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Fungal Biodiversity In The Rhizosphere Arena Of Medicinal Plant

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Abstract:
The rhizosphere of medicinal plants hosts a complex and diverse microbial community, including fungi that significantly influence plant health, growth, and secondary metabolite production. This study investigated the fungal biodiversity in the rhizosphere soils of ten medicinal plants at Barkatullah University campus analyzing soil physico-chemical properties and fungal diversity. Soil samples exhibited alkaline pH (7.9–9.14) and loamy sand texture, favoring diverse fungal communities. Among 96 fungal morphotypes isolated, Ascomycota dominated, followed by Mucoromycota, Basidiomycota, and Oomycota, with Penicillium, Aspergillus, and Rhizopus as the prevalent genera. Diversity indices revealed that Argyreia nervosa exhibited the highest fungal diversity. Soil parameters like moisture content, pH, and clay content showed strong correlations with fungal genera distribution. Secondary metabolites and volatile organic compounds produced by medicinal plants further influenced fungal community composition. This study underscores the importance of rhizosphere fungi in enhancing plant health and secondary metabolite biosynthesis, suggesting potential applications in medicinal plant cultivation and improving their therapeutic properties.
Keywords: *Rhizosphere Fungi, Secondary Metabolites, Medicinal Plants*

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I. Introduction:

The rhizosphere, a narrow zone, adjacent to and influenced by living plant roots is the hot spot of microorganisms and the site of highest microbial activity as well as constitutes complex ecosystem on earth (Sorenson 1997, Kennedy 1999, Hinsinger and Marshner 2006, Pierret et al., 2007, Jones and Hinsinger2008, Hinsingeret al., 2009, Raaijmakers et al., 2009). Rhizosphere microbiome is diverse and enormous (Torsvik et al., 1990) and may be different from bulk soil (Reinhold Hurek et al., 2015). The rhizosphere microorganisms have beneficial, neutral, or detrimental effects on the plant growth and play a major role in maintaining root health, nutrient uptake, and tolerance to environmental stresses, act as biocontrol agents and affect the constituents of plants (Campbell and Greaves 1990, Boehm et al., 1993, Bowen and Rovira 1999, Lemanceau et al., 2017, Reinhold Hurek et al., 2015, Vega –Avila et al., 2015, Gallart et al., 2018, Shaikh and Mokat 2018). The plant and microbes together form holobiont (Liu et al., 2018) which use and compete for different nutrient

The plant rhizosphere is the zone where the roots interact with the surrounding soil environment. As described by Philippot (2013), this region is home to a diverse community of microorganisms and invertebrates that influence plants through direct and indirect interactions. Plants shape the microbial composition of the rhizosphere by releasing root exudates and producing root litter (Veen et.al,2019; Broeckling et.al,2020)

Fungi are one of the most significant and abundant microbial groups in the rhizosphere, playing a key role in carbon and nutrient cycling in ecosystems. Scientific research has uncovered the roles of many fungal species, and new tools have been developed to better understand their function in ecosystems (Nguyen et.al. 2016). Many modern drugs are derived from botanical sources, natural products, or their derivatives, often coming from specific plant parts (De Smet et.al 1994; Newman et.al, 2020). Interestingly, some of these medicinal compounds are not produced by the plants themselves, but by microorganisms in their microbiome.

Medicinal plants can also influence the abundance of pathogenic fungi in the soil and their effects on newly rooted plants. For example, a study by Li et al. (2018) demonstrated that *Atractylodeslancea* has suppressive effects on *Fusarium*. Due to findings like these, several previous studies have explored the fungal rhizosphere communities of various medicinal plants. In 2020, (Abdul Latif Khan et al.) examined the rhizosphere fungal communities of *Adenium obesum*, *Aloe dhufarensis*, and *Cleome austroarabica* , noting a high relative abundance of the phylum *Ascomycota*. The genus *Acremonium* was found to be relatively abundant in the rhizospheres of *A. obesum* and *A. dhufarensis*, while the genus *Corynascus* was more prevalent in the rhizosphere of *C. austroarabica*. Another study, conducted by Villalobos-Flores et al. in (2021), described the rhizosphere fungal community of the medicinal plant *Bouvardia ternifolia*, which showed a high relative abundance of the class *Sordariomycetes* .

The collected soil samples from the rhizospheres of ten different medicinal plants: *Azadirachta indica, Butea monosperma, Cordia dichotoma, Gmelina arborea, Aegle marmelos, Tinospora cordifolia,Argyreia nervosa, Asparagus racemosus,Ocimum sanctum and Celosia argentea* .The study follows on the impact these plants have on the composition of fungal communities in their rhizospheres.

II. Material And Methods:

Study Area:

The present study was conducted at Barkatullah University located in tehsil Huzur of District Bhopal (Madhya Pradesh). Geographically Barkatullah University (formerly Bhopal University) is positioned at latitude 23° 12' 01.83" N and longitude 77° 27' 12.50" E.

Table 1: Properties of selected medicinal plant for the study

S.No.	Medicinal plant	Common Name	Family	Medicinal Property	Isolate code
1	*Azadirachta indica*	Neem	Meliaceae	Antiseptic,ulcer ,smallpox	Ai
2	*Butea monosperma*	Palash	Fabaceae	diuretic activity, anthelmintic	Bm
3	*Cordia dichotoma*	Lasora	Boraginaceae	astringent	Cd
4	*Gmelina arborea*	Khamer	Verbenacea	laxative,stomachic	Ga
5	*Aegle marmelos*	Bael	Rutaceae	anticancer,antimicrobial	Am
6	*Tinospora cordifolia*	Gurbel	Menispermaceae	antimicrobial,anti-osteoporotic,anti allergic ,anti inflammatory	Tc
7	*Argyreia nervosa*	Vidhara	Convolvulaceae	antiseptic,emollient	An
8	*Asparagus racemosus*	Shatavari	Liliaceae	Dyspepsia,bronchitis,	Am
9	*Ocimum sanctum*	Basil	Lamiaceae	anti-inflammatory	Os
10	*Celosia argentea*	Shitivarak	Amaranthaceae	antibacterial,antidiabetic	Ca

Rhizosphere soil sample collection and Analysis:

Rhizosphere soil from the 50 root zone samples was collected up to 11-15 cm depth from each medicinal plant by random sampling method. The physico-chemical properties of all soil samples were analyzed following established protocols (Applebaum,et.al 2022).Soil moisture (%) content was determined gravimetrically by drying soil samples for 24 h at 105 ◦C. Soil pH was determined was measured using pH meter((HI96107 Hannia instruments). Electrical conductivity (as dS* cm^{-1}) was determined as an assessment of soil salinity. Electrical conductivity of the supernatant was determined by an auto-ranging EC/temp.

Isolation of Fungi from rhizospheric soil:

The Rhizosphere soil sample was seriallydiluted in the sterilized water under the sterilized conditions Serial dilutions were made upto 10^{-4} and 0.1ml of each dilution was spread on the Potato Dextrose Agar (PDA). Morphologically different fungal colonies were offer 7 days of incubation on the different plate to maintain the pure culture at 4°C (Cappuccino and Sherman ,2014).

Morphological Identification of Fungi:

Morphological identification was made using both macroscopic and microscopic features. For macroscopic characteristics, the growth pattern, pigmentation and colony appearance were observed during the incubationperiod for identification of fungi. Lactophenol cotton blue dye was used for spore staining. Features such as hyphal type, septate or aseptate features, spores type, sporangia, conidia, and arrangement of sporangiophores and conidiophores were observed through microscope. The fungal genera were identified by using various manuals andmonographs *(*Ellis,1971; Sutton, 1980; Barnett and Hunter, 1987; Domschand Games, 1993; NagRaj, 1993).

Statistical Analysis:

The frequency of fungal harbored in rhizosphere of medicinal plant species were calculated by (Cappuccino and Sherman ,2014).

$$CFUg^{-1} = \frac{No.of\ coloniesXdilution\ factor}{Dry\ weight\ of\ soil\ taken}$$

Diversity indices

Determined a dominant taxon amongst isolated morphotypes from the equations below were used to calculate the Shannon-Wiener index (*H'*), Margalef index(*dM*), Simpson index (*D*), and Pielou evenness index (*J)* (Kausari et al.,2013)

Where *Pi* is the$H1=-\sum i=1SPi\mathrm{In}Pi$

$D=-\sum i=1SPi2$

Isolates number (*Ni*) of a specific taxon divided by the total isolates number (*N*). For a taxon to be considered dominant, *Pi* should exceed Camargo's index (*1/S*) (Kausari et al.,2013; Camargo,1992). The number of fungal taxa is represented by *S*, and *H'* represents the Shannon-Wiener index, and Simpson index (*D*)

III. Results:

Determination of physico-chemical parameters of soil.

The physico-chemical characteristics of the soil of study area of Barkatullah University campus, edaphic characteristics of rhiziosphere soil samples of selected medicinal plants indicated that the soil pH was alkaline (pH 7.9 and 9.14). The soil texture across the plants is predominantly loamy sand, which offers good drainage while retaining adequate moisture, a condition that supports a wide range of fungal species. Moisture content varies significantly, with *Argyreia nervosa* having the highest moisture content (85%) and *Asparagus racemosus* the lowest (20%). This variance affects the fungal diversity, as fungi like Rhizopus and Mucor thrive in moist soils..Clay and sand content vary, with *Tinospora cordifolia* having the lowest clay content (10%) and *Ocimum sanctum* the highest sand content (50%). These soil properties impact water retention and fungal distribution, as fungal growth is often associated with soil's ability to retain water.

Table 2. Physio-Chemical attributes of soils collected from the different rhizospheres of ten plants.

S.No.	Parameters	Am	Tc	Ga	Ai	Bm	Cd	Ca	Ar	Os	An
1	Temperature (C)	19	20.5	19.5	20.1	20	21.5	20.4	20	19	18.5
2	Moisture Content (%)	50	62	72	32	59	45	25	20	55	85
3	Clay (%)	25	10	15	10	12	8	15	11	16	20
4	Sand (%)	40	25	45	30	40	50	35	20	35	25
5	Silt (%)	30	25	30	30	18	30	20	74	15	10
6	Gravel (%)	0	0	15	0	0	10	0	0	0	0
7	Texture	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil	Loamy sand soil
8	EC (dS/m)	0.51	0.89	1.24	0.97	0.32	0.68	0.52	0.38	0.85	0.96
9	pH	8.7	8.2	7.9	8	9.14	8.3	8.5	8.5	8.6	8

Population Density of Soil Fungi:

Enumerated population density of fungi in the rhizosphere soils of the university campus in this study, the average density of the fungi (14×10^5CFUg^{-1} dry soil) in *Ceosia argentea* and *Azadirachta indica* was higher followed by the average density of *Asparagus racemosus* (average 13×10^5 CFUg^{-1} dry soil). Least results were obtained when evaluating the bacterial population density in medicinal plant *Argyria nervosa,* with average value of 4×10^5 CFUg^{1} dry soil.

Table 3: Population density of fungi isolates per dry weight of rhizosphere soil of medicial plants.

S.No.	Isolatecode	Fungal density (CFUg^{-1})
1	Aif	10×10^5
2	Bmf	8×10^5
3	Cdf	9×10^5
4	Gaf	12×10^5
5	Amf	6×10^5
6	Tcf	7×10^5
7	Anf	16×10^5
8	Arf	13×10^5
9	Osf	11×10^5
10	Caf	14×10^5

The total of 96 fungal morphotypes were isolated from 10 medicinal plants. The isolates were classified into four phyla: Ascomycota (dominant), Mucoromycota, Basidiomycota, and Oomycota. The dominance of the Ascomycota phylum in the rhizosphere soil of medicinal plants is significant due to the ecological roles these fungi play in plant growth, health, and the production of bioactive compounds. Many Ascomycota species are saprophytic fungi that break down organic matter in the soil, enriching the soil with essential nutrients and improving soil structure. This process aids in the degradation of plant residues and organic matter, making nutrients more available for plant uptake. Morphological characterization revealed 17 distinct fungal taxa. The predominant genera identified among the isolates were *Mucor*, *Aspergillus*, *Penicillium*, and *Rhizopus*, while genera such as *Sclerotium* and *Emericella* were found at minimal frequencies. Fungal isolates that could not be identified to a specific genus were designated as "unidentified.

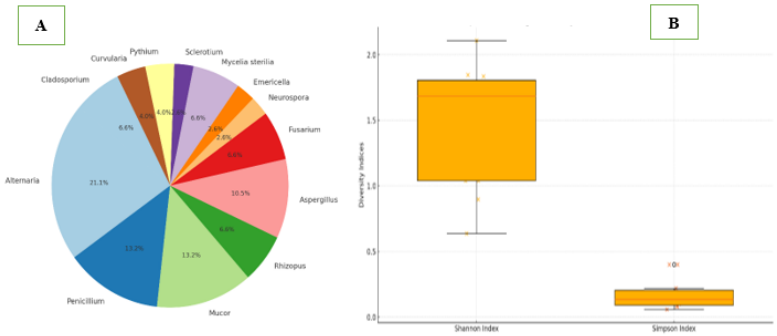

Fig.1 A) Percent abundance of fungal genera in rhizosphere of different medicinal plants. B) Comparison of fungal diversity indices across medicinal plants

The pie chart representing the distribution of fungal genera across the rhizospheres of medicinal plants. The chart visualizes the relative abundance of various fungal genera, with *Aspergillus* (20.9%), *Mucor* (14.5%), and *Rhizopus (*9.1%) being the most abundant genera. Host medicinal plants produce a wide variety of volatile organic compounds that are released into the soil. These compounds can serve multiple purposes, including attracting beneficial microorganisms (such as fungi) and repelling harmful pathogens. Compounds like acetaldehyde, acetone, and ethanol are commonly produced in plant roots and rhizosphere soils, attracting beneficial fungi like *Penicillium* or *Aspergillus.* The diversity indices calculated, such as the Shannon-Wiener index, revealed a high diversity in plants like *Argyreia nervosa*, with a maximum Shannon value of 2.10. The Simpson Index values supported the Shannon index results, with the highest value of 0.93 found in *Argyreia nervosa*. The value of D will always fall between 0 and 1, where 1 represents complete diversity and 0 represent complete uniformity, therefore *Argyreia nervosa* appears to be more diverse in genus abundance than other nine medicinal plant and lowest was recorded in *Butea monospermea*(0.86), within the habitat of ten medicinal plants .

Table 4 Barkatullah University distribution abundance of morphotypes in rhizosphere of medicinal plants

S.No.	Genus	Medicinal plant										Total morphotypes
		Bm	Ga	Ca	Tc	Ai	Ar	Cd	An	Os	Am	
1	*Aspergillus*	3	8	3	1	0	3	0	3	0	2	23
2	*Mucor*	0	1	2	1	2	2	2	1	2	3	16
3	*Penicillium*	6	1	0	1	0	0	0	0	0	0	8
4	*Alternaria*	1	0	1	0	0	0	0	1	0	0	3
5	*Rhizopus*	0	2	1	3	1	0	1	2	0	0	10
6	*Cladosporium*	0	1	1	0	0	0	0	0	1	0	3
7	*Pythium*	0	0	1	0	0	0	0	1	0	0	2
8	*Fusarium*	0	2	0	0	0	2	0	0	1	0	5
9	*Sclerotium*	0	0	0	0	1	0	1	0	0	0	2
10	*Emericella*	0	0	0	0	0	0	0	2	0	0	2
11	*Curvalaria*	0	0	1	0	0	0	0	1	0	0	2
12	*Neurospora*	0	0	0	0	1	0	0	0	0	0	1
13	Mycelia sterlia	0	1	0	1	1	2	0	0	0	0	5
14	*Chaetomium*	0	0	0	0	0	1	0	0	0	0	1
15	*trichophyton*	0	1	0	2	3	1	0	2	0	0	9
16	*Sporothrix*	0	0	0	0	0	0	0	0	0	1	1
17	*unidentified*	0	0	0	0	2	0	0	1	0	0	3
	Total	10	17	10	9	11	11	4	14	4	6	96

Table 5. Morphological characterization of fungi isolates from rhizosphere soil of medicinal plants selected for the study *(***Source:** Ellis, 1971; Sutton, 1980; Barnett and Hunter, 1987; Domsch and Games, 1993;Nag Raj, 1993**).**

S.No	Isolate Code	Colony Morphology	Reverse Colony Color	Microscopic Observations	Probable Organism
1	Bmf1, Bmf5, Bmf6, Bmf8, Gaf5,Tcf1	Colonies are initially white and become dark green or blue green with white periphery	White	Conidia are globuse, greenish and smooth. Septate hyphae, ovate spores and conidial heads composed of continual conidia. Conidiophores show branching, phialides produced in groups from branched metulae, giving brush-like appearance.	*Penicillium sp.*
2	BmBf2.Tcf3	Green, powdery	Light green	Transversely and longitudinally septate (muriform), beaked conidia in acropetal manner.	*Alternaria*
3	BmBf3, BmBf7, GaBf6, GaBf7, GaBf10, GaBf11, GaCf1, GaCf2, GaCf3, GaCf5, TcBf4, CaBf2, CaBf3, CaBf8,Tcf2	Greenish blue, black or green colonies	White	Conidia were small, black, brownish black, green in color. Septate hyphae with rough brown and smooth colorless conidiophores with distinctive conidial heads (flask-shaped).	*Aspergillus sp.*
4	GaBf3, Gabf5	Wooly, white to pink	Red	Extensive septate mycelium and conidiophores in the aerial mycelia were mostly short branched. Macroconidia were formed straight, rare and falcate with 2-3 septate per conidium.	*Fusarium oxysporum*
5	GaBf8, TcBf2, TcBf5, TcBf6, CaBf10	Colonies initially white and become dark gray	White	Non-septate mycelium with root-like rhizoids; black columellate, sporangiophores in clusters.	*Rhizopus sp.*
6	GaBf2, GaBf4, TcBf1, TcBf3, TcBf7, TcBf8, TcBf10, CaBf5, CaBf7, CaBf9	White to dark grey	White	Non-septate mycelium without rhizoids; single columellate sporangiophores.	*Mucor*
7	CaBf1	Greenish black and powdery	Black	Branch conidiophores, conidia variable.	*Cladosporium*
8	CaBf4	Velvety black, fluffy growth on the colony surface	Black	Septate, dematiaceous hyphae producing brown, geniculate conidiophores. The poroconidia are curved slightly to distinctly, transversely septate, with an expanded third cell from the pore of the conidium.	*Curvularia*
9	CaBf6	White	White	Non-septate hyphae, sporangia producing zoospores, oogonia, antheridia, and thick-walled oospores.	*Pythium*
10	Anf11	White	White	Sclerotia are dense, intertwined hyphae with a thick, pigmented outer rind; serves as a survival structure	*Sclerotium*
11	Anf13	White	White	16-spored asci, large, stellate, violet-brown ascospores and lacking an anamorph.	*Emericella*
12	Amf3, Tcf1, Osf2	White	White	Characteristic striations on the spores that resemble axons.	*Neurospora*
13	Ar1, Ai6, Tc4	White	White	Mycelia sterilia are a group of fungi that do not produce any known spores, either sexual or asexual, only hyphae present.	*Mycelia sterilia*
14	Ar2	White	White	conidia are usually globose to ellipsoidal, and they are typically brown or dark in color.The conidia are often produced in clusters at the tips of the conidiophores, and the conidial heads appear to be flask-shaped.The conidia may have 1–3 septa ,giving them a segmented appearance.	*Chaetomium*
15	Ga9 ,Ai11,Ar4,Cd6	white	Lemon yellow	The presence of septate hyphae, macroconidia (cylindrical with septa), and microconidia (tear-shaped), along with the ability to produce arthroconidia and chlamydospores	*Ttichophyton*
16	Amf5	Colonies initially white and become brown	off	The daisy-chain conidia formation and smooth-walled conidia are distinctive microscopic features in the mold form of the fungus.	*Sporothrix*

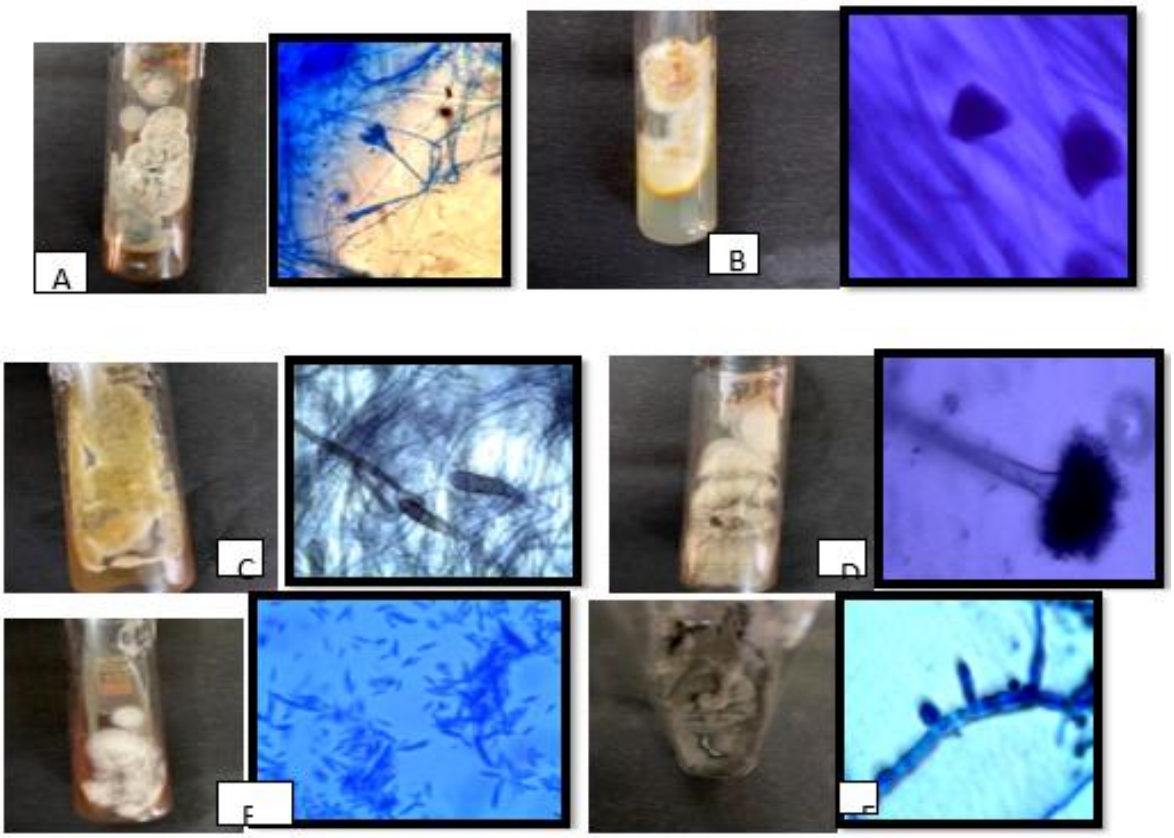

Fig 2: Microphotographs of morphologically identified fungal genus under Lightmicroscope at 40X magnification (A-*Penicillium* sp,B- *Mucor C- Alternaria* sp D *Aspergillus* E *Fusarium* sp,F-*Cladosporium)*

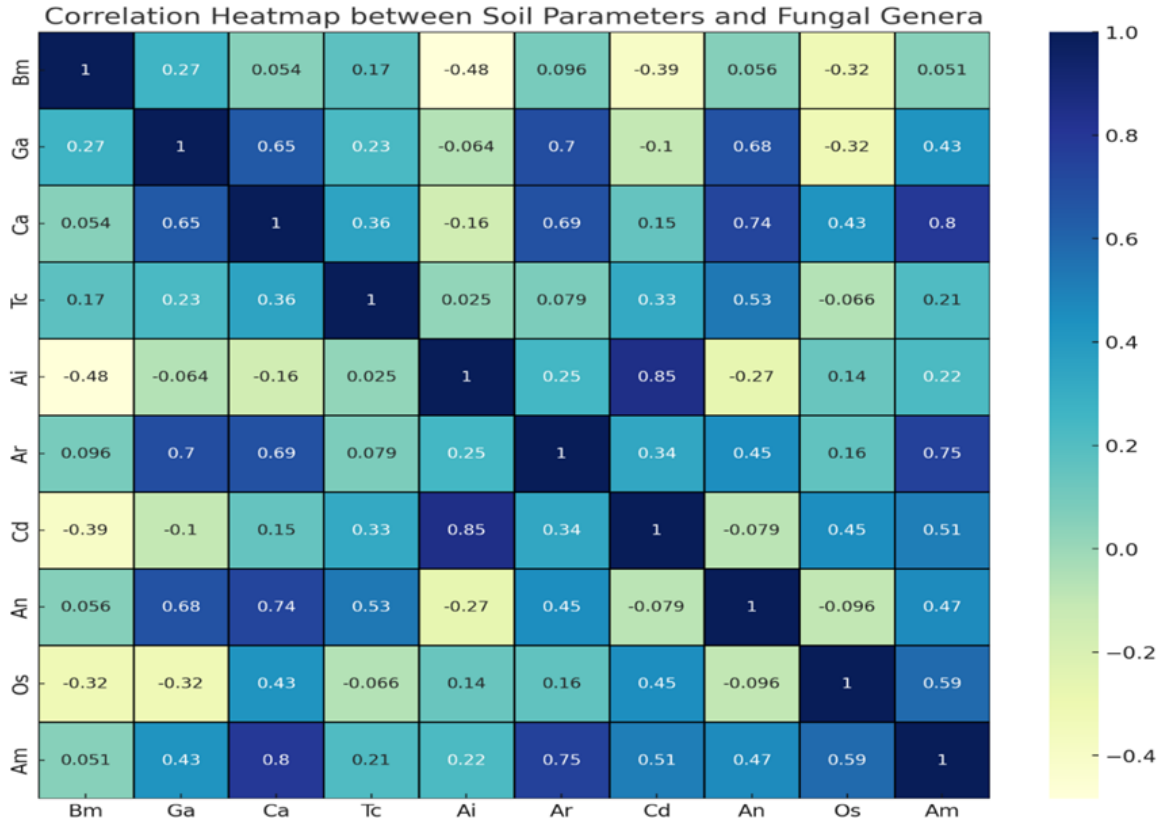

Fig 3 Heatmap depicting the correlation between soil parameters and fungal genera abundance across different medicinal plants.

The heatmap provides a visual representation of the relationships between the soil parameters (e.g., pH, moisture content, clay, sand, etc.) and the abundance of fungal genera.Lighter colors indicate lower values(negative correlation), while darker colors (toward the blue side) indicate higher values ,positive correlation. Fungal genera like *Penicillium* (Bm) and Aspergillus (Ga) show notable positive correlations with Clay (%) and pH.*Fusarium* (Ai) shows a strong positive correlation with Silt (%) and Clay (%), as well as EC (dS/m). Alternaria (Ar) has a positive relationship with Moisture Content (%) and Clay (%)The pH value for each plant species is seen to correlate strongly with certain genera, particularly those more tolerant to alkaline conditions, like *Penicillium* and *Alternaria*.The moisture content and other soil properties also show varying correlations with the abundance of specific fungi, such as *Mucor*, which tends to thrive in more moist soils could contribute to nutrient cycling and plant health by breaking down organic materials. These fungi can influence plant defense mechanisms and enhance the plant's ability to produce secondary metabolites, such as antioxidants and antimicrobial compounds.

IV. Discussion:

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 study is, focused on the diversity of fungi and relationship with soil physicochemical parameters.

The results revealed a significant variation in fungal community composition across the medicinal plants studied. Among the 96 fungal morphotypes isolated, Ascomycota was found to be the dominant phylum, followed by Mucoromycota, Basidiomycota, and Oomycota. The predominance of Ascomycota could be due to its versatile ecological roles, particularly in breaking down organic matter, thus enriching the soil and making nutrients more available for plant uptake.This finding corroborates previous studies by Khan et al. (2020) and Villalobos-Flores et al. (2021), where Ascomycota was the predominant fungal group in the rhizosphere of medicinal plants. Ascomycota includes a wide range of saprophytic, pathogenic, and symbiotic fungi, which play essential roles in nutrient cycling, plant growth promotion, and disease suppression (Liu et al., 2018).

The fungal genera identified, such as *Penicillium*, *Aspergillus*, *Rhizopus*, and *Mucor*, are known for their diverse functional roles in soil ecosystems. *Penicillium* and *Aspergillus*, both dominant in the rhizosphere, are recognized for their ability to produce secondary metabolites with antimicrobial properties, which can protect plants from pathogens (Nguyen et al., 2016). The abundance of *Mucor* and *Rhizopus*, which thrive in moist conditions, further emphasizes the importance of soil moisture content in shaping fungal communities, as evidenced by the higher moisture content observed in *Celosia argentea* (85%) and *Argyreia nervosa* (70%).In line with this, the study found a positive correlation between soil moisture and the abundance of fungi like *Mucor* and *Rhizopus*, which are well adapted to moist environments. Soil pH also emerged as a crucial determinant of fungal diversity, with alkaline conditions (pH ranging from 7.9 to 9.14) correlating with higher abundances of *Penicillium* and *Alternaria*. This finding suggests that fungi tolerant to alkaline conditions are more likely to thrive in rhizospheres with high pH, which is common in the study area.

Furthermore, the diversity indices calculated, including the Shannon-Wiener and Simpson indices, highlighted significant variability in fungal diversity across the studied plants. The Shannon-Wiener index, which measures diversity, was highest in *Argyreia nervosa* (2.10), indicating that this plant's rhizosphere supports a more diverse fungal community compared to others, such as *Butea monosperma*, which had the lowest diversity index (0.86). This suggests that certain plants, like *Argyreia nervosa*, may create a more favorable environment for a wide range of fungal species, possibly due to their root exudates and nutrient availability.

The heatmap analysis further supports the finding that soil parameters, particularly pH, moisture content, and soil texture, have a significant influence on fungal genera distribution. Fungal genera like *Penicillium* and *Aspergillus* were positively correlated with clay content and pH, while *Fusarium* showed a strong positive correlation with silt content and electrical conductivity (EC). This reinforces the idea that soil texture and chemical properties play a key role in structuring the fungal microbiome in plant rhizospheres.

Additionally, the role of secondary metabolites produced by medicinal plants cannot be understated. These metabolites, which include volatile organic compounds (VOCs), influence the microbial community by attracting beneficial microorganisms while deterring pathogenic ones. The VOCs released from the roots of medicinal plants can also play a signaling role, guiding fungal growth towards plant roots. This dynamic interaction between the plants' metabolic outputs and the fungal microbiome is a key aspect of plant-microbe mutualism, contributing to plant health and enhancing secondary metabolite production.

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.

V. Conclusion:

The fungal attribute contributes to plant health and secondary metabolite production. By understanding the relationship between plant-microbe interactions and soil conditions, we can enhance the cultivation of medicinal plants and their medicinal properties

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