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Rhizospheric fungal diversity in the medicinal plants of Rewa District, Madhya Pradesh, India

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ABSTRACT

Rhizospheric fungi play pivotal roles for both soil and plant health. These fungi improving the growth of the plants and enhance the production of secondary metabolites. In the rhizosphere of medicinal plants, a very complex and diverse microbial community is present, including fungi, that significantly influences plant health and secondary metabolite production. Rhizospheric fungi are attracting researchers towards medicinal plants uses due to the presence of bioactive compounds, which consist of alkaloids, peptides, steroids, phenols, quinones, flavonoids, terpenoids, etc. The present study has been undertaken to isolate and identify the soil fungi associated with the rhizosphere of some medicinal plants, *viz.*, *Ocimum sanctum*, *Asparagus officinalis*, *Cymbopogon citratus*, and *Mentha arvensis* of the Rewa district of MP, India. Total eight fungal species were isolated from the rhizosphere of selected medicinal plants. The highest number of fungal colonies was found in the rhizosphere of *Cymbopogon citratus*. Maximum fungal diversity was in the rhizosphere of *Ocimum sanctum*, with six species while minimum was in *Asparagus officinalis* with three species. *Aspergillus fumigatus*, *Aspergillus niger*, and *Penicillium* sp. were more frequent, which were associated with all these plants except *Cymbopogon citratus*. These findings suggested that medicinal plants are associated with different rhizosphere fungi, which are helpful for developing sustainable agriculture practices.

Keywords: Secondary metabolites, Medicinal plants, Fungal diversity, Rhizosphere, Colony-forming unit, Sustainable agriculture

INTRODUCTION

The rhizosphere is a region adjacent to roots of plants. It is a house of different microbes, including fungi, which are beneficial for plant growth and yield. Soil represents a complex ecosystem where various microbial communities have significant roles in soil functionality and health (Khan and Singh, 2024 and 2026). Among them, fungi have significant roles in nutrient cycling and organic matter decomposition via their enzymatic activities (Gaddeyya *et al.*, 2012). Although their association with crop plants is well known, their clean-cut interactions with medicinal plants are especially important in the synthesis of bioactive compounds (Al-Abbasi *et al.*, 2021). The diversity of rhizospheric fungi is very affected by non-living factors, such as the physical properties of the soil, like pH, water levels, and airflow, and farming methods like changing crops and using fertilizers (Khan *et al.*, 2024 and 2025; Dubey *et al.*, 2026). These factors all together influence the population and diversity of microbes and their abilities for plant growth promotion.

Medicinal plants are a large group of commercially important plants. They are commonly used in drugs and pharmaceuticals, cosmetics, perfumes, and the essential oil industry. The production of medicinal plants is not sufficient to fulfill the demand of the market due to pests, pathogens, excessive use of chemical fertilizers and pesticides, and other abiotic stresses. The abiotic factors may deteriorate the soil health and rhizosphere microbiome. All plant roots, including medicinal plants, have a strong and healthy association with different microbes (bacteria and fungi), which enhances plant growth and yield. Their role is like a biofertilizer and biocontrol agent. Rhizospheric fungi also improve soil texture and nutritional value (Vasudha *et al.*, 2013). Hence, there is a need for isolation, identification, and estimation of rhizospheric fungi associated with medicinal plants. Rhizosphere fungi showed healthy interactions between plants and soil, where fungal diversity is much higher and fungal abundance is about 10-20% more than in the rhizoplane (Lynch,

1990; Morgan *et al.*, 2005). Different bioactive compounds secreted by the plant roots that perform many functions. A large number of important organic compounds secreted by plant roots provide nutrition and enhance the microbial population of the rhizosphere (Ortíz-Castro *et al.*, 2009).

The native fungi associated with plant roots enhance nutrient absorption by degrading organic matter (Shah *et al.*, 2021). These microbes not only improve plant health but also improve the production and quality of bioactive compounds in many medicinal plants. The indigenous rhizospheric fungi can be used as beneficial bioinoculant to boost medicinal plants growth without very sophisticated biotechnological processes. This study aims to investigate the population, diversity, isolation, and identification of rhizosphere fungi associated with selected medicinal plants of the Rewa district of Madhya Pradesh, India.

MATERIALS AND METHODS

A systematic survey was conducted during April 2023 of the Rewa district of Madhya Pradesh, India.

Rhizospheric soil sample collection

The rhizospheric soil samples were collected from the medicinal plant cultivated area of Rewa, Madhya Pradesh, India. The rhizospheric soil was taken from a depth of 5-15 cm, placed in sterile polyethylene bags, and transported to the laboratory under controlled conditions for further analysis.

Isolation of rhizospheric fungi

The rhizospheric fungi were isolated by dilution plate technique (Timonin, 1940). The collected rhizospheric soil was serially diluted three times with sterile distilled water. 1 ml of an aliquot taken from the final diluted solution was poured onto PDA plates. For the inhibition of bacterial growth, 1% streptomycin was mixed in PDA. The plates were incubated for 4 to 5 days at 25°C ±2 in BOD. After that, fungal colonies were examined on each plate. The plates were examined, counted, and identified the fungal colonies. For each sample, three plates were plated.

Identification of fungal isolates

The morphology of fungal isolates was examined both microscopically and macroscopically. The macroscopic observation of colony characteristics like color, growth pattern, texture, etc. was observed. For microscopic study, cotton blue-stained slide was prepared and observed under 10X and 40X lenses of the microscope. During microscopic examination, shape, size, colour, hyphae, and conidia, etc. were included. The fungi were identified with the help of Manual of Soil Fungi (Gillman, 1956).

Total 4 medicinal plants (*Ocimum sanctum*, *Asparagus officinalis*, *Cymbopogon citratus*, and *Mentha arvensis*) were selected in our study. The sampling was taken in 5 replicates for each plant, and for each sample, three plates were plated.

Fungal populations were estimated in terms of colony formation unit (CFU). It was calculated with the help of the following formula.

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of sample (mL)}}$$

Where:

Number of Colonies: The count of individual colonies/total number of colonies visible on the single agar plate.

Dilution factor: Times of dilution (1000)

Volume of sample: The amount of volume used during plating (1 ml)

RESULTS

The physico-chemical characteristics of the soil of study area of Rewa District has been presented in Table 1. The pH of rhizospheric soil samples was alkaline (pH 7.1 and 7.8). The soil texture was predominantly loamy sand and loamy, which offers good drainage while retaining adequate moisture. The soil properties provide favourable conditions for the growth of rhizospheric microbes including fungi.

Table 1. Physico-chemical properties of rhizospheric soil of medicinal plants of Rewa District, M. P.

Parameters	<i>Cymbopogon citratus</i>	<i>Ocimum sanctum</i>	<i>Asparagus officinalis</i>	<i>Mentha arvensis</i>
Soil texture	Sandy	Sandy loamy	Sandy loamy	Loamy
pH	7.1	7.5	7.4	7.8
EC (m. mhos.cm ⁻¹)	1.43	1.40	1.47	1.44
Organic Carbon (%)	0.60	0.80	0.50	0.60
Nitrogen (%)	0.281	0.293	0.265	0.286
Phosphorus (mg kg ⁻¹)	36.0	43.0	39.2	40.5
Potassium (mg kg ⁻¹)	52	48	56	51

Table 2. Rhizospheric fungal diversity in the selected medicinal plants of Rewa District, MP

Plant species	Fungal species							
	<i>Rhizopus</i> sp.	<i>Mucor</i> sp.	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Penicillium</i> sp.	<i>Curvularia</i> sp.	<i>Fusarium</i> sp.	<i>Scedosporium</i> sp.
<i>Ocimum sanctum</i>	-	+	+	+	+	+	+	-
<i>Asparagus officinalis</i>	-	-	+	+	+	-	-	-
<i>Cymbopogon citratus</i>	+	-	-	-	-	+	+	+
<i>Mentha arvensis</i>	+	-	+	+	+	-	-	-

All the medicinal plants of Rewa District selected during our study were associated with rhizospheric fungi. Total eight fungal species were isolated from the rhizospheric soil of selected medicinal plants viz., *Aspergillus niger* van Tieghem, *Aspergillus fumigatus* W. Fresenius, *Rhizopus* sp. Ehrenberg,

Penicillium sp. A. Fleming, *Fusarium* sp. F. Link, *Mucor* sp. A. Micheli, *Curvularia* sp. B. Boedijn, and *Scedosporium* sp. A. Saccardo (Table 2, Fig. 1 and 2). Fungal species were identified based on macroscopic and microscopic observation. The identifying characteristics have given in Table 3.

Table 3. Macroscopic and microscopic characteristics of identified rhizospheric fungi

Fungal isolates	Colony morphology and macroscopic features	Microscopic features
<i>Aspergillus niger</i>	Black, powdery colonies with white margins; fast growing	Conidiophore unbranched with foot cell, conidial heads radiate, biseriate, dark brown or black conidia
<i>Aspergillus fumigatus</i>	Velvety, blue- green colonies with white margins	Conidiophore unbranched with foot cell, vesicle flask shaped or globose, conidia mainly globose, green or grey in colour, uniseriate phialides on conidiophores
<i>Rhizopus</i> sp.	Cotton like white colonies that turn grey or black with age	Broad, aseptate hyphae; sporangia large and dark; sporangiospores inside sporangia. Sporangia burst at maturity and formed umbrella like structure
<i>Penicillium</i> sp.	Bluish-green colour with velvety appearance and radial grooves; white border	Conidiophore branched without foot cell, brush like conidiophores; phialides in chain; round or oval smooth conidia are white or cream in colour.
<i>Fusarium</i> sp.	White to pink colonies with cottony texture; may have reddish centre	Septate hyphae, sickle shaped macroconidia, oval microconidia
<i>Mucor</i> sp.	Rapid growing fluffy white colonies becoming grey with age	Coenocytic hyphae; globose sporangia; columella present
<i>Curvularia</i> sp.	Dark brown to black colonies with velvety texture	Curved or lunate shape and multi septate conidia; central cell generally larger
<i>Scedosporium</i> sp.	Greyish white to brown colonies; smooth to powdery surface	Septate hyphae; oval to cylindrical conidia

Aspergillus fumigatus, *Aspergillus niger*, and *Penicillium* sp. associated with *Ocimum sanctum*, *A. officinalis*, *M. arvensis* while *Mucor* sp. and *Scedosporium* sp. were associated with only *O. sanctum*, and *C. citratus*, respectively.

plants: (a) *Aspergillus fumigatus*; (b) *Curvularia* sp.; (c) *Fusarium* sp.; (d) *Mucor* sp.; (e) *Scedosporium* sp.; (f) *Aspergillus niger*; (g) *Penicillium* sp.; (h) *Rhizopus* sp.

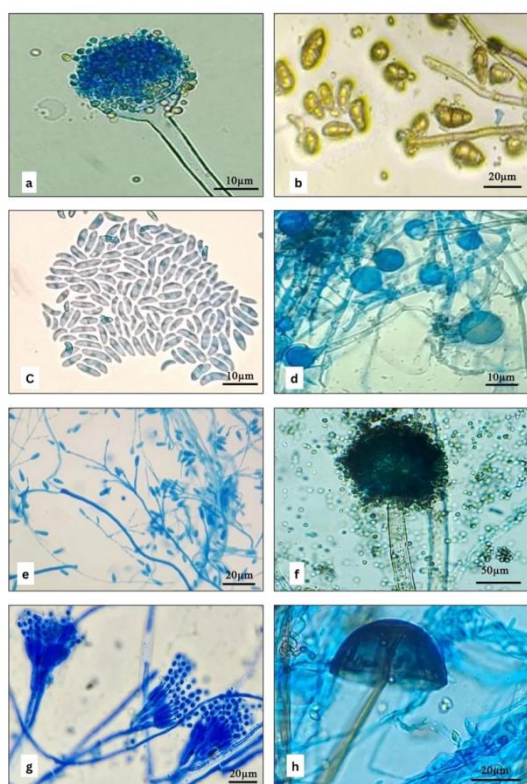


Fig. 1. Microscopic characteristics of fungal species isolated from the rhizosphere of selected medicinal

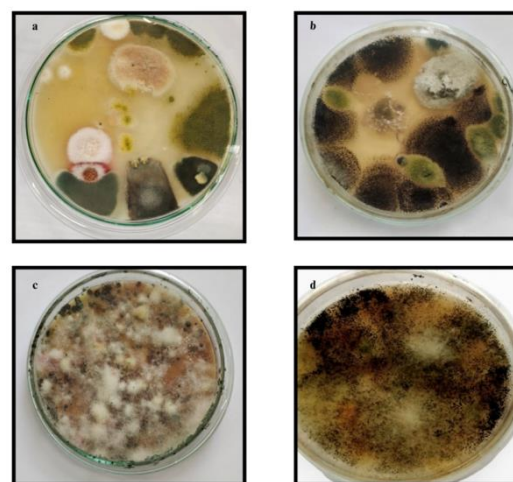


Fig. 2. Growth of rhizospheric fungal colonies on petri plates

Maximum diversity was recorded in the rhizosphere of *O. sanctum*, six different fungi (*Mucor* sp., *A. fumigatus*, *A. niger*, *C. sp.*, *F. sp.*), while minimum diversity in the rhizosphere of *A. officinalis* with only three fungi (*A. fumigatus*, *A. niger*, *P. sp.*).

The fungal colonies showed notable variations in fungal populations across different plant species. *C. citratus* highest fungal colonies (51×10^3 CFU) was in

the rhizosphere of *Cymbopogon citratus*, primarily driven by the abundance of *Rhizopus* sp., (25×10^3 CFU) and *Fusarium* sp. (20×10^3). In contrast, *Mentha arvensis* had the lowest fungal colonies (18×10^3 CFU) with a more even distribution of fungal taxa, particularly *Aspergillus fumigatus* and *Aspergillus niger* (both 8×10^3 CFU). The fungal populations in the root microhabitat of *Ocimum sanctum* and *Asparagus officinalis* were ranging from 21×10^3 to 25×10^3 CFU, dominated by *Aspergillus* sp. and *Penicillium* sp. The fungal colony associated with the selected medicinal plants is presented in Fig. 3.

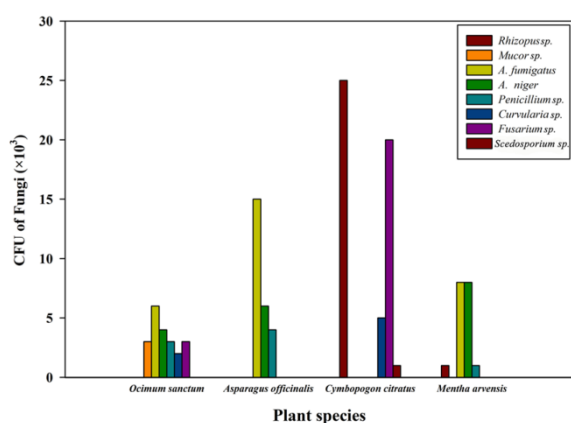


Fig. 3. Total no of fungal species

DISCUSSION

The current study aims to record the rhizospheric fungal diversity associated with four medicinal plants (*Cymbopogon citratus*, *Ocimum sanctum*, *Asparagus officinalis*, and *Mentha arvensis*) of Rewa district, MP, India. In the present investigation, eight major fungal species have been identified, with a predominance of *Aspergillus fumigatus*, *Aspergillus niger*, and *Penicillium* sp. The wide distribution of these fungi in the rhizosphere of plants is due to their adaptability to the changing ecosystem, as the roots of plants secrete a variety of compounds that control the microbial population and foster symbiotic association with beneficial microorganisms (Walker *et al.*, 2003). In the rhizosphere of the plants, the microfungi are of focal importance in nutrient cycling, as they regulate the biological activity of the soil, responding to the

amounts of organic and inorganic content in the soil (Gaddeyya *et al.*, 2012).

Among all the plant species selected during our study, the highest CFU was found in *C. citratus*. This increase in the population of microbes might be associated with the root exudates of the plants, as they are known to contain a complex mixture of organic acid anions, sugars, and secondary metabolites, which increase the population of microbes in the rhizosphere of the plants (Dakora and Phillips, 2002). On the other hand, the highest fungal diversity was found in the plant *Ocimum sanctum*. This may be related to the complex root exudation pattern of the plant species, as the root system of the plant species is known to release complex mixtures of primary and secondary metabolites as chemical signals for the recruitment of specific microorganisms in the rhizosphere (Badri and Vivanco, 2009; Bais *et al.*, 2006). *Mentha arvensis*, on the other hand, had the least fungal infestation. The low number of fungi in this plant may be because it has essential oils like menthol, which hinder fungi from growing (Galovičová *et al.*, 2023).

The rhizospheric microbes help plant growth and recycle nutrients in the ecosystem's rhizosphere compartment by making different beneficial compounds (Khan *et al.*, 2008). Root exudates are chemical compounds that roots release into the rhizosphere compartment. These chemicals attract organisms, which use them as a source of carbon and energy for development (Badri and Vivanco, 2009; Bais *et al.*, 2006).

Rhizospheric fungi is very important part of soil microbiome and responsible for maintaining soil-plant interactions and plant productivity. Similar study was also carried by many researchers, who have suggested the presence of many rhizosphere fungi associated with different medicinal plants and also in different ecosystem across the world (Jena *et al.*, 2015; Caruso *et al.*, 2020; Mir *et al.* 2017). The diversity of fungi associated with the rhizospheric soil

of different host plant growing at different locations also differs (Morgan *et al.*, 2005).

The findings of the present investigation are also supported by Khuseib *et al.* (2020) and Ahmad *et al.* (2021) who have reported the occurrence fungal species in the rhizosphere of different medicinal plants.

The rhizosphere of plants plays a vital role in maintaining the plant's health and enhancing its growth. The diversity and abundance of microorganisms, including fungi, in this microenvironment are influenced by a complex interplay of plant exudates, soil properties, and microbial interactions.

The results revealed a significant variation in fungal community composition across the medicinal plants. The rhizospheric fungi help the plant growth promotion through nutrient cycling, nutrient uptake and protect the plant from biotic and abiotic stresses. (Li *et al.*, 2018).

The rhizospheric fungi, are helpful to perform many useful and diverse functional roles in soil ecosystems. *Penicillium* and *Aspergillus*, both are very frequently associated in the rhizosphere. These fungi have ability to produce secondary metabolites with antimicrobial properties, which can protect plants from pathogens (Nguyen *et al.*, 2016). Alkaline pH of soil is favourable for the growth of rhizospheric fungi in plants.

Overall, this study highlights the intricate relationships between soil properties, plant exudates, and fungal communities in the rhizosphere of medicinal plants. It underscores the importance of maintaining diverse and healthy microbial populations to promote plant growth, disease resistance, and the production of bioactive compounds. It is the need to understand the specific interactions between fungal species and plant metabolites, and how these relationships can be leveraged to enhance the cultivation and medicinal properties of plants.

Ultimately, this study reinforces the notion that rhizosphere fungal community structure is highly host genotype specific, driven to a great extent by the unique chemical makeup of root exudates (Walker *et al.*, 2003). This native host-specific fungal population holds great promise as a tool in the advancement of sustainable agricultural practices of medicinal plants.

CONCLUSION

In our study total eight fungal species were associated with the rhizosphere in selected medicinal plants of Rewa District, MP, India. *Ocimum sanctum* with the highest fungal diversity and minimum in *A. officinalis*. Prevalence of *Aspergillus*, *Fusarium*, and *Penicillium* sp. indicates their versatility in the plant rhizosphere and their activities in soil fertility and plant well-being. *Penicillium* indicates beneficial fungi with probable agricultural uses, and total overall fungal diversity indicates an active role in nutrient cycling and plant defense. Analysis of such fungal communities will improve the cultivation of medicinal plants, soil health, and sustainable agriculture. The native rhizospheric fungi can be used as beneficial bioinoculants to enhance the growth of medicinal plants and production of bioactive compounds without very sophisticated biotechnological processes. Future studies will require investigating their functional roles in plant development and secondary metabolite production.

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