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Biodiversity of Fungi Recorded from Different Chickpea Fields of Madhya Pradesh

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ABSTRACT: Bacteria and fungus that live in the soil contribute significantly to soil fertility and the growth of plants. Soil fungus play an important role in maintaining plant diversity and productivity. Using a biodiversity-ecosystem model, we assessed the effects of root-associated soil fungi on plants. The diversity of microfungi in sixteen soil samples from chickpea crop fields across various agro climatic zones of Madhya Pradesh were examined by using serial dilution plating method with the standard Potato Dextrose Agar (PDA) for both qualitative and quantitative patterns. For analysis of fungi, 11 species were enumerated from the aforementioned soil samples in which *Alternaria* spp. (3.12%), *Penicillium* spp. (11.71%), *Fusarium* spp. (10.15%), *Trichoderma* spp. (17.20), *Rhizopus stolonifera* (6.25%), *Mucor spp.* (2.34%), *Aspergillus Niger* (25%), *Aspergillus flavus* (14.10%), *Aspergillus terreus* (5.46%), *Sclerotium rolfsii* (3.12%) and *Nigrospora* spp. (1.55%) were remarkably prevalent. All agricultural lands were dominated by *Aspergillus niger, Aspergillus flavus, Penicillium* spp., *Fusarium* spp., and *Trichoderma* species because of their high sporulation capacity. Toxins produced by *Aspergillus* species and antibiotics of *Penicillium* species may be attributed for restricting the growth of other fungal species. The *Trichoderma* species function as an antagonist against fungal plant pathogens. This study examines the impact of land use on fungus biodiversity and succession. In conclusion, the authors recommend for a change away from cataloguing fungal species in various soil ecosystems and towards a more global study based on roles and interactions between organisms.

Keywords: Mycoflora, Biodivesity, Rhizosphere.

INTRODUCTION

The earth's ecosystem, majorly includes minerals, gases, enormous numbers of macro and microscopic species, and organic materials, is found in the soil. Many interactions between the soil ecosystem's physical, chemical, and biological components support it (Buscot and Varma 2005). Many biological processes, such as organic matter turnover, symbiotic and non-symbiotic atmospheric nitrogen fixation, denitrification, aggregation, and others, govern the functions that provide different services within ecosystems (Chenu and Stotzky 2002). The confined area of soil next to the root known as the rhizosphere where root activity influences microbial populations. It is well recognized that the rhizosphere is a hub of microbial activity.

In particular context of the effects of climate change and human interference with ecosystem processes, the relationship between the biodiversity of soil fungi and ecosystem function is a matter of utmost concern. Long-term usage of chemical fertilizers may lead to imbalances in the soil's mycoflora and indirectly influence the biological characteristics of the soil, which could result in soil deterioration (Chandrashekar *et al.*, 2014). Depending on the soil depth and nutrient conditions, such as micro and macro nutrients, fungi can make up a larger portion of soil biomass than bacteria. The majority of fungal species in soil are saprophytes, and they play a key role in the breakdown of plant structural polymers including cellulose, hemicelluloses, and lignin, providing carbon and nitrogen sources that improve soil fertility (Prince and Prabakaran 2012).

In addition to facilitating nutrient uptake by plants, soil microbes like fungus also encourage plant growth and stimulate seedling development by secreting chemicals that act like hormones. Because of this, the soil is the most ideal setting and an excellent medium for fungi to flourish in. In general, soils contain 100 to 1500 g of biomass per square meter and 10^5 to 10^6 cfu/g. (Trinci, 1969; Ambikapathy *et al.,* 2001). Some of the fungus may develop quickly on organic substrates in dry, acidic soil with coarse grained, allowing them to diffuse nutrients to unicellular bacteria. Many reports on biodiversity and the effects of diverse fungal species on various soil environments are available on a global scale, indicating that soil fungi are pervasive and live in a variety of soil types. Understanding the mechanism of soil microbes requires more research than the studies of fungal biodiversity in crop plants' rhizospheres and its interactions with microbes. By controlling soil biological activity, microfungi play a key role in the cycling of nutrients (Alexander, 2001; Saravanakumar and Kaviyarasa, 2010). The quantities of organic and inorganic components in the soil directly influence its microbial population. In addition to artificial fertilizers, a variety of herbicides have negative effects on mycoflora, which are crucial for preserving soil fertility and the ecosystem's equilibrium in the soil. Numerous environmental conditions, such as the type of nutrients, moisture, levels of aeration, pH, temperature, etc., affect the members and types of microorganisms that are present in soil (Rani and Paneerselva 2010).

Human population growth in combination with changing patterns of global food consumption under climate change is posing formidable challenge to attaining sustainable global food security (Kumar *et al.*, 2021). However, to attain self-sufficiency and nutritional security, it is imperative to explore the soil microbial diversity and identification of beneficial microbial groups for future use in plant disease management and growth promotion. Plant-soil fungus interactions may influence the link between plant diversity and production through a variety of methods. According to Maron *et al.* (2011); Schnitzer *et al.* (2011); Mommer *et al.* (2018), host-specific diseases can cause conspecific negative density-dependence with negative soil feedback, which becomes less significant as species diversity rises. Therefore, production is increased with diversity, while it is inhibited to a lesser level with high diversity. Among the different pulses, chickpea is one of the major pulses of Madhya Pradesh and being a leguminous crop significantly influence the microbial diversity in the rhizosphere and rhizoplane. Chickpea. In order to understand the seasonal relationship, the impact of crop plants on the soil mycoflora, and the interaction between the soil ecosystem and other physiochemical parameters, the current study was undertaken to analyse the soil mycoflora of various chickpea fields soil samples that are collected during crop cultivation. The results of the current study are thoroughly discussed.

MATERIAL AND METHODS

Collection of soil samples. Rhizospheric soil samples were taken from the various chickpea fields of Madhya Pradesh. One kg sample of soil was collected from each location at a depth of 10-15 cm below the surface, close to the plant's rhizosphere. In sterile polythene bags, the collected soil samples were brought to the lab and kept there at 4ºC until its examination.

Isolation and Enumeration of Fungi from the soil samples. The soil microfungi were isolated and counted using the serial dilution method or the viable plate count method on Potato Dextrose Agar (Waksman, 1922). To prepare microbiological suspensions, 1 g of soil sample was suspended in 10 ml of sterile distilled water (10^{-1} to 10^{-4}). To isolate fungus, dilutions of 10^{-3} and 10^{-4} were utilized. Using the pour plate method, 1 ml of each concentration of the microbial suspension was added to sterile Petri dishes (three of each dilution) before sterile Potato Dextrose Agar (PDA). Before pouring the medium into the Petriplates, one percent solution of streptomycin was added in it to prevent bacterial growth. The Petridishes were subsequently incubated for 5-7 days at 28ºC. The observations were taken daily upto 7 days. The colony forming units (CFU) of fungal isolates were determined. Calculations and statistical analysis were used to analyse each result. (Gaddeyya *et al.*, 2012).

Identification of Mycoflora in Soil. The fungal colonies were developed in the agar plates. A qualitative and quantitative pattern of the fungal colonies was observed. By examining colony characteristics including texture and color, fungus morphology was studied macroscopically. Using cotton blue staining and a light microscope, the hyphal structure, spore shapes and sizes, and spore bearing structures were examined under a microscope.

Quantitative pattern of fungi. Using 1g of soil from each sample, the total number of fungal colonies present in the plates were counted. The percent contribution of each isolate was calculated by using the following formula: (Jayaraman *et al.*, 2018).

$$\text{Per cent Contribution} = \frac{\text{Total No. of CFU of an individual}}{\text{Total No. of CFU of all species * CFU - Colony forming Unit}} \times 100$$

RESULTS

Diversity is the term used to describe the variety of life, which might include bacteria, animals, and plants. Fungi play a significant part in ecological processes on a global scale and are crucial to biodiversity. In the current study, 128 fungal colonies of 11 different genera were found in various fields of chickpea crops in the Jabalpur. The most prevalent fungal genera among the isolates were *Aspergillus*, *Penicillium*, *Trichoderma* and *Fusarium* species (Table 1). They are reliant on the characteristics of the substrate and the temporal area that encourage fungal colonization, growth, and substrate possession.

The proper operation of newly introduced microbial inoculants and their impact on soil health are dependent on soil microorganisms in biogeochemical processes that control plant productivity. Many efforts have been done to investigate the indigenous community's soil microbial diversity, distribution, and behavior in soil habitats. The most prevalent ones were isolated and identified, including *Alternaria* spp. (3.12%), *Penicillium* spp. (11.71%), *Fusarium* spp. (10.15%), *Trichoderma* spp. (17.20), *Rhizopus stolonifera* (6.25%), *Mucor* spp. (2.34%), *Aspergillus niger* (25%), *Aspergillus flavus* (14.10%), *Aspergillus terreus* (5.46%), *Sclerotium rolfsii* (3.12%) and *Nigrospora* spp. (1.55%)(Table 1). A statistical analysis was done to determine the percentage contribution of each fungus species in various chickpea fields (Table 1). Due to their great sporulation capacities, *Aspergillus niger*, *Aspergillus flavus, Penicillium* spp., *Fusarium* spp. and *Trichoderma* species predominated in all agricultural fields. In past also several studies have been conducted for isolation of *Trichoderma* spp. from soil (Kumar *et al.*, 2013a; Kumar *et al.*, 2016) which could not only be proven as a potential bio-control agent in plant disease management (Kumar *et al.*, 2009; Kumar *et al.*, 2013b; Kumar *et al.*, 2014; Kumar and Sahu 2015; Jain *et al.*, 2017; Kharte *et al.*, 2022) but also as significant contributor in plant growth promotion (Kumar and Sahu 2014; Kumar *et al.*, 2019), as biofertilizer (Srivastava *et al.*, 2009) and in bioremediation (Kumar *et al.*, 2015). Other fungal species may be prevented from growing by the toxins produced by *Aspergillus* species and antibiotics produced by *Penicillium* species (Ahmed and Papenbrock 2015). The *Trichoderma* species act as a bioagent against the plant pathogenic fungi.

The above results are in accordance with various reports, the fungi commonly present in diffferent type of soils found as *Aspergillus niger, A. flavus, A. clavatus, A. terreus, Curvularia lunata, Pencillium janthinellum, P. citrinum, Rhizopus nigricans, Trichoderma viride, T. harzianum, Fusarium. oxysporium, F. moniliforme, Mucor, Cladosporium, Verticillium, Acremonium, Chaetomium globosum, Corynespora cassicola, Eurotium chevelari, Botrycis cineea, Helminthosporium sp.* etc. (Wahid *et al.*, 1997; Saravanakumar and Kaviarasan 2010). From Thanjavur district, Tamil Nadu, namely Thanjavur and Thiruvaiyaru, Prince and Prabakaran (2012) isolated 50 different species of phycomycetes and deuteromycetes. From the soils of Thanjavur's sugarcane fields, the two prevalent species were *Aspergillus niger* and *Trichoderma koningii*, which were followed by *T. viride, T. harzianum, T. glaucum, P.chrysogenum, P. citrinum*, and *Botrytis cinerea* in different seasons. *A. niger, Gliocladium virens, T. koningii, A. oryzae, F.oxysporum, P. chrysogenum*, and *T. viride*, on the other hand, were the major species in Thiruvaiyaru soils. A total of 37 species belonging to 16 genera were isolated from the Thanjavur station, and 35 species belonging to 15 genera were isolated from the Thiruvaiyaru station, making a total of fungus in two stations. Similar to that, 22 distinct fungus species were isolated for the current investigation. There are 22 species total, of which 3 are *Penicillium* species and 19 are *Aspergillus* species.

Table 1: Frequency of mycoflora in chickpea crop fields from different Agro-Climatic Zones of Madhya Pradesh.

Sr. No.	Fungal sp. recorded	Average no. of total colonies	% Contribution
1.	*Alternaria* spp.	4	3.12
2.	*Penicillium* spp.	15	11.71
3.	*Fusarium* spp.	13	10.15
4.	*Trichoderma* spp.	22	17.20
5.	*Rhizopus stolonifera*	8	6.25
6.	*Mucor* spp.	3	2.34
7.	*Aspergillus niger*	32	25
8.	*Aspergillus flavus*	18	14.10
9.	*Aspergillus terreus*	7	5.46
10.	*Sclerotium rolfsii*	4	3.12
11.	*Nigrospora* spp.	2	1.55
	Total	**128**	**100**

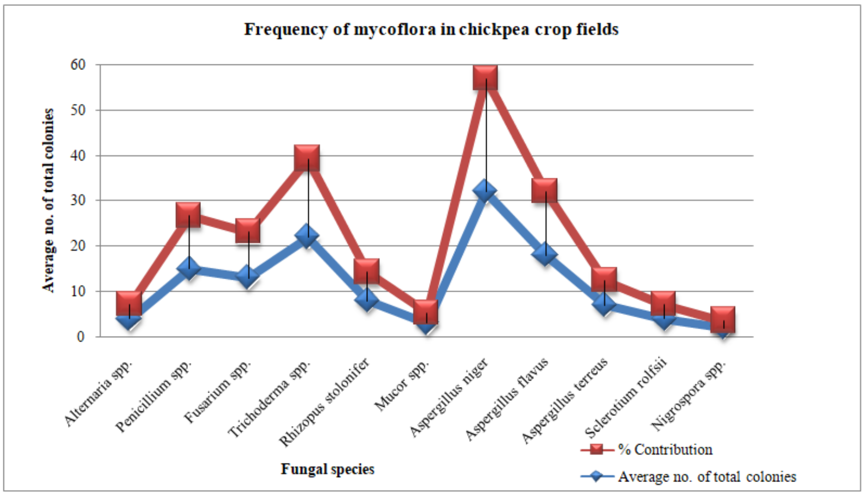


Fig. 1. Percent frequency of fungal genera obtained from various chickpea fields of Madhya Pradesh

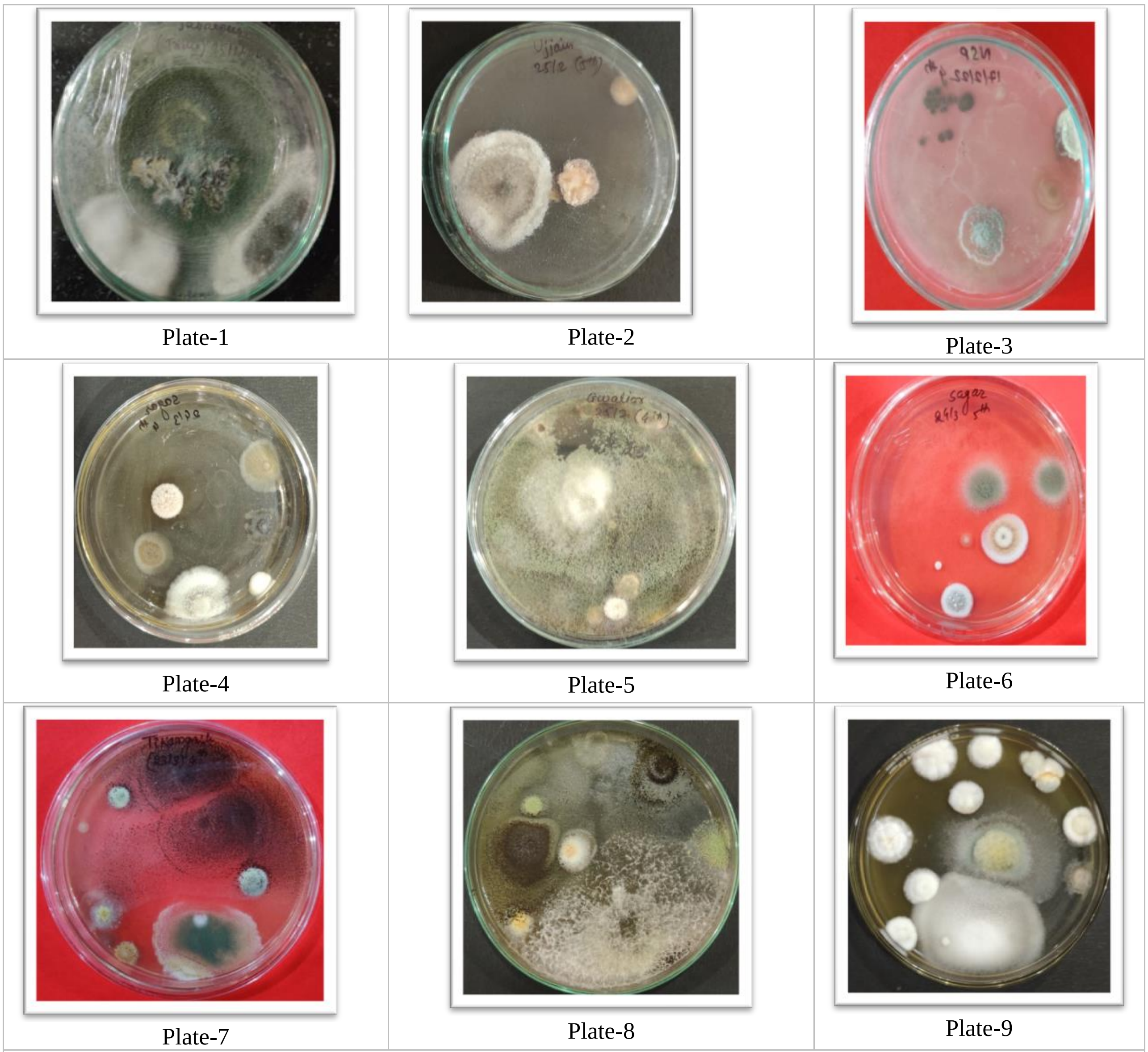
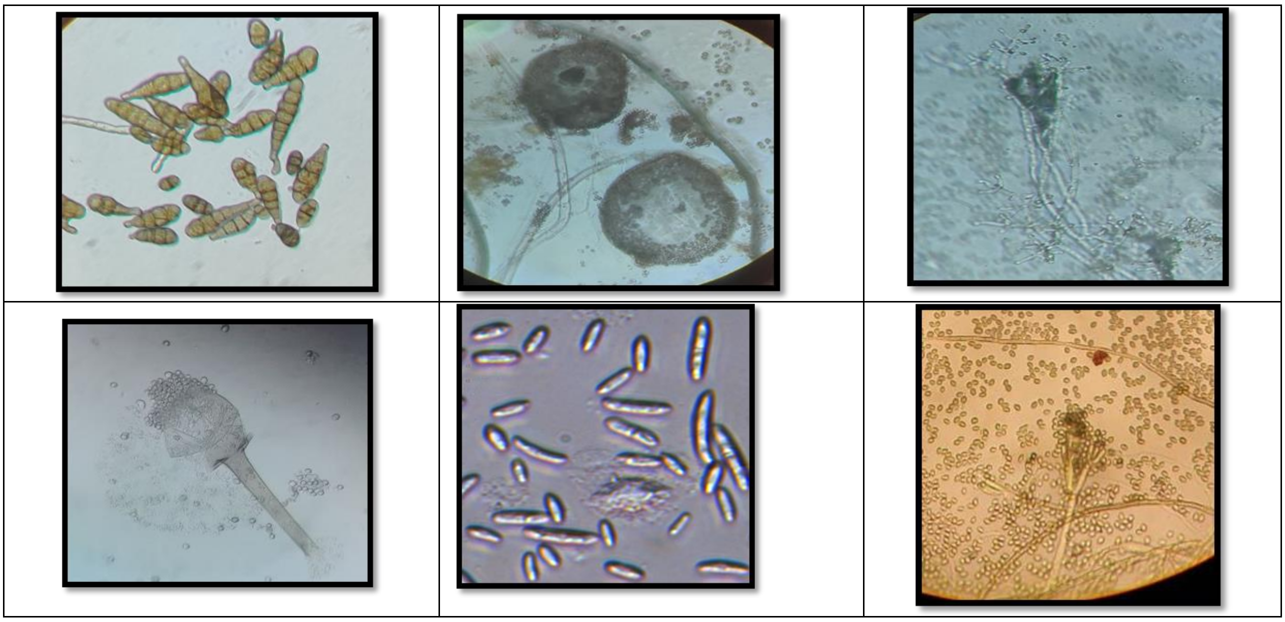


Plate: Diverse fungal colonies obtained on PDA.



a) *Alternaria spp.* b) *Aspergillus* spp. c) *Trichoderma* spp. d) *Mucor* spp. e) *Fusarium* spp. f) *Penicillium* spp.
Plate: Microscopic images of some identified fungal genera.

CONCLUSIONS

The current study demonstrates the diverse nature of fungal biodiversity from several chickpea soil samples with varying population densities, which may be related to differences in soil types, crop cultivation, nutrients in the soil, pH in the soil, moisture in the soil, and soil mycoflora. To understand the relationship between fungal diversity and rhizosphere environment, the soil environment of several agricultural fields with various crop growing techniques was researched. The competing fungus *Trichoderma* inhibited the growth of the pathogenic fungus. It would be fascinating to conduct more research on the diversity of *Trichoderma* species, as well as their significance to agriculture.

FUTURE SCOPE

Soil health issues have a huge impact on environmental sustainability, including agriculture, horticulture, and forestry. Furthermore, soil health is closely related to the production of good food, which affects both human and animal health. More research is needed to determine the optimal strategy to preserve fungal biodiversity in soil, taking into account fungal roles and ecosystem services such as disease management, contaminant detection, and bioremediation. It is critical to have the correct tools and be able to identify species as well as characterise their role in the environment. The ability to compare functional structures across ecosystems and predict responses to environmental changes and interventions would be a significant step forward.

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Conflict of Interest. None.

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