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Identification of Fungal Species from Remote Places of Vindhyachal Forest of Madhya Pradesh for Biological Control of Early Blight

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ABSTRACT: The main goal was to find potential biological control agents for Early Blight, a disease that commonly affects potato and tomato crops. By studying the microbial diversity in these fields, researchers hoped to find environmentally friendly and sustainable solutions for managing this disease. We have collected samples of soil from the rhizosphere (the region of soil directly influenced by root secretions and associated soil microorganisms) and plant root materials as rhizoplane (the surface of a plant root) during October and November 2021. These samples were then serially diluted and streaked over Potato Dextrose Agar media plates, a common method used for the isolation of fungi. The cultured fungi were further analyzed using molecular techniques, specifically Polymerase Chain Reaction (PCR) and sequencing of the Internal Transcribed Spacer (ITS) region. This region is a universally accepted region of fungal DNA for species identification.

The study reported the presence of several important fungi, including *Trichoderma* species, *Penicillium* sp. *Phytophthora infestans, Fusarium oxysporum, Pyrenochaeta lycopersici, Aspergillus flavus, Collectotrichum* spp., *Cladosporium cladosporioides, Periconia byssoides, Aspergillus tamarii,* and *Fusarium* sp. Some fungi among all are known to play a crucial role in the development of healthy plants and protection of potato and tomato plants from other pathogens. This study contributes to our understanding of the microbial diversity in the Vindhyachal forest and its potential use in the biological control of plant diseases. It's a significant step towards sustainable and environmentally friendly farming practices.

Keywords: Biological Control, Early Blight, Fungal species, Molecular identification, ITS region.

INTRODUCTION

Potatoes and tomatoes are prone to a variety of fungal diseases, including Early Blight, which is caused by the fungus *Alternaria solani* and occurs during the maturation phase of these crops, not earlier (Secor and Gudmestad 1999; Mamgain *et al*., 2013). This disease is particularly prevalent in unfertilized or stressed plants and leads to significant crop losses, with symptoms including dark brown to black lesions with concentric rings, primarily observed on older, senescing leaves (Jindo *et al.,* 2021; Chaudhary *et al.,* 2021). Early blight, a widespread and damaging disease affecting potato and tomato crops, is caused by various species of the *Alternaria* fungus. The disease manifests as necrotic lesions on leaves, stems, and fruits, negatively impacting the yield and quality of the crops. Early blight is a global issue, particularly in regions with warm and humid climates. In India, it poses a significant challenge to potato and tomato production, causing yield losses of up to 78% in some instances (Loganathan *et al.,* 2016). Alternaria species, the causative agents of these diseases, are widespread in agricultural soils worldwide and are considered among the most aggressive soil-borne pathogens. These species not only cause plant diseases but can also have detrimental effects on human and animal health due to the over 70 toxins they produce, which are found in various food and animal feed products (Jindo *et al.,* 2021). In recent decades, growers have relied on agrochemicals for crop protection. However, these methods have several concerning effects, including pathogen resistance to chemical agents, harm to non-target organisms, residual toxicity, exposure risks, and other health or environmental hazards. Therefore, there's a growing emphasis on sustainable agricultural practices such as biological control. This eco-friendly, economical, and sustainable strategy involves introducing organisms antagonistic to the pathogen or enhancing naturally occurring antagonistic organisms, resulting in reduced disease severity. It could become a crucial part of an integrated pest management system for controlling Alternaria diseases (Awan & Shoaib 2019).

The use of biological control could be a key strategy in managing Alternaria diseases in potato and tomato crops as part of an integrated pest management system. Biological control, in the context of diseases caused by soil-borne plant pathogens, involves introducing organisms that are antagonistic to the pathogen or

enhancing the presence of naturally occurring antagonistic organisms, leading to a decrease in disease severity.

Currently, the primary method of managing early blight is the use of fungicides. However, these are costly, environmentally damaging, and the pathogen can develop resistance to them. As such, there is a need for alternative, sustainable control methods for early blight, such as biological control (Matrood & Rhouma 2021). This involves using living organisms or their by-products to suppress the growth or activity of a pest or pathogen. Biological control agents (BCAs) can include bacteria, fungi, viruses, nematodes, insects, or plants that antagonize, parasitize, compete with, or induce resistance in the target organism (Das, 2021). The Vindhyachal or Vindhya range, a complex and discontinuous chain of mountain ridges, hill ranges, highlands, and plateau escarpments in west-central India, is rich in flora and fauna and has significant cultural and historical heritage (Roy *et al.,* 2019). Fungal species, potential BCAs for early blight, are of particular interest as they can directly attack and kill the pathogen or produce secondary metabolites that inhibit its growth or sporulation (Kumar *et al.,* 2010).

However, identifying and characterizing fungal BCAs is challenging, requiring morphological, molecular, and pathogenic criteria to distinguish them from the pathogen and other non-target fungi. Furthermore, the effectiveness and consistency of fungal BCAs can vary depending on environmental conditions, the host plant, and the pathogen strain. In this research, our objective was to discover and assess the effectiveness of fungal species, sourced from the secluded regions of the Vindhyachal forest in Madhya Pradesh, India, as a biological countermeasure against early blight. We gathered soil samples from the rhizosphere area and cultivated fungal colonies on plates containing potato dextrose agar (PDA). Following this, we conducted morphological and molecular identification of the fungal species. The identified species were then studied further for their antagonistic properties against species of Alternaria (Rai *et al.,* 2016). These bacteria could play pivotal roles in controlling early blight disease in plants (Dharani *et al.,* 2022).

MATERIALS AND METHODS

Soil and root samples from plants were gathered from the secluded regions of the Vindhyachal forest in Madhya Pradesh. These samples were preserved in sterile plastic bags and brought back to the lab for further examination. For the isolation and identification of fungi, the soil samples underwent a series of dilutions and were then spread on nutrient agar plates. These plates were incubated at 37°C for a period of 24-48 hours, and the fungal colonies that emerged were purified through repeated streaking on fresh plates. The genomic DNA of the fungal isolates was extracted using the phenol-chloroform method. The 18S rRNA gene, a common marker for fungi, was amplified by polymerase chain reaction (PCR) using universal primers. The PCR products were then purified and sequenced using Sanger sequencing. These sequences were compared with the GenBank database using the BLAST tool to identify the fungal species. The genetic similarity among the fungal isolates was determined through phylogenetic analysis of the 18S rRNA gene sequences using the Clustal W software

Table 1: Universal Primer sequence used in present study are given below.

Primer	Sequence	PCR product	Reference
ITS1 (F)	CTTGGTCATTTAGA GGAAGTAA	Near 350 bp	White (1990)
ITS2 (R)	GCTGCGTTCTTCAT CGATGC		

The sequences of DNA were further analyzed using basic local alignment tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for identification.

RESULTS AND DISCUSSION

The study conducted in the Vindhyachal forest of Madhya Pradesh aimed to explore the microbial diversity in the region, with a particular focus on fungal species that could potentially be used as biocontrol agents against Early Blight, a disease affecting potato and tomato crops. Soil samples were collected from the rhizosphere and plant root materials, also known as the rhizoplane, during the months of October and November. These samples were then serially diluted and cultured on Potato Dextrose Agar media plates for the isolation of fungi (Fig. 1). The cultured fungi were further subjected to molecular analysis using Polymerase Chain Reaction (PCR) and sequencing of the Internal Transcribed Spacer (ITS) region. This led to the identification of several important fungi, including. The study reported the presence of several important fungi, including *Trichoderma* species, Penicillium sp. *Phytophthora infestans*, *Fusarium oxysporum*, *Pyrenochaeta lycopersici*, *Aspergillus flavus*, *Collectotrichum* spp., *Cladosporium cladosporioides*, *Periconia byssoides*, *Aspergillus tamarii*, and *Fusarium* sp. Some fungi among (Fig. 2). Some fungi are known to play a crucial role in the development of healthy plants and provide protection to potato plants from various pathogens. The findings of this study highlight the potential of these fungal species in providing an environmentally friendly and sustainable solution for managing Early Blight.

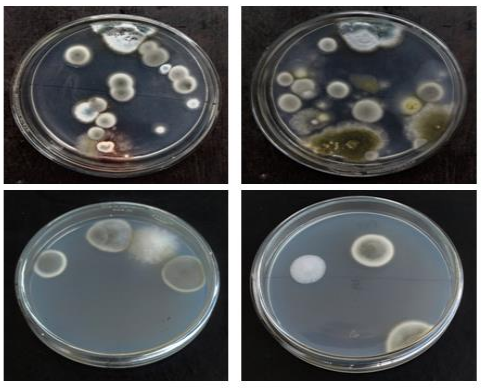

Fig. 1. Representative selected images of mixed fungus species during the present study.

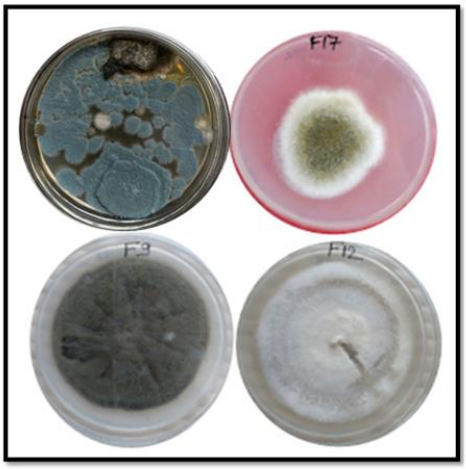

Fig. 2. Representative selected images of Pure culture (F1: Penicillium sp, F12: *Periconia byssoides*, F17: *Aspergillus tamarii*, F19 *Fusarium* sp.) obtained from mixed colony of PDA Plates and validate by ITs region gene sequencing.

Recent studies have also highlighted the potential of endophytic fungi in controlling Early Blight. For instance, a study by Devi *et al.* (2022) reported the potential of endophytic Streptomyces SP. SP5 in controlling Early Blight in tomato and promoting plant growth1. Another study by Attia *et al.* (2022) demonstrated the biocontrol potential of endophytic *Aspergillus terreus* against Early Blight in eggplants. These findings align with the current study, further emphasizing the potential of fungal species as biocontrol agents against Early Blight. Further research is needed to understand the specific mechanisms through which these fungi exert their biocontrol effects and to assess their efficacy in field conditions. In the study by Imran *et al.* (2022), native Trichoderma species isolated from the Jeddah region in Saudi Arabia showed promising antagonistic potential against *A. solani*, both in vitro and in greenhouse and field trials. Similarly, Attia *et al.* (2022) investigated the biocontrol potential of endophytic *Aspergillus terreus* against early blight disease of egg plant. Their study revealed promising results, with *A. terreus* effectively suppressing the severity of early blight and improving the physiological and immunological responses of infected eggplants. The use of endophytic fungi offers a sustainable approach to disease management by harnessing natural plant-microbe interactions. Furthermore, Da Silva *et al.* (2021) investigated the biocontrol potential of different species of Clonostachys against early blight disease in potatoes. Furthermore, Nasr-Esfahani (2022) proposed an integrated pest management (IPM) plan for early blight disease of potatoes, focusing on cultural practices, cultivar selection, and fungicide application. Their findings emphasize the importance of adopting holistic approaches to disease management, integrating various control measures to minimize disease incidence and fungicide use while maximizing crop productivity. The studies highlights the crucial role of certain bacteria in promoting plant health and protecting potatoes from pathogens, including Early Blight (Singh and Bharti 2023). Overall, these studies underscore the importance of exploring diverse strategies, including biological control, soil amendments, and integrated pest management, for sustainable management of early blight disease in tomatoes and potatoes. Future research should focus on optimizing these approaches under different agroecological conditions to develop effective and environmentally sustainable disease management strategies.

CONCLUSIONS

The study reported the presence of several important fungi, including *Trichoderma* species, *Penicillium* sp., *Phytophthora infestans*, *Fusarium oxysporum*, *Pyrenochaeta lycopersici*, *Aspergillus flavus*, *Collectotrichum* spp., *Cladosporium cladosporioides*, *Periconia byssoides*, *Aspergillus tamarii*, and *Fusarium* sp. Some of these fungi are known to play a crucial role in the development of healthy plants and protection of potato and tomato plants from other pathogens. This study contributes significantly to our understanding of the microbial diversity in the Vindhyachal forest and its potential use in the biological control of plant diseases.

FUTURE SCOPE

Looking ahead, this research could lead to the development of effective, environmentally friendly biocontrol agents for Early Blight. The potential applications extend beyond just potato and tomato crops, potentially benefiting a wide range of agricultural practices. Furthermore, the study enhances our understanding of microbial diversity, which could lead to the discovery of more biocontrol agents in the future.

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Conflict of Interest. None.

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