The Pharma Innovation Journal 2023; 12(6): 1793-1797

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 1793-1797

www.thepharmajournal.com
Received: 03-04-2023
Accepted: 08-05-2023

Sravani Gogisetty
Department of Plant Pathology, Odisha University of Agriculture and Technology, Odisha, India

Mihira Kumara Mishra
Professor, Department of Plant Pathology, Odisha University of Agriculture and Technology, Odisha, India

Prabhat Ranjan Mishra
Professor, Department of Entomology, Odisha University of Agriculture and Technology, Odisha, India

Corresponding Author:
Sravani Gogisetty
Department of Plant Pathology, Odisha University of Agriculture and Technology, Odisha, India

Isolation and identification of phylloplane and endophyte mycoflora in *Cajanus cajan*

Sravani Gogisetty, Mihira Kumara Mishra and Prabhat Ranjan Mishra

Abstract
The biological world is dominated by the diversity and complexity of fungus, as well as their unmatched natural beauty. A variety of microorganisms, including filamentous fungus, bacteria, and yeasts, inhabit the complex terrestrial ecosystem known as the phylloplane, which is found on the surface of plant leaves. The mycota that grow on the surface of the leaves are called phylloplane fungi. Endophytic fungi are constantly seen living asymptomatically within plant tissue spaces. In a certain host plant, they develop either intracellularly or intercellularly to finish all or a portion of their life cycle. They have been discovered to be connected to practically every plant that grows in natural environments. They have been chosen to co-evolve with their hosts throughout the course of evolution because of their crucial function in a plant's survival. The traditional approach to stress treatment has been centred around the use of chemicals, which has been shown to be environmentally harmful due to its lingering toxicity. On the other hand, because they are so safe to employ, biological approaches are becoming more and more well-liked in the scientific community. Crop plant phylloplane is a significant source of non-pathogenic microorganisms, some of which have shown effectiveness in treating bacterial and fungal infections. Using both the agar plate and wet chamber techniques, a total of 14 fungal species from nine different genera were isolated from the healthy leaf of the Arhar, *Cajanus cajan*.

Keywords: Arhar, endophytes, mycoflora, phylloplane

Introduction
Cajanus cajan (L.) often known as Pigeon pea, Arhar, Red gram or Tur is a significant edible legume of the semi-arid tropical regions of Asia and Africa (Kumar CV *et al*., 2015) [11]. In a variety of environments, it is grown on 4.75 million acres worldwide (Choudhary AK *et al*., 2014) [5]. It fills a crucial void in smallholder rainfed farmers' sustainable agricultural methods. It holds a significant position in Indian rainfed agriculture. It is an essential part of the nation's varied agroecologies, and is typically interplanted with grains, pulses, oilseeds, and millets. It is the second-most significant pulse crop after chickpea, with an area of over 4.42 million hectares (ha), an output of 2.86 MT, or 16% of all pulse production, and a yield of about 707 kg/ha. Along with numerous uses for various pigeon pea plant components, it is primarily consumed as dry split dhal across the nation. In India, where the majority of the population is vegetarian, increasing the crop's productivity is especially important since it assists in combating protein deficiency (Kumar CV *et al*., 2015) [11]. Due to the complimentary nature of the essential amino acids, when wheat or rice is coupled with red gram, the biological value increases significantly. Riboflavin, Lysine, Niacin, Iron and Thiamine are particularly abundant. Additionally, it is known to increase the fertility of the soil by fixing nitrogen at a rate of 40 kg per hectare and releasing the soil-bound phosphorus (Choudhary AK *et al*., 2014) [5]. It is good for dryland farming and is typically grown as an intercrop with various crops because it is a drought-resistant crop.
The term "phylloplane" refers to the complete leaf surface, whereas "phyllosphere" refers to the complete aerial habitat of plants. The phylloplane serves as a niche for a variety of microbial communities, making it a significant ecosystem from an ecological and monetary perspective (Susmita *et al*., 2021) [12]. Bacteria, filamentous fungi, yeast, and phylloplane fungi are just a few of the microorganisms that can be observed on the outer layer of plant leaves, which is a wide and varied terrestrial environment. Phylloplane fungi, unlike endophytes, saprobes, and harmful fungi, have received scanty research. The phylloplane or leaf surface, serves as a favourable environment for the development of antagonistic microbes that can outcompete the pathogen for resources and prevent pathogen growth by secreting antibiotics or toxins (Yadav *et al*., (2011) [13] and Blakeman (1982) [4].

Specific fungi, yeasts and bacteria live in the diverse environment known as the phyllosphere of plants. Their activity is influenced by a variety of interactions between abiotic and biotic environmental variables (Behrendt *et al*., 1997, 1999) [1, 2]. High variety of fungi can be found in plant-associated microhabitats like internal plant tissues, leaf surfaces and organic residues (Bhat, 2009) [3].

The definition of "endophytic fungi" describes fungi that form a mutually beneficial symbiotic connection with their host plant and live there throughout their entire or portion of their life cycle without creating any negative effects or diseases (Hyde *et al*., 2019) [8]. They are covert and subtle residents in various plant tissues and intercellular spaces (Juan Wen *et al*., 2022) [9]. They are organic elements of the plant microecosystem that have a positive impact on the physiological functions of the host plant in a few ways, including the production of hormones like indoleacetic acid, biosynthesis and nutrient acquisition for plant growth and development, secretion of stress-adaptor metabolites to defend the host plant from the incursions of herbivorous animals and pathogens, and enhancing the host's flexibility to abiotic stressors (Hyde *et al*., 2019) [8]. Endophytic fungus can develop a variety of chemical structures and effective, low-toxic novel secondary metabolites that were once believed to be produced by the host plants (Juan Wen *et al*., 2022) [9].

The plant-beneficial microorganisms are significant bioresources for agriculture because they can promote plant development and improve nutrient uptake by solubilizing and mobilizing (K, P and Zn), fixing nitrogen, and producing siderophores (microbes-mediated bio-fortification of Fe in different crops). Added agricultural yields, contamination removal, disease inhibition, and the production of new compounds can all be significantly increased by beneficial organisms. The biological nitrogen fixation, the biological control of plant pathogens by the production of antibiotics, antibacterial or antifungal, Fe-chelating substances, the production of plant growth regulators like gibberellic acids, IAA, and cytokines, the improvement of mineral bioavailability and the development of acquired host resistance are all possible outcomes of beneficial microbes (Natesan *et al*., 2020) [10].

Since research on the phylloplane microbiology of red gram is still in its initial stages, an effort has been made in the current study to identify and define the dominant red gram leaf surface microflora. For acquiring information on microbial interactions and general phyllo-plane ecology, records on the subjective and objective estimation of microbial associations in the red gram phylloplane are also necessary. The current analysis offers the opportunity for studying the microbial abundance, species richness, and diversity related to this commercially significant legume crop in terms of microbial conservation and resource usage.

Materials and Methods

Sample collection

Fresh Arhar leaves in the upper, middle, and lower portions of the plants were picked at the College of Agriculture, Odisha University of Agriculture and Technology. In order to prevent further contamination, healthy and mature leaf samples had been collected, placed in sterilized polythene bags, tied, and taken to the lab where they were maintained at room temperature for further analysis.

Potato Dextrose Agar Media

After being peeled, the potato tubers were weighed for roughly 200 g. A sterilized knife was used to cut the tubers into little pieces. Approximately 1000ml of distilled water was added to a conical flask before the potatoes were added. For 20 minutes, the contents were boiled. The filtrate was collected after the supernatant was decanted and strained through muslin cloth. In order to dissolve the components, dextrose (20 g) and agar (15 g) were added to the extract. Distilled water was added to the medium to bring it up to 1 litre, and chloramphenicol (150 mg/l) was added to inhibit bacterial growth. A 5.6 pH adjustment was made to the medium. Lastly, the medium was cotton-plugged and autoclaved for 15 minutes at 121 °C (Garg *et al.*, 2019) [7].

Isolation of Fungi

The collected leaf samples were cut into small pieces with sterilized blade. These small pieces were kept in Petri plates filled with PDA. Five samples per each plate were taken. Some samples were kept in abaxial side and some other in adaxial side on PDA. These plates were incubated at 25 °C for 7 days.

Purification and Preservation of the isolated fungal colonies

After incubation, the growth of microorganisms was examined in the plates. All the morphologically separated visible colonies of fungi from each plate were transferred to new PDA plates and purified with repeated sub culturing. Pure cultures were identified with their scientific names and they are also transferred to cultural slants containing PDA and can stored at 4ºC. These fungal isolates were identified to species / genus levels with the help of Indian Type Culture Collection (ITCC), literatures, spore identification and online identification keys.

Characterisation of fungal isolates

Hyphal tip isolation was performed and the fungal isolates were allowed to grow on PDA. For growth patterns the pure cultures thus obtained were observed and tentative inferences were noted.

Slide identification

On a clean microscopic slide, a drop of Lactophenol blue stain was placed. Later a very small amount of hyphae was taken and uniformly spread with the aid of a needle. After proper spreading, a cover glass was kept on the sample. Ensure no air bubbles were present under the cover glass, then under the microscope this slide was examined and various observation points were noted._Slide was described based on hyphae/mycelium appearance and morphology, spores, form, size of spore bearing structures and number of spores in spore bearing structures.

Interpretation of results and identification

By using the standards of Methuen hand book of colour, fungal colony colour was evaluated. They were later classified up to species / genus levels.

Results and Discussion

From the present study, altogether 14 fungal species belonging to nine genera were isolated and identified from the healthy leaf of the Arhar, *Cajanus cajan* by employing both

agar plate and moist chamber methods. Phylloplane and endophytic fungi isolated from *Cajanus cajan* were shown in Table-1. *Alternaria* sp, *Aspergillus flavus*, *Aspergillus niger*, *Collectotrichum sp*, *Chaetomium sp*, *Fusarium solani*, *F. oxysporum*, *F. proliferatum*, *Penicillium sp*, *P. digitatum*, *P. expansum*, *Paecilomyces marquandii*, *Hypocrea* sp, *Neonectria* sp were identified from phylloplane of Arhar.

Table 1: Fungi were isolated and identified from the healthy leaf of Arhar using both agar plate and moist chamber methods

S.No	Name of the Fungi	Potato Dextrose Agar Medium (PDA)		Moist Chamber Method	
		Endophyte	Ectophyte	Endophyte	Ectophyte
1.	*Aspergillus flavus*	+	+	-	-
2.	*Aspergillus niger*	+	+	+	+
3.	*Colletotrichum sp*	+	+	+	+
4.	*Chaetomium sp*	+	-	+	-
5.	*Fusarium solani*	+	-	-	-
6.	*Penicillium sp*	+	-	+	-
7.	*Penicillium digitatum*	+	-	+	-
8.	*Penicillium expansum*	-	+	-	+
9.	*Alternaria sp*	-	+	-	+
10.	*Fusarium oxysporum*	+	-	-	-
11.	*Fusarium proliferatum*	+	-	-	-
12.	*Neonectria sp*	+	-	-	-
13.	*Hypocrea sp*	+	+	+	+
14.	*Paecilomyces marquandii*	+	+	-	-

In this study, fungi exhibit significant diversity alongside Arhar plants. The size of leaf pieces, cultivation techniques, and methodologies used may all have an impact on the variety of fungi. Between the wet and dry seasons, there were also notable differences in the frequency of certain fungus species. In the dry season, colonization occurred less often and in lower numbers than during the wet season. The more rain there is during the rainy season, the more likely it is that fungus spores will spread (Colado JG *et al.*, 1999) [6]. By utilizing light microscopy, it is possible to directly observe the quantity of fungal colonies as well as the percentage cover by phylloplane fungi.

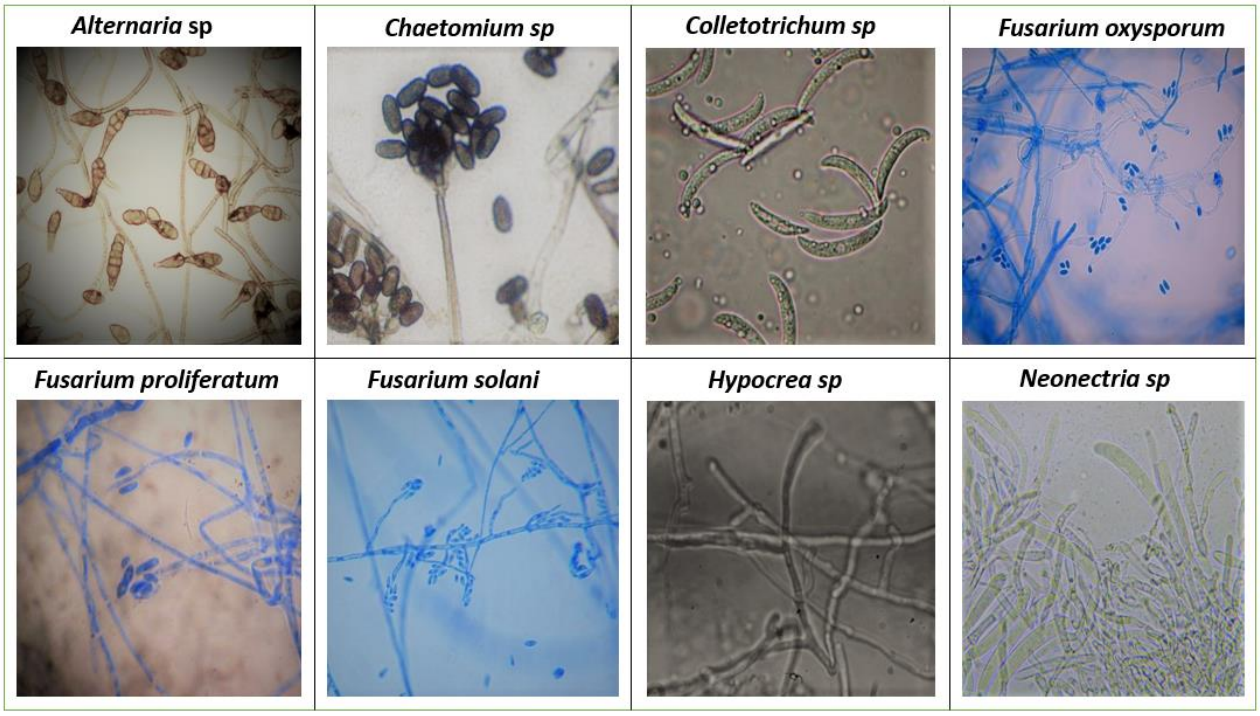

Fig 1: Representative microscopic pictures of *Alternaria sp*, *Chaetomium sp*, *Colletotrichum sp*, *Fusarium oxysporum*, *F. proliferatum*, *F. solani*, *Hypocrea sp*, *Neonectria sp*

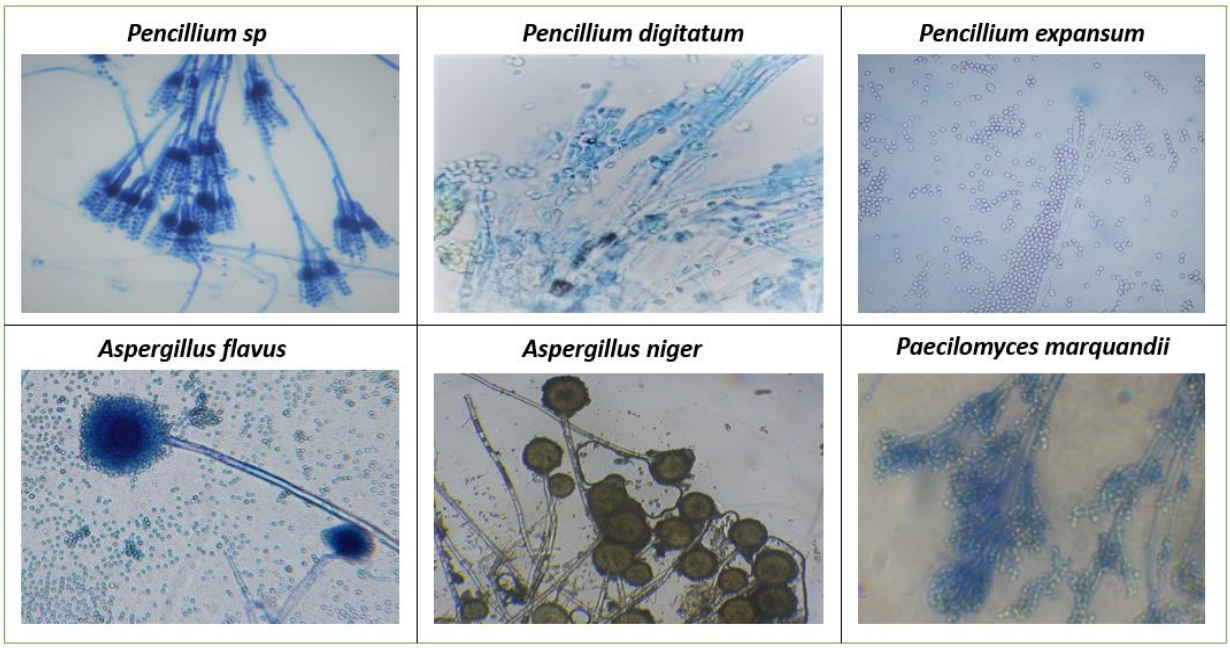

Fig 2: Representative microscopic pictures of *Pencillium* sp, *P. digitatum*, *P. expansum*, *Aspergillus flavus*, *A. niger*, *Paecilomyces marquandii*

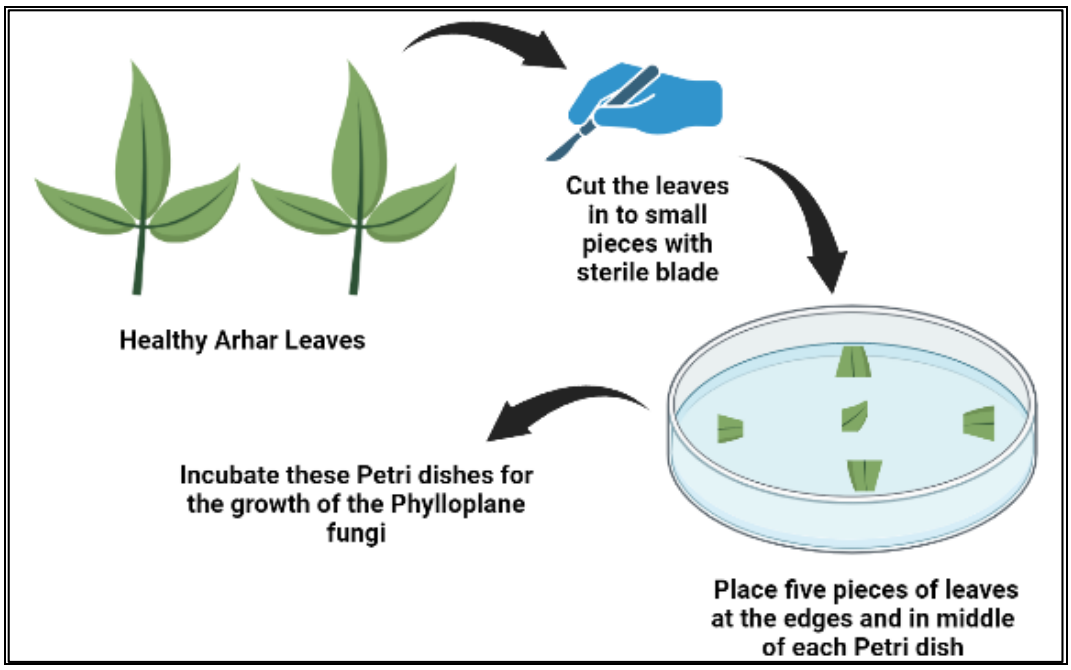

Fig 3: Isolation of Phylloplane mycoflora from healthy Arhar leaves by leaf printing method

Conclusion

In the present investigation isolation of 13 phylloplane and endophytic fungal species were recorded. Among which *Aspergillus*, *Collectotrichum* and *Hypocrea* were the most dominant fungal species in both PDA and moist chamber method. The results of the current study highlight the importance of the Arhar leaf surface as one of the most appropriate niches for a variety of various microbial communities. For the isolation of the phylloplane mycoflora in Arhar, we used the leaf printing method because it has been shown to be effective in the laboratory when cultivating microbial isolates from habitats like leaf surfaces. It is necessary to conduct additional research on the microbial relationships, ecology, and functions in the Arhar phylloplane to comprehend the microbial mechanisms that play a role in nutrient management and prevention of disease.

Transparency Declaration

The author declares no conflicts of interest.

Acknowledgment

The authors are thankful to the Dean, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India for providing the necessary facilities to undertake the study.

References

1. Behrendt U, Müller T, Seyfarth W. The influence of extensification in grassland management on the populations of micro-organisms in the phyllosphere of grasses. Microbiological Research. 1997;152:75-85.
2. Behrendt U, Ulrich A, Schumann P, Erler W, Burghardt J, Seyfarth W. A taxonomic study of bacteria isolated

from grasses: A proposed new species *Pseudomonas graminis* sp. nov. International Journal of Systematic Bacteriology. 1999;49:297-308.
3. Bhat DJ. Fascinating microfungi (Hyphomycetes) of the Western Ghats-India. Broadway Book Centre, Goa, India; c2009, p. 222-243
4. Blakeman JP. Potential for biological control of plant diseases on the phylloplane. Annual Review of Phytopathology. 1982;20:167-192.
5. Choudhary AK, Kumar RV, Saxena KB. Red gram hybrids in India: A 40 year journey of research. Indian Farming. 2014;63(12):11-13.
6. Colado JG, Platas I, Gonzaloz Pelaez F. Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. New Phytologist. 1999,144:525-532.
7. Garg KS, Pal M, Keerti Jain. Studies on Phylloplane Mycoflora of Some Medicinal Plants. Journal of Drug Delivery & Therapeutics. 2019;9(4-s):904-907.
8. Hyde KD, Xu JC, Rapior S, Jeewon R, Lumyong S, Niego AGT, *et al*. The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity. 2019;97:1-136.
9. Juan Wen, Samuel KO, Shu Wang, Jianchen Wang, Lei Xie, Yinan Ran, *et al*. Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. Journal of Fungi. 2022;8(2):205.
10. Natesan SK, Ramamoorthy SK, Gopal SK, Rajaram SK, Kalimuthu Arjunekumar. Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. Plant Microbiomes for Sustainable Agriculture. 2020;25:113-172.
11. Sameer Kumar CV, Singh IP, Suyash BP, Myer GM, Vijay Kumar R, Saxena RK, *et al*. Recent advances in Pigeonpea [*Cajanus cajan* (L.) Millspaugh) Research. In ICRISAT Open Access Repository; c2015.
12. Susmita G, Navodit G, Rita Singh M. Phylloplane microbes impact host physiology: A review. Journal of Plant Protection Research. 2021;61(3):213-221.
13. Yadav SL, Mishra AK, Dongre PN, Singh Rashmi. Assessment of fungitoxicity of phylloplane fungi against *Alternaria brassicae* causing leaf spot of mustard. Journal of Agricultural Science and Technology. 2011;**7**(6):1823-1831.