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Fungal pathogen affecting medicinal plant in India: Diversity and management strategies

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Abstract
Due to the fact that medicinal plants possess effective therapeutic properties, they have become an essential component of conventional healthcare systems. On the other hand, these plants are susceptible to a number of fungal infections, which lessen their capacity for development, production, and medicinal effectiveness. This study was conducted with the intention of gaining a deeper understanding of the endophytic fungus that is present on native medicinal plants, as well as their distinctive characteristics and any potential effects that they may have on the health of the plant. Under carefully monitored conditions in the laboratory, we collected a variety of plant components, including bark, stems, and leaves, from ten different kinds of medicinal plants. Identification of endophytic fungus was accomplished by the use of the lactophenol cotton blue staining method, which was based on the macroscopic and microscopic characteristics of the fungi. For the purpose of isolation, a medium consisting of potato dextrose agar (PDA) was used. A total of 133 unique fungal isolates were obtained from the 51 samples. It was discovered that certain fungal genera, including Aspergillus, Penicillium, Fusarium, Alternaria, Nigrospora, Rhizopus, and Verticillium, were present in rather high numbers. There were no fungi discovered in Chinese wisteria; nevertheless, the most fungal isolates were discovered in Azadirachta indica (Neem), which was one of the plants that was put through the examination process. The study highlights the wide range of fungal endophytes that are connected with medicinal plants. This is in addition to the ecological value of these endophytes, as well as their potential role in the management of plant diseases and the production of bioactive chemicals.

Keywords: Endophytic fungi, medicinal plants, fungal diversity, plant–microbe interaction, *Aspergillus*, *Penicillium*, fungal pathogens, bioactive compounds, antimicrobial activity, disease management

Introduction

Medicinal plants have been used for a very long time in traditional medicine, and those plants have been used for both the treatment and prevention of sickness. It is possible that the development, production, and therapeutic usefulness of these plants might be affected by a number of microbial illnesses, particularly those that are caused by fungus. Among these bacteria, endophytic fungi stand out as a separate and very diverse population that inhabits plant tissues without causing any symptoms. The plants that are infested by these fungi create complex interactions with them, and they colonise the plant's internal tissues without causing any symptoms. Fungal organisms that spend a portion or the whole of their life cycle inside the tissues of plants are referred to as endophytic fungi. The majority of these fungi do not cause any harm to plants, and they are widely distributed and polyphyletic. Researchers have discovered endophytic fungus in almost every plant species that has been investigated up to this point. The research that has been done on the endophytic fungal variety of the roughly 420,000 plant species that are found across the world has only covered a very small part of these individuals. The diversity and ecological relevance of this group of organisms is shown by the fact that fungal endophytes make up around seven percent of the estimated one and a half million fungal species that exist in the globe.

In addition, it is well known that endophytic fungi are capable of producing a large range of natural chemicals that exhibit biological activity and fascinating structures. A significant number of the secondary metabolites that are generated by these microorganisms include anticancer, antibacterial, antifungal, and antioxidant properties. The following types of metabolites are included in this category: alkaloids, peptides, steroids, terpenoids, flavonoids, phenols, and quinones. In recent times, there has been a significant amount of interest in endophytic fungus as a potential source of bioactive compounds that may be used in the fields of biotechnology, medicine, and agriculture. The presence of endophytic fungus in medicinal plants has the ability to influence the metabolism of the plant as well as the chemical synthesis that is responsible for pharmacologically relevant effects. During the course of their mutualistic interactions with host plants, these microorganisms have the potential to enhance the plants' capacity to flourish, withstand stress, and defend themselves against infectious microbes. As an example, it has been shown that medicinal plants such as Neem, Tulsi, Peepal, and Turmeric all contain a variety of endophytic fungal populations, which may be the reason why these plants are so successful.

Tulsi, also known as *Ocimum sanctum*, is one of these therapeutic plants that has a significant place in both the culture and the medical practices of India. The anti-inflammatory, stress-relieving, and asthma-fighting benefits of this substance have been widely described in the literature of Ayurveda. There are a number of plant diseases that have been demonstrated to be vulnerable to the potent antibacterial and antifungal actions of the extracts and essential oils of this plant, particularly eugenol. These diseases include *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Aspergillus species*. In light of the significance of endophytic fungus in both ecology and medicine, the purpose of this study was to compile a list of the many microorganisms that are found in connection to a

few local medicinal plants and to also determine their range. It was necessary to examine a number of plant components, such as the bark, stems, and leaves, in order to identify the presence of fungal colonisation. The identification of the isolated fungus based on their macroscopic and microscopic features allowed for the investigation of the variety of the fungus as well as its potential role in the process of managing plant diseases and maintaining plant health.

Objectives

1. To separate and identify endophytic fungi and fungal diseases connected to certain medicinal plants.
2. To assess the distribution of fungal isolates in various plant sections and examine their diversity and microscopic characteristics.

Material and Method

A modified and standardized approach was used in order to successfully isolate and identify endophytic fungus from native medicinal plants in the Bilaspur, which is located in the state of Chhattisgarh. The samples were given a moderate washing under running water in order to eliminate any dust or debris that may have been present. After the stem samples were meticulously cleaned, they were then sliced into lengthy sections that measured between 0.5 and 1 centimeter. In a similar manner, the leaves were prepared under aseptic circumstances and then chopped into pieces that measured 2-3 millimeter by 0.5-1 centimeter. It was determined that the percentage of sodium hypochlorite (NaOCl) used for surface sterilization varied from 1% to 13%, depending on the kind of tissue. Following an initial treatment with 75% ethanol for a duration of one minute, three distinct groups of plant material were submerged in sodium hypochlorite and then subjected to a second treatment with 75% ethanol for a duration of thirty seconds further.



Fig 1: The bark, stem, and leaves of several medicinal plants are used to isolate endophytic fungus.

Any residual sterilant were removed from the stem and leaf explants by washing them in deionized sterile distilled water. This was done in order to sterilize the explants. This was followed by placing them on sterilized tissue paper and allowing them to dry. After this, they were cultured on Petri plates using a medium consisting of potato dextrose agar (PDA) that was treated with 100 g/mL of streptomycin. The petri dishes were kept in an incubator at a temperature of 27±2 degrees Celsius for a period of 15 days, during which time they were monitored daily for any signs of contamination. After that, they were placed in a low-light environment and covered with parafilm. After that, the fungal cultures that had formed from the plant tissue samples were transferred to separate PDA plates and allowed to grow at room temperature for a period of time. In order to determine the identity of the isolated fungus, the lacto-phenol cotton blue wet mount method was used in conjunction with the compound microscope. In the world of fungus, the term "mycelia sterilia" refers to fungi that are unable to produce sporuli. This was done so that the characteristics of the colony could be evaluated, and the mycelia were moved to PDA agar media. It was decided to keep the fungal isolates at a level of four for the sake of future study and preservation.

Observation Table

Table 1: Endophytic fungus separated from several sections of native medicinal plants

S. No.	Host Plant	Site of Isolation	No. of Samples	No. of Fungi Isolated
1	Neem (*Azadirachta indica*)	Leaf, Stem and Bark	7	20
2	Jatropha (*Jatropha gossypiifolia*)	Leaf, Stem and Bark	5	19
3	Tulsi (*Ocimum sanctum*)	Leaf, Stem and Bark	6	10
4	Peepal (*Ficus religiosa*)	Leaf, Stem and Bark	4	18
5	Eucalyptus (*Eucalyptus tereticornis*)	Leaf, Stem and Bark	4	11
6	Geloy (*Tinospora cordifolia*)	Leaf, Stem and Bark	3	9
7	Chinese Wisteria (*Wisteria sinensis)*	Leaf, Stem and Bark	3	—
8	Turmeric (*Curcuma longa*)	Leaf, Stem	5	13
9	Moringa (*Moringa oleifera*)	Leaf, Stem and Bark	6	15
10	Ashoka (*Saraca asoca*)	Leaf, Stem and Bark	8	18

Result and Discusssion

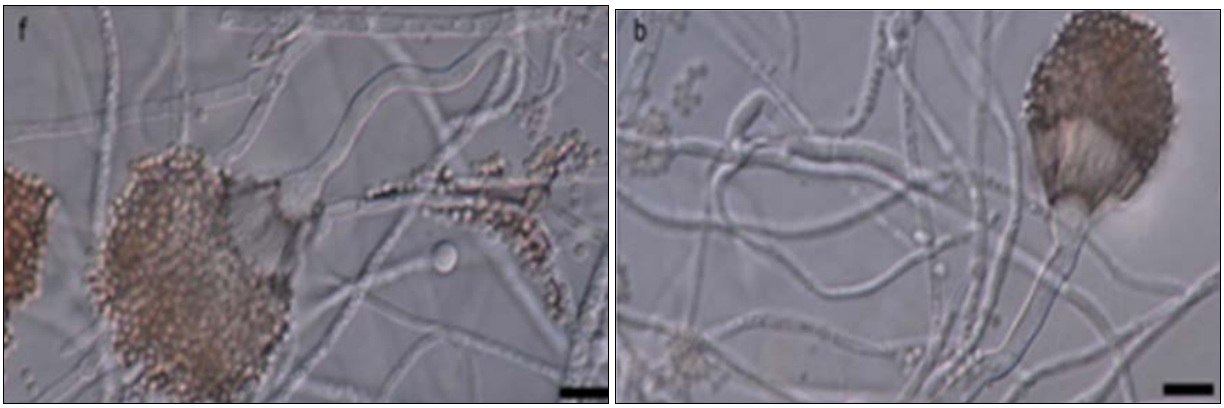
After extracting 133 fungi, comprising both common and unusual species, from 51 samples of 10 different plants, antimicrobial activity was evaluated (Table 1). The fungus included both common and rare species. There were a few moulds among the fungus, such as *Rhizopus, Mucor, and Rhizomucor;* however, the great majority of the fungi were ascomycetes, including *Candida, Aspergillus, Penicillium, and Nigrospora. Aspergillus, Alternaria, Pencililum, Fusarium, and Verticillum* species were the most common types of fungi that were found to be infesting the plants in this region. According to the findings of the present study, endophytic fungi were found to be very widespread in neem plants, but Chinese wisteria plants did not contain any endophytic fungus. The fungal specimens were grown at a temperature of 27 degrees Celsius for a period of seven days. There were two distinct runs of the experiment that was conducted in a fully randomized manner. All of the following were measured: the color of the medium, the color of the back of the petri dish, the color of the

mycelium, the aspect of the borders of the colonies, and the average diameter of the colonies in centimeters. The microscopic characteristics of the endophytic fungi that were isolated were investigated using a compound microscope that was used for the examination. The microscopic properties of the fungus that were isolated were taken into consideration using the lacto-phenol cotton blue wet mount technique. The results of the microscopic examination of a number of different fungal species are shown in Table.

Table 2: Microscopic features of several endophytic fungus seen with a compound microscope

S. No.	Microscopic characteristics	Fungi Isolated
1	Colony colour green.Vegetative structure consists of multicellular mycelium with long septate branching hyphae. Conidiophores produce spherical conidia. Conidia arise from phialides giving a brush-like appearance. Sexual spores (ascospores) occur inside asci within ascocarps.	*Penicillium*
2	Moderate colony growth with peach, white, salmon pink or violet colour. Produces macroconidia and microconidia. Macroconidia are hyaline, curved and septate with a foot cell; microconidia are oval to cylindrical. Chlamydospores may also be present. Macroconidia size ranges 27–60 × 3–5 µm.	*Fusarium*
3	Rapid colony growth with white to dark brown or black mycelium. Conidiophores arise from the substratum with globose vesicles. Phialides and metulae produce chains of rough, globose conidia measuring 4–5 µm.	*Aspergillus*
4	Colonies greyish-white with felty patches and sparse aerial mycelium. Conidial masses are salmon pink. Conidia are falcate with obtuse ends measuring 15.5–26.5 × 4–5 µm. Appressoria are clavate to circular.	*Colletotrichum*
5	Colonies appear fuzzy white initially with dark brown concentric rings. Conidiophores are pale brown and arise from substrate. Conidia measure about 31.875 µm × 8.125 µm and are produced in chains.	*Alternaria*
6	Colonies white initially, turning brown or black due to heavy sporulation. Conidia are solitary, spherical or broadly ellipsoidal, smooth, black and 10–16 µm in diameter. Conidiophores are branched and smooth.	*Nigrospora*
7	Colonies dark greyish-brown with simple rhizoids. Sporangiophores up to 400 µm long arise in groups. Spherical sporangia reach 100 µm diameter containing numerous spores.	*Rhizopus*
8	Septate hyaline hyphae present. Conidiophores branch in whorls (verticillate pattern). Phialides surround conidiophores and produce conidia.	*Verticillium*
9	Conidiophores long and unbranched. Conidia smooth, hyaline, pear or club-shaped measuring 8–10×12–18µm. Conidia overlap forming a zig-zag pattern at the tip of the conidiophore.	*Trichothecium*





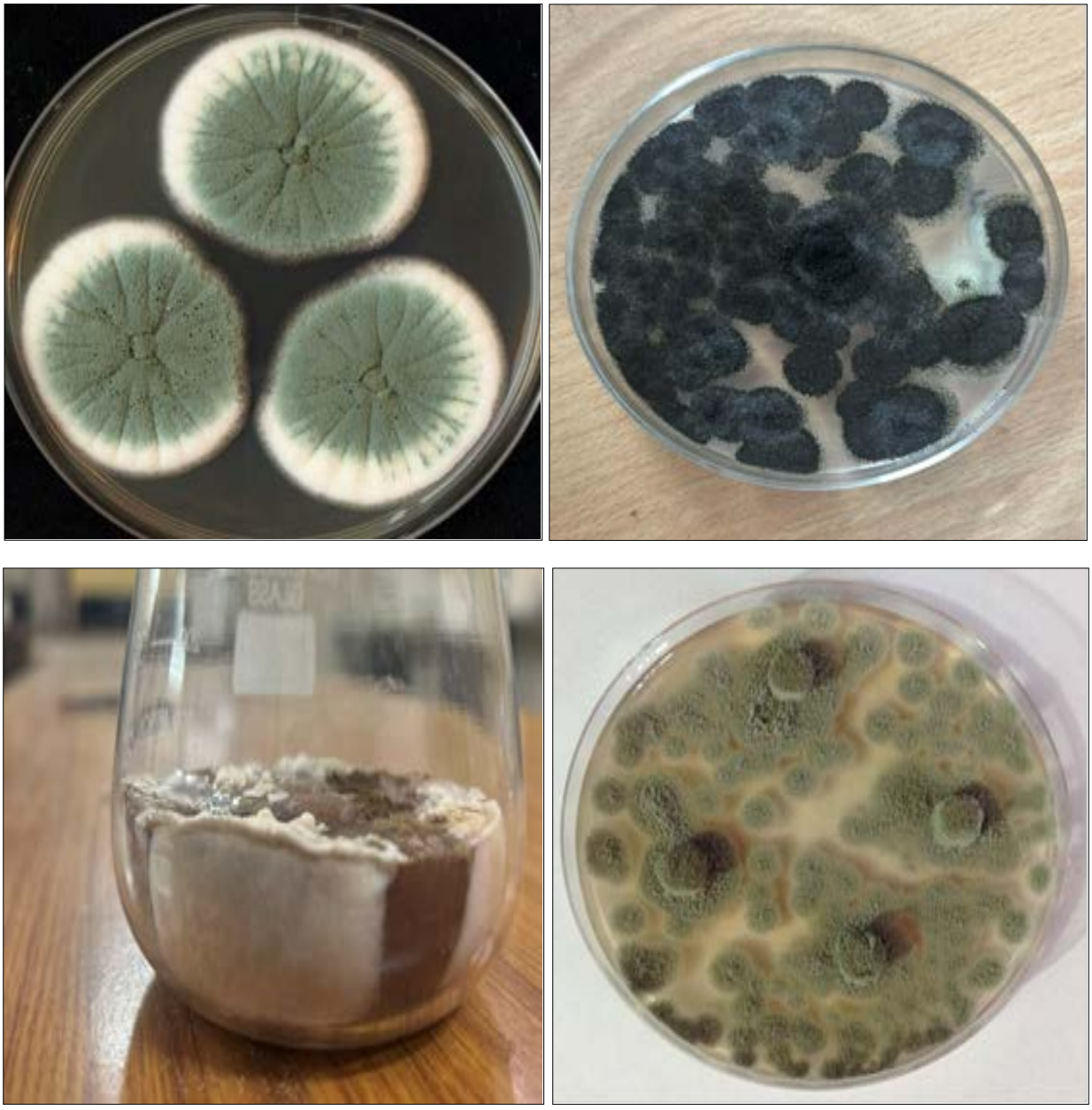


Fig 2: Macroscopic (a, b) and (c, d) microscopic structures Aspergillus terreus, the most prevalent endophytic fungus found, and its microscopic morphological features.

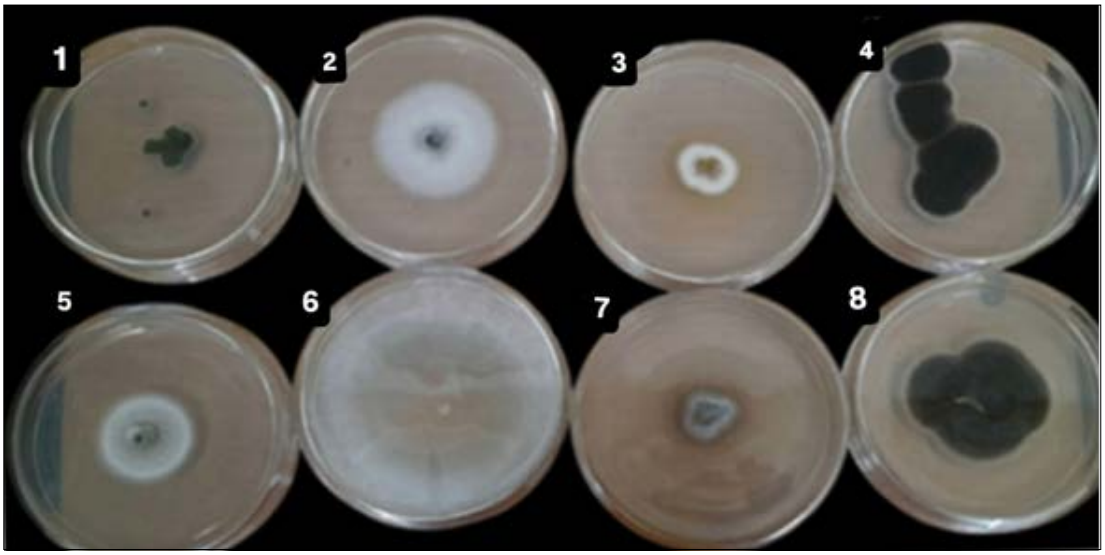


Fig 3: Pictures of endophytic fungus that have been separated from plants at the macroscopic level. (1) Penicillium (2) Fusarium (3) Verticillium (4) Aspergillus (5) Colletotrichum (6) Nigrospora (7) Rhizopus (8) Alternaria.

In recent years, pharmacological research has been focusing on medicinal plants, which are the foundation of traditional medicine. It is well-documented that endophytic bacteria are present in every single plant species that has ever been explored. Because of the symbiotic relationships they have with higher animals and the helpful substances they create for those hosts, endophytic fungi are among the most fascinating and diverse types of organisms. This is due to the fact that they produce chemicals that host higher animals. On the other hand, research on the endophyte variety and bioactive chemical production of only a few number of plants has been conducted. There has been a significant amount of study conducted on the diversity of endophytic organisms, their taxonomy, reproduction, the ecology of hosts, and the consequences that they have on hosts. In light of the fact that endophytes are capable of growing in a wide variety of peculiar environments and occupy their own unique position within the biosphere, they are currently considered to be an excellent source of bioactive compounds.

Both facultative and obligate endophytic bacteria are possible kinds of endophytes. It is not always the case that facultative endophytes spend their whole life cycles inside of plants; there are instances in which they will reside outside of the plant and establish a link with the soil in the immediate rhizosphere of the host plant. Nevertheless, obligatory strains are present in plants throughout the whole of their life cycles. These bacteria multiply via consecutive generations of plants through a method known as vertical transmission. Additionally, they modify the products of plants and the metabolic processes that they carry out in order to ensure their own survival. Bacteria perform best in habitats, such as plants, in which they are able to satisfy all of their nutritional requirements via the process of mutualism. Endophytes are microorganisms that live in plants and create bioactive metabolites. These endophytes have the potential to be a source of beneficial therapeutic compounds that may be acquired from nature. Endophytic fungi (EFs) are one of the endophytes that have been investigated the most because of their ability to produce biostimulants that are necessary for the production of essential oils, new chemicals, antibacterial and antifungal substances, antioxidants, and anti-carcinogenic molecules. In addition to their functions as biological control agents and stress-strengthening agents for plants, they may also enhance the development of the host plant by boosting the efficiency with which nutrients are dissolved in the rhizosphere of the plant. Recently, there has been a surge in the scientific community's interest in comprehending, elucidating, and making use of the plant-microbe interaction. This increased attention may be attributed to the potential benefits that endophytic fungalplant interactions have. The production of the same biomolecule by both the plant and the microorganisms participating in the symbiotic interaction between plants and endophytes is one of the potential outcomes that might occur as a result of such a relationship. *Myrtucommulones, camptothecin, paclitaxel, and deoxypodophyllotoxin* are some examples of these.

These compounds are obtained from plants such as *Myrtus communis, Taxus brevifolia, Camptotheca acuminata, and Juniperus communis*. All of these plants are used to produce these compounds. This modification, in theory, enhances the microbe's chances of surviving in the milieu that it is exposed to inside the plant tissues. There are three types of symbiotic relationships that endophytes have with the plants that they live on: parasitic, commensal, and mutualistic. Endophytes have a special ecological function of maintaining a delicate balance. Unique metabolites are produced by endophytes, and the interaction between the two is mostly controlled by chemical substances. These organisms are the topic of growing attention in the scientific community as a result of the key role that they play in the creation of natural goods, especially in situations where the plant is crucial for the manufacture of healthcare products. Both the metabolome of the plant and the endophyte metabolites that are released into the tissues of the plant have the potential to play a role in the therapeutic capabilities of the plant. This is the reason why this is the case. One of the primary motivating factors behind the recent developments in biotechnology has been the identification of novel bioactive compounds derived from fungal endophytes. Notwithstanding this, fungal endophytes continue to comprise a negligible portion of the overall population.

Management Strategies for Fungal Pathogens

Diverse species of fungi like *Aspergillus, Fusarium, Alternaria, Penicillium, and Rhizopus* on medicinal plants show the importance of effective disease management strategies to keep the plants healthy and productively. Cultural, biological and chemical means have been used to combat fungal infections in medicinal plants.

1. Cultural Management

Medicinal plants benefit greatly from cultural practices to prevent and control fungal infections. Aspects such as proper sanitation, removal of affected plant debris, and growing crops in rotation with one another help to reduce the spread of fungal pathogens. Numerous studies have indicated that by utilizing crop rotation and benefiting from improved field sanitation, medicinal plant crops can expect to see a reduction in fungal infections of approximately 25-40%. Additionally, adequate spacing between plants creates improved airflow, which reduces the humidity around plants and limits the development of fungi and their spores. Another means of minimizing the infestation of fungi on medicinal plants is through the use of pathogen-free planting stock and optimally managing the irrigation of the crops.

2. Biological Control

Using good microbes to manage harmful fungi is an environmentally friendly method of controlling fungal disease in agriculture and horticulture. Many lots of fungi that act to stop disease are able to do so by competing with the fungus for food, by producing antifungal substances, or by using mycoparasitism. The addition of species of the fungus *Trichoderma* to soil have been shown to reduce *Fusarium* and *Rhizoctonia* disease in many of the medicinal plants tested by 50-70%. There is also significant data to suggest that other bacterial bioagents such as *Pseudomonas fluorescens* and *Bacillus subtilis* produce antibiotics and enzymes that (i) prevent fungal growth and (ii) increase plant resistance.

3. Plant-Based Antifungal Compounds

Extracts made directly from medicinal plants have shown potential for use as natural antifungal agents. For instance,

essential oils produced from the plant *Ocimum sanctum*, which has been demonstrated to contain a high concentration of eugenol, have been shown to impede the growth of paths such as *Rhizoctonia solani*. Another plant, the neem tree (*Azadirachta indica*), has exhibited at least a 30%-60% decrease in fungal growth in laboratory trials. Plant-derived antifungals may represent an eco-friendly alternative to conventional synthetics.

4. Chemical Control

When there is a lot of fungal disease present in medicinal plants then Chemical fungicides are also used to manage this. Examples of fungal disease control Fungicide sprays such as *Carbendazim*, *Mancozeb*, and *Copper Oxychloride* have all worked very well to manage Leaf Spot, Wilt, and Root Rot due to fungal pathogens. Under controlled situations where fungicides are used, the incidence of disease can be reduced by as much as 60% - 80%. However, excessive application rates of chemical fungicides should be avoided because of both pollution of the environment and the potential development of resistance by the pathogen populationsIn general, a combination of cultural practices, biological control agents, and limited chemical use is considered the best practice for managing fungal pathogens on medicinal plants, and is known as Integrated Disease Management (IDM) approach.

Conclusion

According to the findings of this study, the endophytic fungus that is associated with a wide variety of medicinal plants presents a pretty diversified landscape. For the purpose of this study, 133 fungal isolates were obtained from 51 samples of ten distinct kinds of medicinal plants. When it came to the fungal population, the species of *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Nigrospora*, *and Verticillium* were the ones that were discovered the most often. *Azadirachta indica* showed the highest frequency of fungal isolates among the plants that were examined, which suggests that it is home to a rich population of endophytic fungus. On the other hand, *Chinese Wisteria* did not show any signs of having a fungal presence. The physical characteristics of the isolated fungus were confirmed by microscopic examination, which was made possible by the lactophenol cotton blue staining. The findings indicate that medicinal plants are natural habitats for a diverse range of fungal communities, some of which may play an important role in maintaining the health of the plant, maintaining ecological balance, and producing bioactive metabolites. Further investigation into these fungal isolates may result in the development of novel antimicrobials as well as long-term strategies for the management of illnesses that affect medicinal plants.

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