



Isolation and Identification of Fungal Isolates from the Rhizospheric Region of a Legume Crop-*Cicer arietinum* L.

Priyanka, Maneesh Kumar Lomas and Bhawna Bajpai

Department of Botany, Chaudhary Charan Singh University, Meerut, India

Abstract

The legume crop, *Cicer arietinum* L., commonly known as chickpea, belongs to the family Fabaceae. It is the world's second-most significant legume in terms of total pulse production. Present study was conducted on identifying and isolating the legume crop (*Cicer arietinum*) rhizosphere mycoflora. Saini village, Mawana road, Meerut was the source of the rhizosphere soil samples utilised in this study. Utilizing a hoe, the entire plant (shoot and root) was removed from the field. Serial dilution plate technique was used for isolation of fungal strain of test crop plant and using potato dextrose agar medium, sample inoculation was completed. A total of 128 fungal isolates of different fungal species were isolated. These 128 fungal isolates belonged to eleven species. Out of eleven species, eight species were identified viz. *Aspergillus niger*, *A. flavus*, *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Trichoderma* spp. and *Rhizopus* spp. *Aspergillus niger* had the highest percentage frequency of occurrences of 19.53% and *Trichoderma* spp. had the lowest percentage frequency of occurrence of 2.34% respectively. Conclusively, despite the significant amount of research that has previously been done on the soil's microflora, it must be acknowledged that no one has yet been able to paint a complete picture of all of the interactions between the many groups of living beings in the soil. This will require cooperation amongst plant scientists, environmentalists, and agronomists to build the rhizotron system, wherein molecular and biochemical biology investigations might be carried out on the spot, in order to develop fresh approaches to investigating rhizosphere ecology under the current environmental circumstances.

Keywords: Legume crop, Rhizospheric fungi, Soil, Serial dilution plate technique.

Introduction

Chickpeas contribute for around 12% of global pulse production, with Asian countries accounting for more than 70%. The yield of *Cicer arietinum* L. in India is noted about 70% of the world yield (Aswathi, *et al.*, 2019). Australia (6%), Pakistan (5%), Turkey (4%), Myanmar (4%), Ethiopia (3.5%), Iran (2%), Mexico (2%), Tanzania (1%), and Malawi (0.5%) are the remaining *Cicer arietinum* L. producing countries. Chickpeas are also high in minerals (calcium, phosphorus, magnesium, zinc, and iron), unsaturated fatty acids, fibre, and β -carotene. It is a good source of carbohydrates and

proteins, which together constitute about 80% of the total dry seed mass. The starch content of chickpea cultivars have been reported to vary from 41% to 50% (Kishor, *et al.*, 2017). *Cicer arietinum* L. is a crop grown in 59 countries that grows in dry and semiarid environments. The need of pulses has direct proportion to population growth. To occupy need of human beings, it is necessary to introduce the cultivars by increasing the yield of *Cicer arietinum* L. through different breeding programs (Ali, *et al.*, 2018; Shengu, *et al.*, 2018). In chickpea, the major limitations in attaining optimum grain yield are deficiency

of wide heredity variation in sowing *Cicer arietinum* L. germplasm market price than desi-type (Benkadri, et al., 2018).

The rhizosphere is the area of the soil next to the root that exhibits intensive microbial-activity and is where the growth of microorganisms is promoted by the organic nutrients produced by cell death and exudates from the root, including sugar, organic acid, amine compounds, and other substances. Rhizospheric fungi, the most common plant-fungus association; comprises about 150 species, belonging to the order *Glomales* of *Zygomycotina*, the arbuscular mycorrhizal. Symbiosis is an association between most terrestrial plants and a class of fungi (*Glomeromycota*) that occurs in the roots of host plants, the fungus receives carbohydrates (sugars) and growth factors. They can be found in many types of soil, but they are more common in areas where chemical fertilisers are not employed. They have the ability to increase soil fertility. Colonization of roots by fungus has been proven to increase crop plant productivity in drought-stressed soils. Ultimately fungi improve soil structure by binding soil particles together. Rhizospheres include a variety of organic compounds that are rich in microorganisms, particularly fungi. Species richness is often high in the Rhizosphere, while it is noticeably low in the rhizoplane (Shinkafi and Gobir, 2018). The main objective of this study was to isolate and identify root-associated fungi associated with the legume crop *Cicer arietinum* L.

Materials and Methods

Rhizosphere soil samples of chickpea crop used in the case study were collected from Saini village, mawana road, Meerut, Uttar Pradesh. It was collected; the plants with root were dug out of the field using a hoe and transported to the Pathology laboratory of Botany Department, CCS University Meerut. The samples were collected in clean polythene bag for further processing and analysis. The Rhizosphere soil and its adhering soil were carefully cut off and placed in a 250ml conical flask containing 100ml of sterile distilled

water. Similarly, 1g of Rhizosphere soil was placed in a test tube with 9ml of sterile distilled water. The prepared suspension was labelled and vigorously shaken. To obtain a 10⁻⁹ dilution, 1ml of stock suspension and inoculates were pipetted into 9ml of sterile distilled water in labelled test tubes and forcefully shaken. To obtain a 10⁻¹ dilution, 1ml of the first dilution was pipetted into another labelled test tube. They were repeated until a dilution of 10⁻⁹ was obtained. In PDA plates, 0.1ml of 10⁻⁴ and 10⁻⁵ dilutions were inoculated and swabbed to ensure equal distribution of inoculums in the medium. In addition, 0.5ml of each stock suspension was immediately inoculated into PDA agar plate and swabbed as previously described. All plates were labelled and incubated for 5 to 6 days at room temperature and later fungal colonies were grown (Shinkafi and Gobir, 2018). After 7 days, fungi from petriplate were identified using LCB (Lactophenol, cotton blue) stain, putting a drop of fungal stain over the clean slide and delicately brushed (spread). A drop of lacto phenol, cotton blue was applied on the fungus stain, and a cover slip was placed on top of the slide. Then, using x40 objectives, inspected under the microscope. The fungal strains obtained were identified using standard keys (Gilman, 1957; Nagamani, et al., 2006).

Results

In the present investigation, a total of 128 fungal isolates of different fungal species were isolated from the rhizospheric soil of *Cicer* (Figure 1). These 128 fungal isolates belonged to 8 identified species and 3 unidentified species. The morphological characteristics of the isolated fungus were shown in Table 1. Out of identified 8 species, 3 species belonged to class Eurotiomycetes viz. *Aspergillus niger*, *A. flavus*, *Penicillium* spp., 2 species belonged to Dothideomycetes viz. *Alternaria* spp., *Cladosporium* spp., 2 species belonged to class Sordariomycetes viz. *Fusarium* spp., *Trichoderma* spp. and one species i.e. *Rhizopus* spp. belonged to class Phycomycetes.. In the present investigation, the maximum number (25) and percentage (19.53%) of fungal isolate was recorded in *A.*

niger while minimum number (3) and percentage (2.34%) of fungal isolate was recorded in *Trichoderma* spp. (Table 2).

Table 1: Morphological and cultural characteristics of the isolated fungal strains

S.No.	Fungus isolated	Colony texture
1.	<i>Aspergillus niger</i>	Colonies consisting of a compact white yellowish with dense of dark brown to black.
2.	<i>A. flavus</i>	Yellowish green on upper surface and reddish on the lower surface of petriplate.
3.	<i>Cladosporium</i> spp.	Blackish- brown, powdery texture
4.	<i>Alternaria</i> spp	Black to grey colony, wooly tufts.
5.	<i>Rhizopus</i> spp.	White, then gray, dense fluffy growth
6.	<i>Penicillium</i> spp.	Grey in color
7.	<i>Fusarium</i> spp.	Pale yellow colonies, dark brown outer zone
8.	<i>Trichoderma</i> spp.	Green powder like appearance

Table 2: Frequency of occurrences of the isolated fungal strain

S. No.	Fungal isolates	Number of Occurrences	Percentage of occurrences (%)
1.	<i>Aspergillus niger</i>	25	19.53
2.	<i>A. flavus</i>	18	14.06
3.	<i>Cladosporium</i> spp.	17	13.28
4.	<i>Alternaria</i> spp	15	11.72
5.	<i>Rhizopus</i> spp.	13	10.16
6.	<i>Penicillium</i> spp.	12	9.38
7.	Unknown-1	8	6.25
8.	<i>Fusarium</i> spp.	6	4.69
9.	Unknown-3	6	4.69
10.	Unknown -2	5	3.91
11.	<i>Trichoderma</i> spp.	3	2.34
	Total	128	100

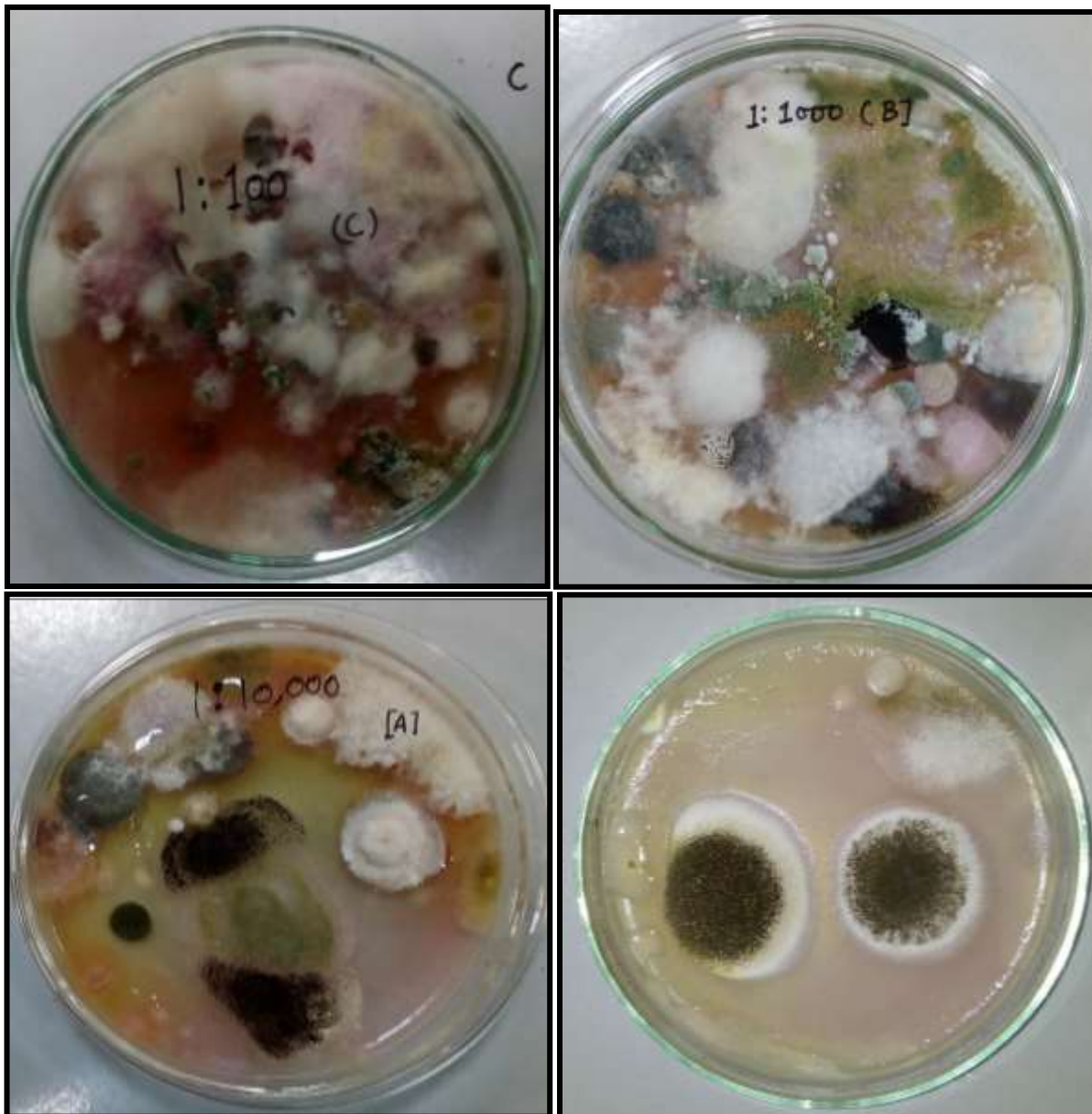


Figure 1: On the rhizospheric soil of cicer, different colonies of fungal strains were isolated and grown using the serial dilution plate technique

Discussions

Based on the findings, eight fungal species were found, each with a positive or negative effect on crop plants. *Aspergillus niger* is more common and has a higher proportion. *A. niger* play a specific role in soil fertility maintenance by acting as an all-purpose decay agent from which new life develops. *Trichoderma* spp. is the least common and has a lower proportion, demonstrating plant development and biocontrol capabilities (Rinu, et al., 2012). *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium* isolates from rhizosphere soils have been shown to

promote IAA synthesis in host plants (chickpea, rice, and wheat), resulting in higher growth and yield (Abri, et al., 2015; Kumar, et al., 2017; Ozimek, et al., 2018). Besides IAA, gibberellic acid (GA), also called as diterpenoid acid, is a phytohormone that affects seed germination as well as root and shoot development (Gillespie-Sasse 1991). Moreover, the treatment of wheat and cucumber plants with *Cladosporium* sp. increased their growth because it induced GA production (Hamayun, et al., 2010), it has been hypothesized that GA production plays an important role in the host colonisation of legume (pea) plants (Vadassery, et al., 2008).

Furthermore, *Trichoderma* spp. treatment stimulated the production of cytokinins (most importantly zeatin), which improved development in Arabidopsis and melon (Speakman and Kruger, 1984; Martinez-Medina, 2014). Various groups of mycorrhizal fungi (*Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*, and others) have a beneficial effect on crop plant growth (Dewan and Sivasithamparam, 1989; Meera, et al., 1994; Shivanna, et al., 2005; Murali, et al., 2013). In contrast, *Penicillium* spp. in the native rhizosphere inhibited the growth of native zinnia and tomato plants (Meera, et al., 1994). The growth and yield of maize, wheat, barley, chickpea, brome grass, lupine, pea, clover, and ryegrass were also enhanced by non-sporulating fungi called SBF (sterile black fungus), SRF (sterile red fungus), and SDF (sterile dark fungus) that reside in the native rhizosphere soil of plants (Dewan and Sivasithamparam, 1989). It has been noted that treating seeds with a conidial suspension of *Aspergillus niger*, *Trichoderma* and *Penicillium* enhances chickpea growth (Abri, et al., 2015). A study found that seed treatment with T. talc-based bioformulation significantly boosted the root length, fresh weight, and total biomass of chickpea and rice plants and retained a good amount of conidial suspensions compared to seed priming with charcoal, sawdust, or cow dung (Singh, et al., 2013; Doni, et al., 2017). Numerous significant plant parasitic fungi, in addition to the saprophytic fungi that serve as scavengers, exist in the soil and attack economically significant plants through their roots or at the ground level (Smith, 2000). The parasitic fungi identified for this investigation included a *Rhizopus* species, which under the appropriate conditions has the potential to cause a number of diseases. According to Shinkafi and Gobir, (2018) study, a certain strain of *Rhizopus*, such as *Rhizopus oryzae*, is used in the production of alcoholic drinks in some regions of Asia and Africa, highlighting the significance of the two in the industry. Fruits and vegetables are reportedly also affected. It has also been reported that strawberries, tomatoes, and sweet potatoes can develop fruit rot due to *Rhizopus stolonifer*.

Additionally, it is used to make fumaric acid and cortisone for industrial use (Zheng, et al., 2007). Agamy, et al., (2013) reported that, *A. solani* inhibits overall growth and significantly lowers output by decreasing the photosynthetic and pigment content.

Conclusions

The result of this study indicated that 128 fungal strain of different fungal species were isolated. These 128 fungal isolates belonged to eleven species. Out of eleven species, eight species were identified viz. *Aspergillus niger*, *A. flavus*, *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Trichoderma* spp. and *Rhizopus* spp. *Aspergillus niger* had the highest percentage frequency of occurrences of 19.53% and *Trichoderma* spp. had the lowest percentage frequency of occurrence of 2.34% respectively. Even though there has been a tremendous amount of research done on soil microflora, it must be acknowledged that no one has yet been able to provide a comprehensive picture of all life in the soil and the interactions between various groups of living things. However, because to the difficulties of studying subsurface processes in a way that is both regulated and realistic, our understanding of how they interact is incomplete.

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