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Study on soil fungal diversity of laokhowa wildlife sanctuary, Kathalguri, Assam

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Abstract
The present study was aimed to access the diversity of soil fungi of Laokhowa Wildlife Sanctuary, Kathalguri, Assam. Survey and collection were carried out during October 2023 to April 2024 from four random sites of the sanctuary. Fungal colonies were isolated by serial dilution followed by potato dextrose agar (PDA) plating methods. From the present study, a total of 25 fungal isolates were recorded out of which 15 isolates were identified. The identified species are distributed under the Divisions Ascomycota with 3 genera and Mucormycota with 2 genera. The dominant fungal genera were *Aspergillus* with 7 species and followed by *Penicillium* with 4 species. Pure cultures of all the fungal isolates were deposited in Department of Botany, University of Science & Technology Meghalaya and Meghalaya.

Keywords: *Aspergillus Penicillium*, pure cultures, serial dilution, soil fungi

Introduction
Fungal diversity is a crucial component of overall microbial and biological diversity. The notable role of microorganisms in maintaining and sustaining human and environmental health is exceptionally remarkable. Fungi are found in abundance in the environment including soil, litter, water, air, as endophytes, symbionts and pathogens. Soil serves as an excellent medium for sustaining fungal diversity attributed by rich soil organic matter from decomposition, availability of mineral nutrients and exudates from plant roots. According to Dubey and Maheshwari (2004) [5] a gram of productive soil may contain 10^5 to 10^6 of fungal cells. Among the different types of soil, forest soil holds high fungal diversity. Study on global terrestrial ecosystems showed that ecosystem stability correlated positively with the richness of fungal decomposers, but negatively with the richness of fungal plant pathogens (Wang *et al*., 2022) [13]. Moreover, research has shown that soil biodiversity can have an equal or irreplaceable effect on ecosystem stability with plant diversity (Yang *et al*., 2021) [14]. Thus studies on composition of soil biodiversity are of prime importance for understanding and maintaining health and viability of ecosystem.

Northeast India comprised of eight states and the region is distinguished for its diverse and eminent biological wealth. Along with other Northeastern states, Assam is a part of Eastern Himalayan which is a constituent unit of global biodiversity hot spot (Moghe, 2011) [9]. Due to rapid loss in forest cover, there prevails an urgent need to conserve and document the status of biological diversity in this region with emphasis on protected areas which remain largely unexplored. The state's forest covers support ample diversity of floras and faunas and at the same time provide other numerous valuable forest products. The total forest cover of Assam stood at 39% in 1987, which regrettably came down to 35% in 2016 (Forest Survey of India, 2016) and with increasing rate of urbanization, it is expected that forest cover is reducing at a rapid rate.

Most of the protected areas of Assam, including Laokhowa Wildlife Sanctuary suffer from encroachment and settlement issues (Bora, 2004; Phukan and Sarma, 2004) [1, 12]. Laokhowa Wildlife Sanctuary is located at the flood-plain forests of Assam and it forms an intrinsic part of the Laokhowa and Burhachapori ecosystem of the Southern bank of river Brahmaputra (Phukan and Sarma 2004) [12]. The flood-plain forest is unique and an endangered ecosystem (Junk and Welcomme, 1990) [7]. According to the nagaon.assam.gov.in (last accessed 17th April, 2025).

Laokhowa Wildlife Sanctuary is primarily a flood-plain grassland area attached to woodlands and numerous wetlands on the bank of river Brahmaputra. A couple of studies on soil physic-chemical properties and floristic diversity of Laokhowa Wildlife Sanctuary has been conducted in the past (Nath and Sarma, 2008; Deori and Thalukdar, 2017) [11, 2]. However, study on soil microbial or fungal diversity from Laokhowa Wildlife Sanctuary is not known till date, to the best of our knowledge. Hence the present study was conducted to obtain preliminary data of the soil fungal diversity of Laokhowa Wildlife Sanctuary.

Materials and Methods

- **Study area and sample collection:** Laokhowa Wildlife Sanctuary is one of the protected areas of Assam and is situated in Northern part of Nagaon district under the Nagaon Wildlife Division. The sanctuary is located between 92º37'91" to 92º47'23" E longitude and 26º28'32" to 26º32'14" N, at an elevation ranging from 35 to 60 meters above sea level (MASL).
- **Sample collection:** Collection of soil samples were carried out during October 2023 to April 2024. The soil samples were collected from different depths of soil ranging from 2-10 cm from four random sites.
- **Sterilization and establishment of fungal cultures:** All the experimental glassware's were sterilized in hot air oven at temperature of 120°C for 60 minutes. Potato dextrose agar (PDA) was used for establishment of mix as well as pure fungal cultures. The prepared PDA was sterilized using autoclave. Serial dilution plate method (Johnson and Curl, 1972) was used for inoculation of fungal isolates. The inoculated plates were incubated in incubator for 7 days at 25±2ºC to allow fungal growth. For establishment of pure cultures, streptomycin was added to PDA medium before pouring. After 5 to 7 days pure cultures of the fungal isolates were obtained and the petri plates were transferred to refrigerator and maintained at 4°C.
- **Identification:** Identification of the fungal species was done consulting Diba *et al*. (2007) [4], Nagamani *et al*. (2006) [10] and mycobank.org database (Last Accessed 12 April, 2025).

Result and Discussion

From the present investigation, a total of 25 fungal isolates were recorded out of which 15 isolates were identified. The identified species are distributed under the Divisions Ascomycota with 3 genera and Mucormycota with 2 genera. The dominant fungal genera were *Aspergillus* with 7 species and *Penicillium* with 4 species (Table 1; Fig. 1). Two *Mucor* species and one each species of *Cladosporium* and *Rhizopus* were identified. The identified fungal species were: *Aspergillus awamori, A. clavatus, A. flavus, A. fumigatus, A. kanagawaensis, A. Niger, A. ochraceus, Penicillium adametzi, P. chrysogenum, P. citrinum, P. glabrum, Mucor circinelloides, M. hiemalis, Cladosporium cladosporioides* and *Rizhopus microspores*.

Three *mycelia sterilia* (MS): MS1, MS3 and MS3 were also isolated. Seven fungal isolated could not be identified due to time constrain. Pure cultures of all the fungal isolates were deposited in Department of Botany, University of Science & Technology Meghalaya and Meghalaya, India. The photographs of selected mix and pure cultures and microscopic reproductive structures are shown in Figure 2. Reproductive structures of selected fungi are shown in Figure 3. The present study showed that Laokhowa Wildlife Sanctuary harbors various fungal species with *Aspergillus* and *Penicillium* as the dominant genera.

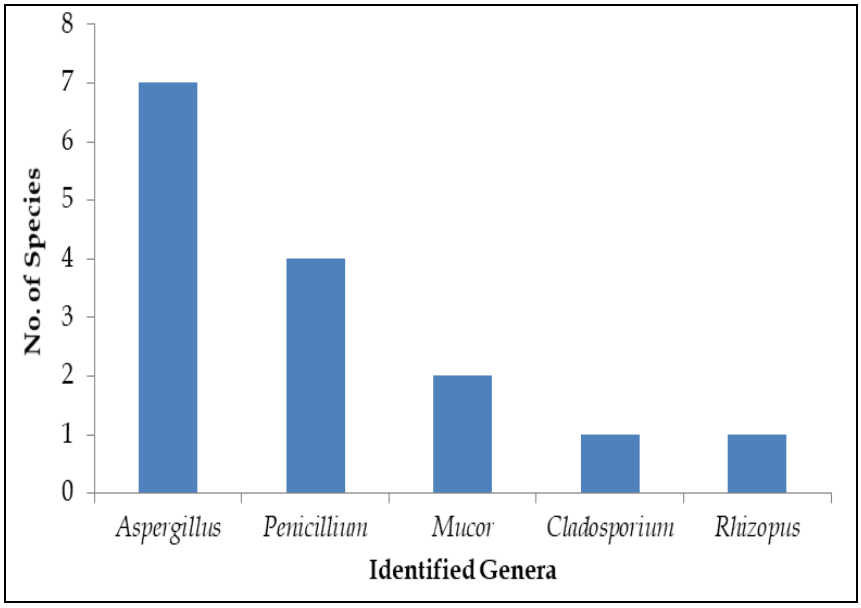

Fig 1: Number of species identified under the 5 genera

Table 1: List of identified fungal species

Division	Family	Genus	Species
Ascomycota	Aspergillaceae	*Aspergillus*	*A. awamori, A. clavatus, A. flavus, A. fumigatus, A. kanagawaensis, A. niger, A. ochraceus*
		Penicillium	*P. adametzi, P. chrysogenum, P. citrinum, P. glabrum,*
	Davidiellaceae	*Cladosporium*	*C. cladosporioides*
Mucormycota	Mucoraceae	*Mucor*	*M. circinelloides, M. hiemalis,*
		Rizhopus	*R. microspores*

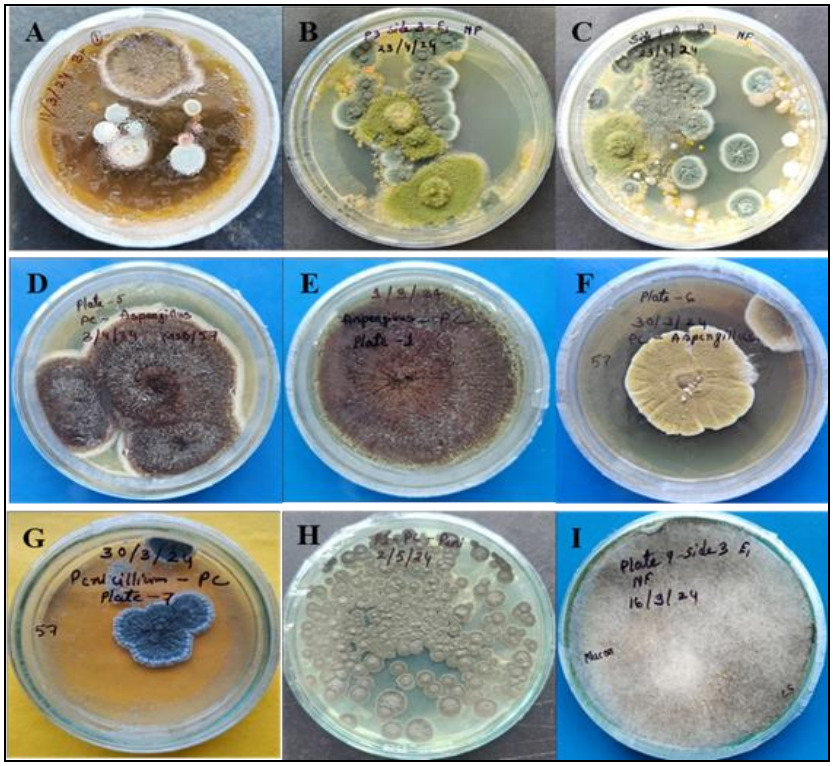

Fig 2: A-C: Mix culture plates; D-I: Pure cultures of *Aspergillus fumigatus*, *A. Niger*, *A. ochracous*, *Penicillium chrysogenum*, *P. Citrinum* and *Mucor hiemalis* respectively

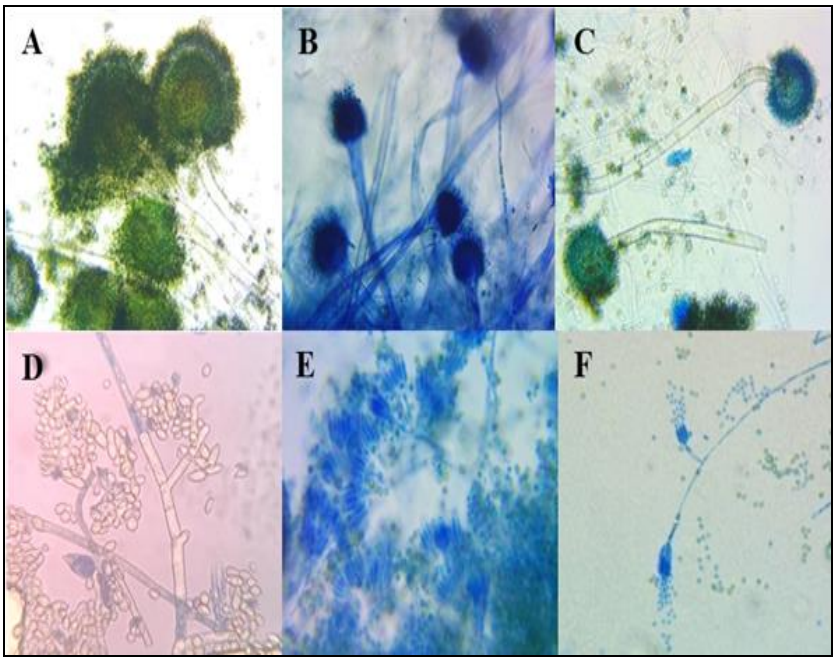

Fig 3: A-F: Reproductive structures of *A. flavus*, *A. Niger*, *A. ochracous*, *C. cladosporioides*, *P. chrysogenum* and *P. citrinum* respectively.

Aspergillus and *Penicillium* are two of the most dominant fungal groups and can be isolated from various sources including soil, food, air, litters, etc. The abundance of these fungi has also been reported by numerous researchers including Kayang (2006) [8] and Devi (2017) [3]. There has been report that soil fungal diversity as strong link with plant diversity as compared to bacterial diversity (Wang *et al*., 2022) [13]. Therefore, studies on soil fungal diversity along with forest tree diversity and their relationships will allow us to get a better understanding forest-trees and fungal diversity relationship in the future. The present study is a preliminary study and it is recommended to conduct detail researches on fungal diversity of Laokhowa Wildlife Sanctuary in the near future.

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Conflict of Interest: The authors have no conflict of Interest to declare.

Financial Support: Not available

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