SEASONAL VARIATION AND DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM DIFFERENT PARTS OF *Andrographis paniculata* (BURM. F.) WALL. EX NEES

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Abstract

A total of 18 species of endophytic fungi were isolated from *Andrographis paniculata* plant. The fungi were: *Aspergillus flavus, A. niger, A. terreus, Cladosporium* sp., *Colletotrichum* sp., *Curvularia chonburiensis, Curvularia lycopersici, Fusarium solani, Fusarium udum, Fusarium* sp. 1, *Lasiodiplodia theobromae, Monodictys paradoxa, P. chrysogenum, P. oxalicum, Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3, and *Scytalidium lignicola.* In summer, 18 species of fungi were isolated from *A. paniculata* while 13 fungal species were isolated in winter season. The highest value of Shannon and Simpson diversity index was found in the leaf in summer season and the lowest value in the stem in winter season. Maximum species richness of endophytic fungi was recorded in leaf in Margalef's index and Menhinick index in winter season. Minimum species richness of endophytic fungi was recorded in stem in Margalef's index and Menhinick index in summer season.

Key words: Endophytic fungi; *Andrographis paniculata*; Species richness; Species diversity.

INTRODUCTION

The term "endophytic fungi" pertains to fungi that establish a mutually beneficial symbiotic relationship with plant tissues, either throughout their entire life cycle or partially, without causing harm or disease to the host (Hyde *et al.* 2019, Patchett and Newman 2021). Plant colonization by various endophytic microorganisms is common, particularly in perennials, encompassing diverse polyphyletic groups thriving asymptomatically in healthy tissues such as stems, leaves, and roots (Stone *et al.* 2004, Demain 2014, Faeth and Fagan 2002). It is estimated that all terrestrial plants host one or more species of endophytic fungi (Dos *et al.* 2022). Despite their ubiquity in nature, the distribution and diversity of endophytic fungi remain largely unknown, owing to their intricate morphological structures and lifestyles often dependent on other organisms.

Andrographis paniculata, commonly known as "King of Bitters" or kalmegh (in Bangladesh), is an annual herb of the Acanthaceae family, reaching a height of half to one meter. Widely recognized for its medicinal properties, *A. paniculata* has been traditionally used across Asia, America, and Africa for centuries to treat various ailments (Okhuarobo *et al.* 2014). Utilized in traditional herbal medicine in multiple countries, including Bangladesh, China, India, Malaysia, and Thailand. The plant serves as an antidote for snakebites, treats dyspepsia, influenza, dysentery, malaria, respiratory infections, and various other conditions (Chopra 1980, Jarukamjorn *et al.* 2010). Leaf extracts are used to address infectious diseases, fever, colic pain, loss of appetite, irregular stools, and diarrhea, while the roots are employed for their febrifuge, tonic, stomachic, and anthelmintic properties (Saxena *et al.* 1998, Chopra 1980).

The endophytic fungi associated with medicinal plants exhibit significant abundance and diversity, depending on the host organism. Recent interest in endophytic fungi has surged due to their potential synthesis of pharmacologically active compounds with broad biotechnological applications. This study aims to elucidate the diversity of endophytic fungi in *A. paniculata* concerning different plant parts and assess seasonal variations in endophytic fungal populations relative to their abundance.

MATERIAL AND METHODS

Plant materials

Matured and healthy leaf, stem and root samples of *Andrographis paniculata* (Burm. f.) Wall. ex Nees were collected from the botanical garden of Curzon Hall Campus, University of Dhaka. The samples were collected in summer and winter seasons. The samples were collected from March 2021 to January 2022 (In summer, the samples were collected from March to July 2021 and in winter it was collected from November 2021 to January 2022).

Isolation of endophytic fungi

Fungal isolation was done using the "Tissue planting method" (CAB 1968) on PDA (Potato Dextrose Agar) medium. The samples were treated within 48 hours after collection. The preserved leaf, stem and root samples of *A. paniculata* were rinsed in running tap water, cut into small segments about 3×3 mm^2 in size with the help of a sterilized scissor/blade under aseptic conditions and was immersed in a 2 - 4% aqueous solution of sodium hypochlorite (NaOCl, Clorox solution) for 1 to 1.5 minutes. Then the samples were rinsed out three times with sterile distilled water and allowed to surface dry on sterilized filter papers inside Petri plates under aseptic conditions. The surface sterilized plant segments were then placed on sterilized Petri plates containing potato dextrose agar medium (PDA). Petri plates containing PDA medium with inocula were incubated at 25±2ºC temperature in the incubation chamber. Any hyphae that extended from the leaf fragments were used in a successive culture with PDA.

Identification of fungi

Morphological studies of the isolated fungi were done to identify the fungi primarily depending on the colony colour, texture, shape, diameter, surface appearance following standard literatures (Thom and Raper 1945, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Barnett and Hunter 1972).

Molecular identification of the isolates was performed using the internal transcribed spacer (ITS) region. PCR amplification was conducted using ITS4 as reverse primers. The PCR was initiated by an initial denaturation step at 94°C for 5 minutes following 30 cycles of 94, 54 and 72°C each for 30 sec, with a final extension step of 5 min at 72°C and ended with 4°C. Sequence alignment and editing were done with the BioEdit Sequence Alignment program and compared against the sequences already available in the databases using the programme BLASTn (http://www.ncbi.nlm.nih.gov/BLAST, Nessa *et al.* 2023).

Data analysis

Determination of percent frequency of the isolated endophytic fungi: The percent frequency of the fungi was calculated following the standard formula given below:

$$Percent\ frequency = \frac{Total\ number\ of\ colonies\ of\ the\ same\ fungi}{Total\ number\ colonies\ of\ different\ fungi} \times 100\%$$

Analysis of diversity indices: Three indices were used to estimate species diversity, species richness and species evenness following Ludwig and Reynolds (1988).

RESULTS AND DISCUSSION

A total of 18 species of endophytic fungi were isolated from *A. paniculata* plant following 'Tissue planting' method. The isolated fungi were: *Aspergillus flavus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Colletotrichum* sp., *Curvularia chonburiensis*, *C. lycopersici*, *Fusarium solani*, *F. udum*, *Fusarium* sp. 1, *Lasiodiplodia theobromae*, *Monodictys paradoxa*, *P. chrysogenum*, *P. oxalicum*, *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3, and *Scytalidium lignicola* (Table 1).

Table 1. Endophytic fungi associated with leaf, stem and root of *Andrographis paniculata.*

Name of fungi	Leaf	Stem	Root
Aspergillus flavus	+	+	+
A. niger	+	+	-
A. terreus	+	+	+
Cladosporium sp.	+	+	-
Colletotrichum sp.	+	-	-
Curvularia chonburiensis	-	+	-
Curvularia lycopersici	+	-	-
Fusarium solani	+	-	+
Fusarium udum	-	+	+
Fusarium sp. 1	+	-	+
Lasiodiplodia theobromae	+	-	-
Monodictys paradoxa	+	-	-
Penicillium chrysogenum	+	-	+
P. oxalicum	-	+	+
Penicillium sp. 1	+	+	+
Penicillium sp. 2	+	+	-
Penicillium sp. 3	-	-	+
Scytalidium lignicola	+	-	-

'+' and '–' represent the presence and absence of fungi, respectively.

In summer, the highest frequency percentage of *Fusarium solani* (17.46%) was isolated from the healthy leaves of *A. paniculata* and *Penicillium* sp. 2 showed lowest percentage frequency (1.59%). Whereas in winter, *Penicillium* sp. 1 showed highest frequency percentage (28.38%) and the lowest frequency was (2.22%) in *A. flavus. Aspergillus niger*, *A. terreus*, *Fusarium solani* and *Monodictys paradoxa* were found in summer season. During summer season the highest frequency percentage (21.11%) was found in the stem of *Fusarium udum* and the lowest frequency percentage in *A. terreus* (5.59%).

In winter season, the highest frequency percentage was (58.79%) found in *Penicillium* sp. 1 and the lowest frequency percentage was (2.22%) found in *Cladosporium* sp. *Aspergillus. niger*, *A. terreus*, and *Curvularia chonburiensis* were found only in summer season. In root, the highest frequency percentage was found in *Fusarium solani* (23.59%) during summer season and the lowest frequency percentage in

Penicillium sp. 4 (2.22%). In winter season, the highest frequency percentage was found in *P. oxalicum* (43.521%) and the lowest frequency in *A. flavus* (2.78%). *Apergllus terreus*, *Fusarium solani*, and *F. udum* were found only in summer season (Fig. 1 and 2).

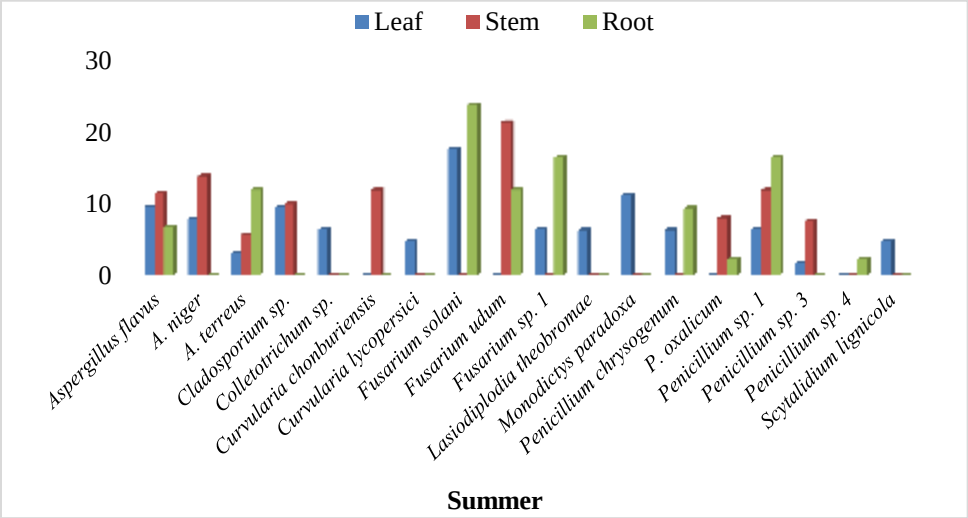

Fig. 1. Percent frequency of endophytic fungi associated with the leaf, stem and root of *Andrographis paniculata* during summer.

The highest value of Shannon diversity index (2.51) and Simpson diversity index (0.92) was found in the leaf in summer season, which indicates high endophytic fungal species diversity in present study and the lowest value of Shannon diversity index (1.32) and Simpson diversity index (0.65) was found in the stem in Winter season which indicates low endophytic fungal species diversity in present study.

Table 2. Shannon diversity index (H) and Simpson diversity index (D) of endophytic fungi.

Parts of plant	Season	Shannon diversity index (H)	Simpson diversity index (D)	Shannon Evenness index (E)	Margalef index (R_1)	Menhinick index (R_2)
Leaf	Summer	2.51	0.92.	0.953	3.1258	1.75
	Winter	2.05	0.86	0.890	3.4563	2.135
Stem	Summer	2.13	0.87	0.970	2.0247	1.2481
	Winter	1.32	0.65	0.737	2.2155	1.4796
Root	Summer	2.01	0.87	0.916	2.127	1.3725
	Winter	1.44	0.75	0.805	2.3521	1.6432

The highest value of Shannon evenness index was found in the stem in summer season (0.970) which indicates the number of endophytic fungal species within this plant was fairly constant and the lowest value was found in the stem in winter season (0.737) which indicates the number of endophytic fungal species within this plant part during winter was not constant. Maximum species richness of endophytic fungi was recorded in leaf with 3.4563 in Margalef's index and 2.135 in Menhinick index in winter season indicating the increase of endophytic fungal biodiversity in present study. Minimum species richness of endophytic fungi was recorded in stem with 2.0247 in Margalef's index and 1.2481 in Menhinick index in summer season indicating the decrease of endophytic fungal biodiversity (Table 2).

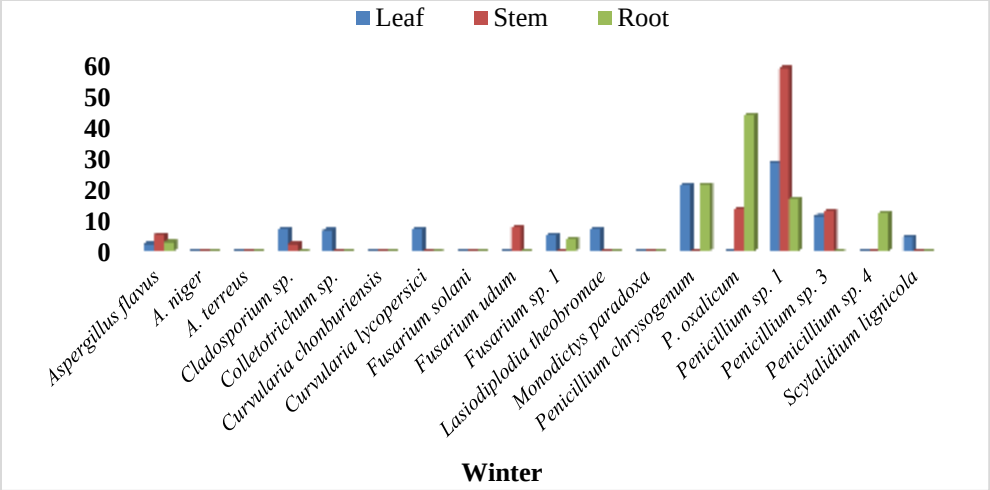

Fig. 2. Percent frequency of endophytic fungi associated with the leaf, stem and root of *Andrographis paniculata* during winter.

This study reveals that the diversity of endophytes varies among different plant parts, demonstrating seasonal fluctuations. Specifically, 18 distinct species were identified during the summer, while 13 were observed in winter. Notably, the leaf exhibited the highest diversity (H), with a greater number of endophytes isolated during the summer season. This suggests a potential impact of climate on endophytic dispersal, aligning with previous findings (Schulz and Boyle 2005). Furthermore, our results indicate a seasonal disparity in the number of morphotypes attributed to endophytic fungi. Present findings contribute valuable insights into the dynamic relationship between plants and endophytes, shedding light on the influence of plant parts and seasonal variations in endophytic fungal communities.

REFERENCES

Barnett, H. L. and B. B. Hunter. 1972. *Illustrated Genera of Imperfect Fungi*. 3rd ed. Burges Pub., Co. Minneapolis, USA. 185 pp.

Benoit, M. A. and S. B. Mathur. 1970. Identification of species curvularia on rice seed. *Proc. Int. Seed Testing Ass. Proc*. **35**(1): 1-23.

Booth, C. 1971. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 273 pp.

CAB. 1968. *Plant Pathologist's Pocket Book*. 1st ed. The Commonwealth Mycological Institute, England. 267 pp.

Chopra, R. N. 1980. *Glossary of Indian Medicinal Plants*. Council for Scientific and Industrial Research. New Delhi, India. 18 pp.

Demain, A. L. 2014. Importance of microbial natural products and the need to revitalize their discovery. *J. Ind. Microbiol. Bio. Technol*. **41**:185-201.

Dos, R. J. B. A., A. S. Lorenzi and H. M. M. do Vale. 2022. Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. *Arch Microbiol*. **204**(11): 675.

Ellis, M. B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 608 pp.

Ellis, M. B. 1976. *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 507 pp.

Faeth, S. H. and W. F. Fagan. 2002. Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integr. Comp. Biol*. **42**: 360-368.

Hyde, K. D., J. C. Xu, S. Rapior, R. Jeewon, S. Lumyong, A. G. T. Niego, P. D. Abeywickrama, J. V. S. Aluthmuhandiram and R. S. Brahamanage. 2019. The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Divers*. **97**: 1-136.

Jarukamjorn, K., S. Kondo, W. Chatuphonprasert, T. Sakuma, Y. Kawasaki and N. Emito. 2010. Gender-associated modulation of inducible CYP1A1 expression by andrographolide in mouse liver. *Eur. J. Pharm Sci*. **39**: 394-401.

Ludwig, J. A. and J. F. Reynolds. 1988. *Statistical Ecology-A Primer on Methods and Computing*. John Wiley and Sons, Inc. New York, USA. 337 pp.

Nessa, F., S. Shamsi and M. A. A. Noman. 2023. Morphological and molecular charactriization of endophytic fungi isolated from *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Centella asiatica* (L.) Urban. *Bangladesh J. Taxon*. **30**(1): 111-122.

Okhuarobo, A., J. E. Falodun, O. Erharuyi, V. Imieje, A. Falodun and P. Langer. 2014. Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pac. J. Trop. Dis*. **4**(3): 213-322.

Patchett, A. and J. A. Newman. 2021. Comparison of plant metabolites in root exudates of *Lolium perenne* infected with different strains of the fungal endophyte *Epichlo festucae* var. *lolii*. *J. Fungi.* **7**: 148.

Saxena, S., D. C. Jain, R. S. Bhakuni and R. P. Sharma. 1998. Chemistry and pharmacology of *Andrographis* species. *Indian Drugs*. **35**: 458-467.

Schulz, B. and C. Boyle. 2005. The endophytic continuum. *Mycol. Res.***109**: 661-686.

Stone, J., J. D. Polishook and J. F. White. 2004. Endophytic fungi. In: G. M. Mueller, G. F. Bills and J. F. White (eds.). *Biodiversity of Fungi*. Elsevier, Amsterdam., pp. 241-270.

Thom, C. and K. B. Raper. 1945. *A Manual of the Aspergilli*. Williams and Wilkins, Baltimore, MD., USA. 373 pp.

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