of Gujarat.

MATERIAL AND METHODS

Study area and soil sample collection

Rhizosphere and Non-Rhizosphere soil samples of Pigeon Pea [*Cajanus cajan* (L.) Millsp.] were collected from Arad village of Halol taluka, Panchmahal district, Gujarat state. It is located at 22.5072° N and 73.4718° E. Soil samples were collected in the month of September during rainy season. The rhizospheric soil and non-rhizospheric soil collection was done on the same day. The temperature of the region ranges from 28°C to 35°C during the rainy season. The annual rainfall recorded for this region is 200 mm to 330 mm. The soil type in this region is red and black type. The other major crops grown in the region are paddy, wheat, maize, groundnut and cotton which are cultivated in Rabi/kharif season. Soil samples were collected from 3 to 4-inch depth from the surface layer for nonrhizospheric soil.

Isolation of mycoflora from soil

The rhizospheric and the non-rhizospheric mycoflora was isolated by plating the soil suspensions on sterile Potato Dextrose Agar (PDA; pH 5.5) plates. For this, 1gm of the soil sample was suspended in 10ml of sterile double distilled water and this was serially diluted further (10^{-1} to 10^{-6}). For plating, 0.1 ml of each dilution was spread on PDA supplemented with 1% streptomycin to inhibit bacterial growth. Plates of all dilutions were were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for seven days. The experiment was repeated twice with the same soil sample.

Staining and identification of fungi

Identification of the isolated fungi was performed by studying the colony morphology and the sporulation pattern which was examined microscopically. For this, the plates were incubated for longer period till the sporulation was visible. A small portion of the fungal colony was aseptically transferred on a glass slide using sterile forceps and stained with lactophenol/ cotton blue stain and observed under 40X objective of compound microscope. Photographs were taken with the help of Am-scope camera. The fungal species were identified with the help of Prof. Arun Arya from department of botany, The M S University of Baroda, India.

Data analysis

The similarity between the mycoflora of the rhizospheric and non-rhizospheric soil was compared using Sorensen index of similarity (IS) (Wolda 1981) using the formula given below:

$$IS = \frac{2C}{A + B} \times 100$$

Where, A = the total number of species found in soil sample A (Rhizosphere soil); B = the total number of species found in soil sample B (Non-rhizosphere); C = the number of species common in both the samples.

Further, the fungal diversity of the rhizospheric soil sample was evaluated by the Shannon-Wiener index (H') (Spellerberg & Fedor 2003) and calculated according to the formula:

$$H' = - \sum_{i=1}^n p_i \ln p_i$$

Where, p_i is the proportion of number of colonies of the i -th species to the total number of colonies when $i = 1, 2, 3, \dots, n$; $E_H = H' - N$ where N= total number of species.

For all data analysis MS-excel software was used.

RESULTS AND DISCUSSION

Fungal diversity in the rhizospheric and the non-rhizospheric soil

A comparative analysis of the rhizospheric and the non-rhizospheric soil sample was performed in order to identify the *C. cajan* rhizosphere specific fungi in the Halol area of Guajrat, India. For this, the rhizospheric and non rhizospheric soil samples were collected in the month of September during rainy season assuming the soil will be enriched with fungi after the first rain in the month June. A uniform soil suspension of both the samples were spread on fungal selective media, PDA (pH 5.5) (Fig. 1). The bacterial growth was inhibited by antibiotics, streptomycin incorporated in the medium. Microscopic analysis of fungi isolated from both the soil samples revealed presence of 6 different fungal species in the *C. cajan* rhizospheric soil and 3 fungal species in the non-rhizospheric soil sample (Figs. 2 & 3). Mixed cultures were further subcultured to obtain pure culture of the

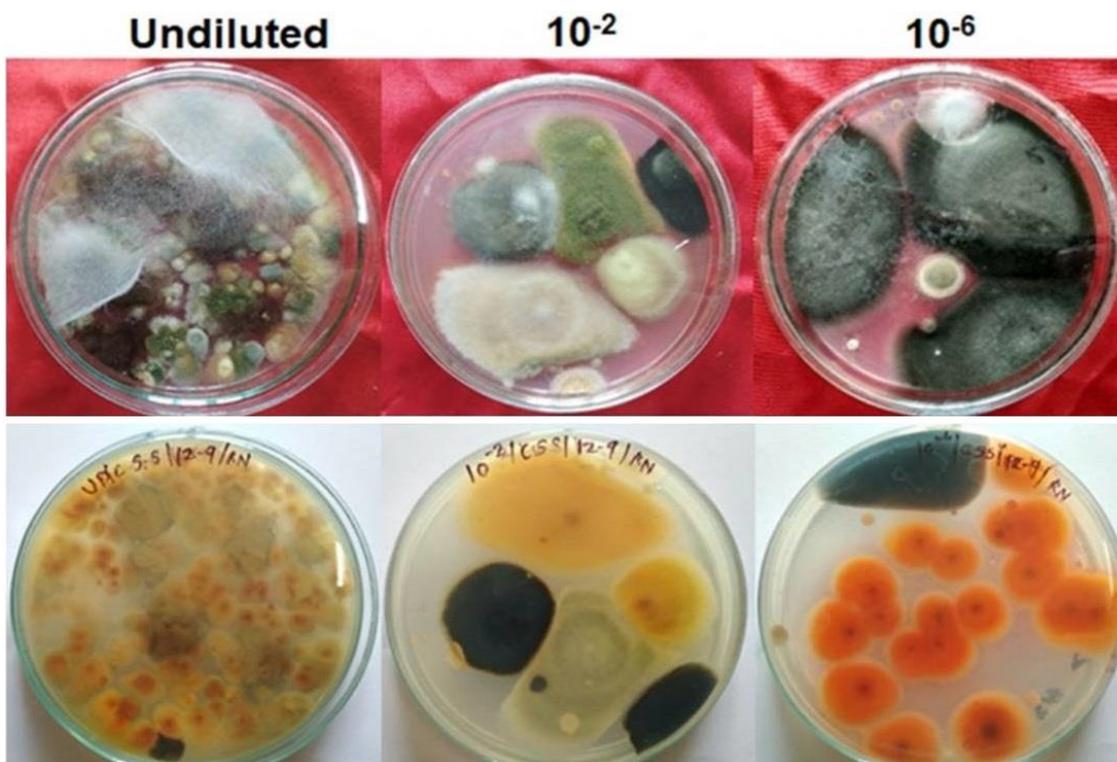


Figure 1. Fungal colonies grown on PDA plates after seven days of incubation.

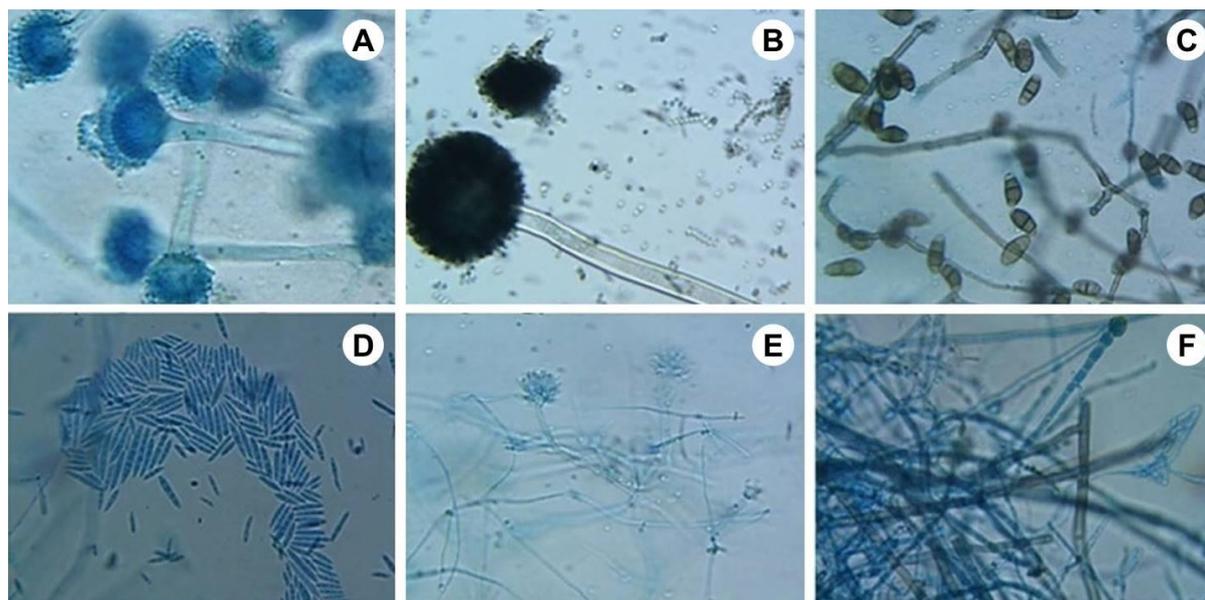


Figure 2. Fungal species identified in the rhizospheric soil sample: A, *Aspergillus flavus* Link; B, *Aspergillus niger* van Tieghem; C, *Curvularia* sp.; D, *Fusarium* sp.; E, *Syncephalastrum* sp.; F, Unidentified fungi-2.



Figure 3. Fungal species identified in the non-rhizospheric soil sample: A, *Aspergillus niger* van Tieghem; B, *Rhizopus stolonifer* Vuillemin; C, Unidentified fungi-1.

fungus isolates. The fungal species were identified on the basis of colony characteristics and sporulation pattern. The different species identified as common in the two experimental replicates belonged to the genera *Aspergillus*, *Rhizopus*, *Fusarium*, *Curvularia* and *Syncephalastrum* (Figs. 2 & 3). One of the species isolated from the non-rhizospheric soil remained unidentified but it appears to have yeast-like cellular structures. The majority of the fungal species belonged to the Ascomycota family. The details of each fungal species are given in table 1. The most common soil fungi identified were organic matter decomposers like *Rhizopus* and *Aspergillus*. The decomposers like *Aspergillus* sp., were common to both the rhizospheric and the non-rhizospheric soil. *Rhizopus* was found only in the non-rhizospheric soil. However, Jalander & Gachande (2011) reported that *Rhizopus* sp. are also found to be associated with a few varieties of *C. cajan* grown in Telangana state of India. There were also many phytopathogenic fungi such as *Curvularia* and *Fusarium*. Out of these two plant pathogens, *Fusarium* remains dormant the soil and when the *C. cajan* crop is grown in the next season, the pathogen revives and causes serious necrotrophic wilt disease in this crop (Gordon 2017). The present study indicates that the rhizospheric soil of *C. cajan* from Halol is enriched with decomposer fungi and *C. cajan* specific fungal pathogen.

Table 1. Different species identified in rhizospheric and non-rhizospheric soil of *Cajanus cajan* L. Millisp. in Halol region of Gujarat.

Fungal species identified	Rhizosphere	Non-Rhizosphere	Family
<i>Aspergillus niger</i> van Tieghem	+	+	Trichocomaceae
<i>Rhizopus stolonifer</i> Vuillemin	-	+	Mucoraceae
<i>Aspergillus flavus</i> Link	+	-	Trichocomaceae
<i>Curvularia</i> sp.	+	-	Pleosporaceae
<i>Fusarium</i> sp.	+	-	Nectriaceae
<i>Syncephalastrum</i>	+	-	Syncephalastraceae
Unidentified fungi-1	-	+	
Unidentified fungi-2	+	-	

Note: Symbol '+' and '-' indicates presence or absence of particular species respectively.

Similarity index rhizospheric and nonrhizospheric soil mycoflora

In order to calculate the similarities of the fungal communities between the two soil types, we used Sorensen's index of similarity for comparison (Wolda 1981). If Sorensen's index is higher than 55% it indicates the soil samples have similar fungal communities and a value lower than 35% indicates a low similarity between the samples under study (Puangsombat *et al.* 2010). In our experiment, we have obtained Sorensen's index as low as 22.2%, which indicated that there are more differences in the fungal community than similarity (Table 2). Plant exudates secreted around the root system is one of the key factors that select the microbial pattern in the rhizospheric region (Deshmukh *et al.* 2016). The non-rhizospheric soil lacks the root exudates thus the composition is mainly dependent on the soil's physical and chemical properties. The similarity between the two soil types was contributed by the decomposers of *Aspergillus* sp. which was common to both the soil samples and the dissimilarities were due to the phytopathogen and other saprophytic fungi present only in the rhizospheric soil.

Table 2. Diversity and abundance of fungal species in rhizospheric and non-rhizospheric soil of *Cajanus cajan* L. Millisp. in Halol region of Gujarat.

	Rhizosphere (A)	Non-rhizosphere (B)	Common in (A & B = C)
Number of species	6	3	1
Sorensen index of similarity (IS)	$IS = \frac{2C}{A+B} \times 100$		22.2%

Diversity index for rhizospheric mycoflora

We have calculated the diversity index (H) for only rhizospheric soil as the number of species obtained is more than the non-rhizospheric soil. The Shannon-Wiener diversity index (H') is used to calculate the diversity of any ecosystem (Spellerberg & Fedor 2003). A lower E_H value indicates more diversity while higher E_H value indicate less diversity. The E_H value obtained is 0.3 which is closer to zero indicating high fungal diversity in the *C. cajan* rhizospheric soil.

CONCLUSIONS

Overall our study revealed that all the mycoflora of the *C. cajan* rhizospheric soil from agricultural field in

Halol area is rich in fungal diversity and the predominant species were either decomposers like *Aspergillus* sp. or *C. cajan* phytopathogens. Unlike Jalander & Gachande (2011), we didn't find many species of fungi in *C. cajan* rhizosphere namely, *Penicillium* sp., *Trichoderma* sp. *Rhizopus* sp. etc. This may be due to the variation in soil composition and the climatic conditions of Halol which is very different from Telangana state of India. We have reported presence of *Syncephalastrum* in the *C. cajan* rhizospheric soil which was not reported before. The present study is in conjuncture with previously reported studies that the rhizospheric soil has more fungal diversity than the non-rhizospheric soil. The root exudates released by *C. cajan* root might be responsible for the dissimilarities between rhizospheric and nonrhizospheric mycoflora. Thus, our observations add new information to the scientific data available on *C. cajan* rhizospheric mycoflora.

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