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ISOLATION AND IDENTIFICATION OF FUNGI FROM RHIZOSPHERIC SOIL SAMPLE OF COMMON SELECTED MEDICINAL PLANTS FROM BETUL

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ABSTRACT:

This research is based on isolation and identification of fungi from the Rhizospheric soil of different medicinal plants. Rhizospheric soil samples, used for the study were collected from Betul. A total number of sixteen fungi were isolated from rhizospheric soil of different medicinal plants. In this paper, the isolated and identified fungi are Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus oryzae, Aspergillus varsicolor, Rhizopus oryzae, Tricophyton rubrum, Microsporum gypsum. Aspergillus fumigates and Aspergillus Niger had the highest percentage frequency of occurrences. Rhizopus oryzae and Rhizopus stolonifera had the lowest percentage frequency of occurrence respectively. Conclusively Inspite of immense amount of work already accomplished in the investigating the micro flora of the rhizospheric soil it must be admitted that no one has yet been able to give a clear picture of all life in the soil and of all interaction of different groups of living things. Rhizospheric soil ecology under the natural condition of different fungi from different medicinal plants are needed and will required collaboration between plant biologists, ecologists, and soil scientist to develop the rhizotron system.

KEYWORDS:

A. niger: Aspergillusniger, A. flavus: Aspergillus flavus, A. fumigatus: Aspergillus fumigatus, A. nidulans: Aspergillus nidulans, A. oryzae: Aspergillus oryzae, A. varsicolor: Aspergillus varsicolor, R. oryzae: Rhizopus oryzae, T. rubrum: Tricophyton rubrum, M. gypseum: Microsporum gypseum.

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INTRODUCTION:

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesise hundreds of chemical compounds for functions including defence against insects, fungi, diseases, been placed on the treatment rather than prevention of diseases. However, there exists in the literature considerable report in recent times on research work on the use of medicinal plants and their constituents in disease prevention. A World Health Organisation (WHO) Expert Group defined Traditional Medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing.

Betul is a part of Madhya Pradesh in India and is a famous place, which has a moderate climate.

The rhizospheric is the soil zone around the roots in which microbial biomass is impacted by the presence of plant roots. The ability of the rhizosphere to stimulate microbial activity has been long known. The rhizosphere, which is the volume of soil surrounding the plant root, is influenced by root activities such as exudation of reactive carbon compounds and uptake of mobile nutrients and water. Roots have evolved to adapt to their surrounding environment by optimizing their functional architecture to use resources in heterogeneous soils. Thus, the co-evolution of rhizosphere and plant roots play a major role in soil physical, chemical, and biological processes that sustain biodiversity, provide soil carbon sequestration, and cycle nutrients in natural and agricultural systems.

The indigenous fungal isolates obtained from the rhizosphere soil can be used as they are said to solubilize the insoluble zinc, phosphorous, potassium etc. They are known to control the different fungal pathogens and thus promoting the plant growth and health. The microorganisms that are predominantly present in the rhizosphere have been shown to play a role in the transport of mineral nutrients, secretion of secondary metabolites, and mitigation of abiotic and biotic stresses. During microbial association with the host plants, bacteria and fungi produce various extracellular enzymes that convert the macromolecules into transportable simpler products that can be distributed throughout the plant cells. In addition to the initiation of the host-symbiosis process, some of these exozymes hinder the plant pathogenic infections and boost abiotic stress tolerance. The plant, on the other hand, facilitates a suitable niche for distinct microbes to grow and reproduce while mutually sharing beneficial exudates and nutrients. Such interactions between the microbial communities and medicinal plants have been minimally investigated, particularly in arid ecosystems.

OBJECTIVES OF PROPOSED WORK:

1) Isolation of fungi from rhizospheric soil sample of common selected medicinal plants from Betul.

2) Identification of fungi from Rhizospheric soil sample of common selected medicinal plants from Betul.

REVIEW OF LITERATURE:

Pharmaceutical Biology

Caroline Arruda, Mohamed Abd El-Salam, Jairo Kenupp Bastos

Inflammatory disorders are common in modern life, and medicinal plants provide an interesting source for new compounds bearing anti-inflammatory properties. In this regard,

Brazilian medicinal plants are considered to be a promising supply of such compounds due to their great biodiversity.

To undertake a review on Brazilian medicinal plants with corroborated anti-inflammatory activities by selecting data from the literature reporting the efficacy of plants used in folk medicine anti-inflammatory, including the mechanisms of action of their extracts and isolated compounds.

International Scholarly Research Notices 2010

S Jayasurya Kingsley, Emmanuel S Sathish, D Devapriya

Hexane, ethyl acetate, ethanol and methanol extracts of Psidium guajava, Terminalia chebula, Mimusopselengi and Achyranthes aspera were tested against the dental caries causing bacteria Streptococcus mutans and fungus Candida albicans isolated from caries infected patients. All the four extracts of P. guajava showed activity against both S. mutans and C. albicans. Maximum zone of inhibition was observed in ethyl acetate of P. guajava. The four extracts of T. chebula and M. elengi showed antibacterial activity against S. mutans. M. elengi extracts and ethanol extract of T. chebula did not show any antifungal activity against C. albicans. Except for the hexane extract of A. aspera, the other three extracts showed activity against the tested microbes. The ethyl acetate P. guajava leaf extract showed the minimum inhibitory concentration (MIC) against S. mutans to be <0.076 mg/ml in both MHB and BHI. The P. guajava ethyl acetate extract was subjected to GC-MS.

RevistaBrasileira de Farmacognosia, Alyne MR de Carvalho, Francisca CF Sousa

Medicinal plants have been used in traditional medicine for several thousand years all over the world. In this sense, information from Brazilian ethnic groups on folk medicine has contributed to the discovery of pharmacological activities from various plant-derived agents potentially leading to the innovative drugs. The Caatinga (semi-arid) vegetation is a highly threatened biome, covering a vast area in north eastern Brazil and has suffered from strong human influence for many decades. Many plants species found in the Caatinga have been widely used in folk medicine and for commercial manufacturing of phytotherapeutic products. Thus, the present review aims to disseminate to the scientific community some known species of medicinal plants found in the Caatinga that have been studied and analyzed in pharmacological scientific assays. Among the species that stood out for their local importance and multiplicity of uses were: Amburana cearensis (umburana-de-cheiro), Anadenanthera colubrina (Vell.) Brenan (angico-branco), Anacardium occidentalis L. (cajueiro), Bauhinia forficata Link (mororó), Cissus sicyoides L. (insulina-vegetal), Myracrodruonurundeuva Allemão (aroeira-do-sertão) and Zingiber officinalis L. (gengibre). The present study shows that several herbal constituents from Caatinga plants, whose pharmacological actions have been well characterized, may be relevant candidates for future and innovative therapeutic development.

MATERIAL AND METHODOLOGY:

Sample Collection:

Collection of 20 Samples from 20 Different Place of Betul Madhyapradesh. Details are given below:

S.NO	SAMPLE NO.	SAMPLE AREA
1.	AS01	Khedi
2.	AS02	Ranipur
3.	AS03	Dharakhoh
4.	AS04	Betul Bazar
5.	AS05	Bhadush
6.	AS06	Hamalapur
7.	AS07	Gauthana
8.	AS08	Jamathi
9.	AS09	Bharatbharti
10.	AS10	Sonaghati

S.NO	SAMPLE NO.	SAMPLE AREA
11	AS11	Badora
12	AS12	Sohagpur
13	AS13	Khedala
14	AS14	Padhar
15	AS15	Sihari
16	AS16	Aamdhana
17	AS17	Mahadgav
18	AS18	Umari
19	AS19	Malakpur
20	AS20	Ronda

Sterilization of glassware

All the glass wares were first soaked and washed thoroughly with tap water and detergent solution and then rinsed with several changes of distilled water in order to completely remove traces of detergent and air dried completely before sterilizing them in steam oven at temperature of 121°C for 15minutes and then allowed to cool down at room temperature before usage. The entire working surface was also disinfected with ethanol to reduce contamination.

Materials and reagents

The material and reagents used in the laboratory for this research work includes; Potato dextrose agar (PDA), ethanol, Petri dishes, conical flasks, syringes, cotton wool, lacto phenol cotton blue, sterilized knife, glass rod, test tubes, streptomycin, nutrient agar, aluminum foil, masking tape, distilled water, face mask, hand gloves, filter paper, microscope, Cork borer, slide and cover slip.

For isolation, fungal media with antibiotics should be used to suppress bacterial contamination. **Sabouraud's dextrose agar and potato-dextrose agar** are commonly used media. Cultures are routinely incubated at 25° to 30° C for up to 4 weeks. Isolation of zygomycetous fungi can be difficult.

Procedure:
Sterile slide—> add the molten agar and allow to solidify —> cut the material making two half —-> place cover slip ——> seal the coverslip with wax or petroleum jelly making small area free at the side if cut —-> buried in a soil gently in a tray à allowed to incubate for few days ——-> remove gently ——> remove coverslip and observe under microscope.

Isolation of fungi

1. The sample is diluted serially by 10^{-1} by using sterile distilled water.

2. The diluted sample is taken in the micropipette and pours on PDA Agar (Potato Dextrose Agar plate).

3. Incubate the plate on 24°C for 2 to 4 days. Incubate the same plate on 24°C for 2 to 4 days.

4. Then, Observe the colonies of fungi on the PDA plate

Direct Plating Method: -
In direct plating, fungi are placed directly on solidified SDA media. In most situations, particles should be surface disinfected before plating, as this removes the inevitable surface contamination arising from dust and other sources, and permits recovery of the fungi actually growing in the media. This process provides an effective measure of inherent fungi quality. Surface disinfection should be omitted where surface contaminants become part of the downstream fungi. Even here, surface disinfection before direct plating provides the most realistic appraisal. Results from direct plating analyses are expressed for fungi. The technique provides no direct indication of the extent of fungal invasion in individual. However, it is reasonable to assume that a high percentage infection is correlated with extensive invasion in the fungi.

Soil dilution plating:

- If hyphen cannot be dissected from field material for identification, they must be induced to grow cut as visible colonies onto an artificial culture medium.
- The dilution plate method, in which a dilution series is prepared from soil suspension and selected dilution incorporated in an agar medium (PDA-SDA).
- After few days of incubation, colonies will appear in varying densities and the number of spores present in the original sample can be calculated

Cover slip culture technique for fungi identification:

- This technique is simple, less time-consuming technique which produces high quality permanent mounts and is suitable for clinical isolate identification, student teaching, examination of fungi at different stages of their development without disturbing the arrangement of spores and hyphal structure.

- It is advantageous over slide culture technique that if the first preparation fails to demonstrate adequate sporulation, there are still left to be examined of weekly intervals.
- The ability of aerial mycelia to adhere to a glass surface has been utilized as a basis of this technique.

Procedure of coverslip culture method:

- Insert 4-5 cover slips on petri-plate containing POA at an angle of 45^{o} ——> inoculate organism at the point of media and coverslip ——> incubate the plate at room temperature for 7 days ——> after incubation remove the coverslip gently and mounted with lactophenol cotton blue and observe under microscope.

Microslide culture technique for fungi identification:

Procedure of microslide culture technique:

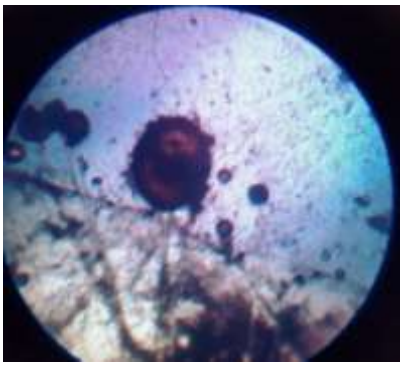
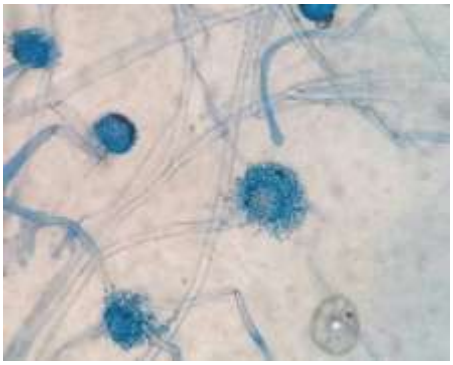
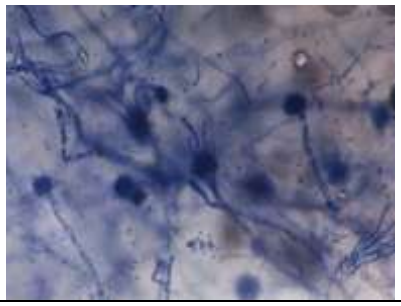
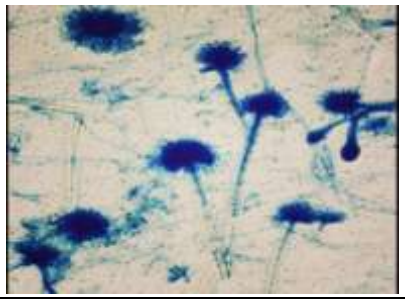
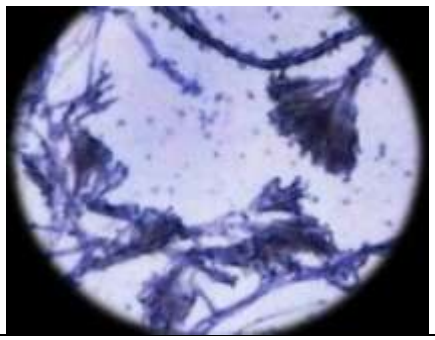
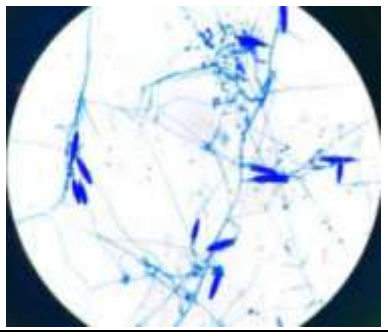
- Take sterile petri dish—-> remove its lid ——> place sterile filter paper over it and add distilled water to moisten the filter paper ——> put glass slide over glass rod ——> add $5mm^2$ PDA agar on glass slide from agar plate ——> inoculate spores of fungi on that agar and cover with coverslip ——> incubate at room temperature for 7 days ——> remove coverslip and mounted in next slide and observe under microscope.

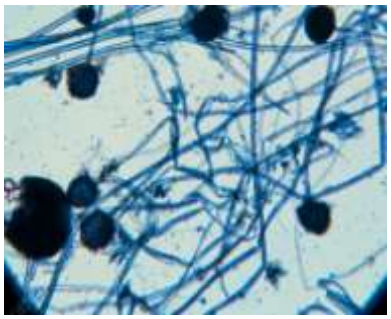
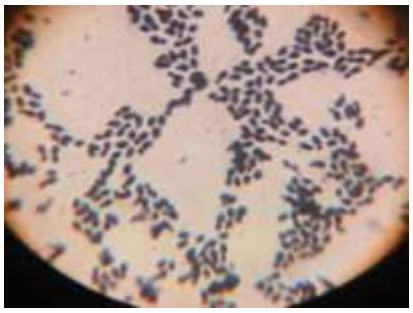
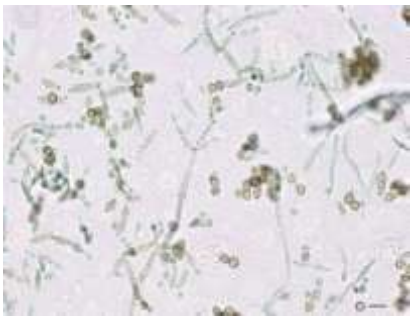
RESULT:

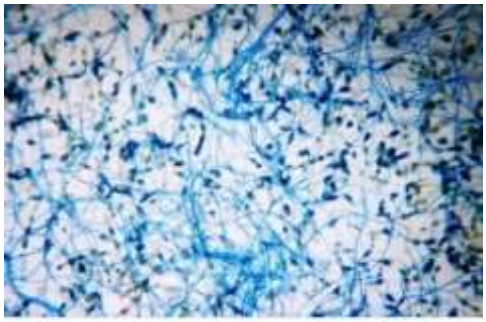
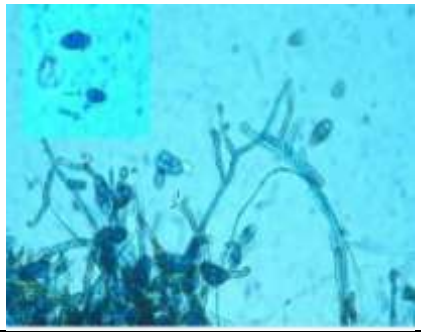
S. No	Botanical Name Tree	Local Name tree	Samples	Method	Isolates
1.	Ficusbenghalensis	Bargad	Rhizospheric soil	Direct plating	A. niger, A. flavus, Rhizopus oryzae, M. gypsum
2.	Azadirachaindica	Neem	Rhizospheric soil	Direct plating	Trichophyton rubrum, A. fumigatus, Alternaria spp.
3.	Ficusreligiosa	Peepal	Rhizospheric soil	Direct plating	Rhodotorulla spp, Cladosorium spp.
4.	Annonasquamosa	sitaphal	Rhizospheric soil	Direct plating	R. oryzae, A. nidulans, A. flavs
5.	Terminaliaarjuna	Arjuna	Rhizospheric soil	Direct plating	Mucor spp., R. oryzae
6.	Mangiferaindica	Mango	Rhizospheric soil	Direct plating	Alterneria spp., Rhodotorula, M. gypsum, A. niger, A. flavs
7.	Aeglemarmelos	Bel	Rhizospheric soil	Direct plating	R. oryzae, Rhodotorula
8.	Bombaxceiba	Semal	Rhizospheric soil	Direct plating	Penicillium spp, R. oryzae,
9.	Moin	Moin	Rhizospheric soil	Direct plating	R.oryzae, M. gypseum
10.	Madhucalongifolia	Mahua	Rhizospheric soil	Direct plating	A. niger, A. flavs, T. rubrum, Fusarium spp.

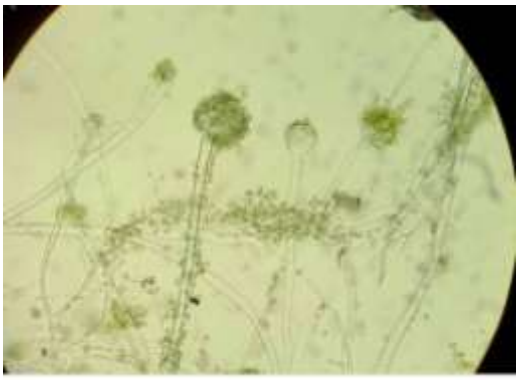
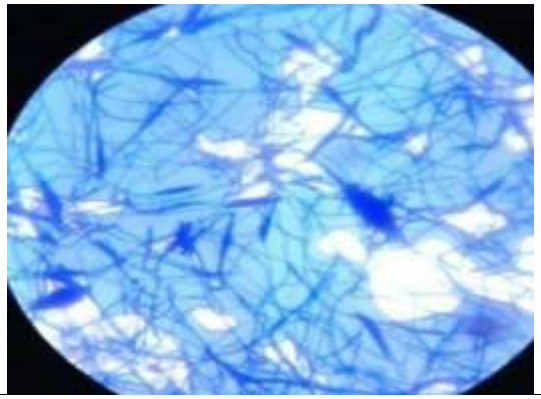
S. No	Botanical Name Tree	Local Name Tree	Samples	Method	Isolates
11.	Ficusracemosa	goolar	Rhizospheric soil	Direct plating	A. fumigatus, R. oryzae, Penicillium spp.
12.	Buteamonosperma	Palaash	Rhizospheric soil	Direct plating	R. oryzae, Fusarium spp.
13.	Cuminisyzygium	Jaamun	Rhizospheric soil	Direct plating	A. niger, A. flavus, Rhizopus spp.
14.	Milletti a pinnata	Karanji	Rhizospheric soil	Direct plating	Cladosporium spp. A. flavus
15.	Bauhinia variegata	Kachanaar	Rhizospheric soil	Direct plating	A. flaves, A. Oryza Rhizopus spp.
16.	Senegaliacatechu	Khair	Rhizosphericsoil	Direct plating	A. niger, R. oryzus
17.	Cassia fistula	Amalataas	Rhizospheric soil	Direct plating	Fusarium spp. A. fumigatus,
18.	Tamarindusindica	Imalee	Rhizospheric soil	Direct plating	Curvularia spp. A. oryzae
19.	Phyllanthusemblica	Aanvala	Rhizospheric soil	Direct plating	A. nidulans, A. flavesR.oryzae
20.	Simaroubaglauca	Lakshmeetara	Rhizospheric soil	Direct plating	A. niger, A. flavus, R. oryzae,

Images of fungi microscopic morphology are given below:

Aspergillus Niger 	**Aspergillus Flavus** 
Aspergillus Fumigatus 	**Aspergillus Versicolor** 
Penicillium spp. 	**Trichophyton Rubrum** 

Aspergillusnidulans 	**Fusarium spp.** 
Rhizopusoryzae 	**Mucur spp.** 
Rhodotorula Spp. 	**Cladosporium spp.** 

Alternaria spp. 	**Curvularia spp.** 

Aspergillus Oryzae	MicrosporumGypseum
	

DISCUSSION

1 Aspergillus niger

A. Colony Morphology: -Surface is black with white border; thallus is deep ,visibly composed of longwise to cream, erect hyphae with clusters of black conidia at the apices and reverse is white to cream

B. Microscopic Morphology-. Septet hyphae. Conidiophores are long, smooth, and may be brownish near to the top of conidia.

2. Aspergillus flavus

A. Colony Morphology: - Surface is yellow-green to olive, often with specks of yellow and white border may be present. Texture is velvety to cottony with reverse is usually yellowish to ten.

B. Microscopic Morphology- Septatehyphae; conidiophores are long and when fully mature, the walls are characteristically rough spiny specially at the apex. Conidia is haring smooth or slightly walls

3. Aspergillus fumigatus

A. Colony Morphology: - Surface is velvety or powdery, various shades of green at prime with a narrow white border; colony turn dark gray with age.

B. Microscopic Morphology: - Septatehyphae conidiophores are smooth; the phialides are uniseriate, close together, forming only on the upper two-thirds of the vesicle, parallel to the axis of the conidiophores. Conidia are round, smooth, or slightly rough.

4. Aspergillus versicolor

A. Colony Morphology: - Surface is suede like, often having radial grooves; color varies but most commonly green or ten with spots of yellow, orange or pink. Reverse may be white, yellow, orange or red.

B. Microscopic Morphology: - Septatehyphae conidiophores smooth and of, medium length .Small conidial heads are occasionally form that, resembling penicillin. Conidia are round, slightly or clearly rough and 2-3.5um in diameter.

5. Penicillium spp.

A. Colony Morphology: -Surface at first is white, then becomes very powdery and bluish-green with a white border. Some fewer common species differ in color and texture. Reverse is usually white, but may be red or brown.

B. Microscopic Morphology: - Hyphae are septate with branched or unbranched conidiophores that have secondary branches known as metulae.

6. Trichophyton rubrum

A. Colony Morphology: - Surface is granular or fluffy, white. Reverse is deep red or purplish; occasionally it is brown, yellow-orange, or even colourless.

B. Microscopic Morphology: - Septate hyphae. Tear-shaped microconidiausually found single all along the sides of the sides of the hyphae and may be abundant.

7. Aspergillus nidulans

A. Colony Morphology: - Surface velvety, green; buff to yellow or purplish brown white border is found if cleistothecia are present; white border. Reverse buff, is brownish orange, or deep reddish purple.

B. Microscopic Morphology: - Septate hyphae; conidiophores are smooth, short and brown, darkening with age. Phialidesbiseriate with metula, forming only upper half of the vesicle. Conidia are round, smooth or slightly rough, and 3-4µm in diameter.

8. Fusarium spp.

A. Colony Morphology: - At first white and cottony, but often quickly develops a pink or violet centre with a lighter periphery.

B. Microscopic Morphology: - Septate hyphae. There are two types of conidiation: (1) unbanked or branched conidiophores with phialidas that produce large (2-6 ×4-8 µm), oval, one- or two-celled conidia singly or in clusters resembling those of Acremonium spp.

9. Rhizopus oryzae

A. Colony Morphology: - Quickly covers agar surface with dense growth that is cotton candy-like; colonies are white at first and then gray or yellowish brown. Reverse is white to pale shades of gray or brown.

B. Microscopic Morphology: - Broad hyphae with no or very few septa. Numerous stolons run among the mycelia, connecting groups of long sporangiophores that usually are unbranched.

10. Mucur spp.

A. Colony Morphology: - Quickly covers agar surface with fluff resembling cotton candy; white, later turns gray or greyish brown. Reverse is white.

B. Microscopic Morphology: - Hyphae are wide and practically nonseptate. Sporangiosphores are long and often branched and bear terminal round, spore-filled sporangia.

11. Rhodotorula spp.

A. Colony Morphology: - Usually pink to coral, but can also be more orange to red. Colony is yeast like, soft, smooth, moist, and sometimes mucoid.

B. Microscopic Morphology: - On Cornmeal-Tween 80 agar at 25˚C for 72 h, budding cells are round to oval or elongate (occasionally a few rudimentary pseudohyphae are seen. A Capsule is sometimes formed.

12. Cladosporium spp.

A. Colony Morphology: - Surface is greenish brown or black with greyish velvety nap, becoming heaped and slightly folded. Reverse is black.

B. Microscopic Morphology: - Hyphae are septate and dark; conidiosphores are dark and branched, vary in length, and usually produce two or more conidial chains. Conidia are brown, round to oval (3-6 ×4-12µm), and usually smooth.

13. <u>Alternaria spp.</u>

A. Colony Morphology: - Surface is at first greyish white and woolly and later becomes greenish black or brown with a light border. Eventually become covered by short, greyish and aerial hyphen. Reverse is black.

B. Microscopic Morphology: - Hyphae are septate and dark. Conidiosphores are septate, of variable length, and sometimes have a zigzag appearance. Conidia are large and brown, have both transverse and longitudinal septations.

14. <u>Curvularia spp.</u>

A. Colony Morphology: - Colony is dark olive-green to brown or black with a pinkish gray, surface. Reverse is dark.

B. Microscopic Morphology: - Hyphae are septate and dark. Conidiophores are simple or branched and bent or knobby at points of conidium formation (sympodial geniculate growth). Conidia are large usually contain four cells, and eventually appear curved.

15. <u>Aspergillus oryzae</u>

A. Colony Morphology: - Colonies growing rapidly pale greenish-yellow or with different shades of green and typically haring dull brown shades with age.

B. Microscopic Morphology: - Conidial heads radiate to loosely columnar. Conidiosphorestipes are hyaline. And Vesicles are subspherical Conidiogenous cells are uniseriate and biseriate.

16. <u>Microsporum gypseum</u>

A. Colony Morphology: - Colonies are rapidly growing rapidly, powdery, cinnamon-tan. Reverse is yellowish-buff and sometimes with pinkish tings.

B. Microscopic Morphology: - Macroconidia are found in large clusters, rather thin-walled and regularly verrucose.

<u>CONCLUSION:</u>

Fungal communities exert important influencing forces on plant growth and health. However, information on the dynamics of the fungal community structure of the worldwide economic medicinal plants are limited. In the present study, next-generation sequencing of nuclear ribosomal internal transcribed spacer-1 (ITS1) was performed to characterize the fungal communities. The medicinal plants were grown in rhizospheric soil (RS) that had been continuously grow medicinal plant. The fungal species richness, diversity, and community composition were analyzed and compared among the rhizospheric soil resources and developmental stages. We found that the fungal community structures were different between the rhizosphere and bulk soil and the difference were significantly varied. Our results suggested that different sixteen fungal community structure variation may have been primarily influenced by the interaction of medicinal plant with different soil resources. We also found that the community composition of the fungi varied significantly during different developmental stages. In addition, we observed those fungi which insights can lay a

foundation for deep research into the dynamics of pathogenic fungi and nutrient absorption of rhizospheric soil. This research illustrates the characteristics of the fungal communities and provides important information for understanding the potential influences of the said fungal communities on the growth and health of rhizospheric soil along with its medicinal plants.

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