**Morphological Characterization of Fungal Endophytes Isolated from the leaves and roots of Banana plants (*Musa* sp.)**

**ABSTRACT**

In recent years, fungal endophytes are being studied extensively for their crucial role in plants growth promotion as well as their role in antagonistic activity against many plant pathogens. In the present investigation, focus was made to study fungal endophytes, to isolate and morphologically characterized and identify from the leave and roots of wild and locally cultivated bananas from the Chumoukedima district, state Nagaland, India. Altogether 58 fungal endophytes were isolated and out of these, 43 isolates were from the leave samples and 15 isolates from the root samples. Out of these, 50 isolates were from the wild banana plants (38 isolates from the leaf samples and 12 from the root samples) and 8 isolates from the cultivated banana plants (5 from the leaf samples and 3 from the root samples). Based on the morphological studies, seven isolates were identified as *Penicillium* sp., five isolates as *Trichoderma* sp., five isolates as *Diaporthe* sp., four isolates as *Fusarium* sp., three isolates as *Aspergillus* sp., three as *Colletotrichum* sp. and one each of *Apiospora* sp. and *Botrytis* sp. A total of 20 isolates were identified as Mycelia sterilia. A total of eight genera were identified that belonged to the Phylum Ascomycota.

***Keywords:*** *Fungal endophytes, banana, isolation, characterization.*

1. **INTRODUCTION**

Fungal endophytes are microscopic organisms that inhabits the internal tissues of different parts of plants and does not cause any harm to the host plant. Microorganisms like fungi and bacteria are known to exist as endophytes in plants (Carbungco et al., 2017). In agriculture, endophytes can be used to improve the performance of crops since they are able to colonize the internal tissues of plants, their ability to promote growth of plants and in controlling diseases of plants (Yuan et al., 2009). Banana belongs to the genus *Musa* (Musaceae, Zingiberales), and is one of the most important fruit crops. In India, banana rank first among the fruit crops and occupies third in terms of area. Banana (*Musa* spp.) continues to control fruit market in the world, with cultivation taking place in more than 135 nations (FAO, 2021). Its production and cultivated areas have grown throughout the years (FAO, 2021). Despite it being holding a major share in the market, there are several diseases that impacts the growth and production of banana throughout the growing regions in the world. Many of the current methods in managing the important diseases of banana are laborious and non-economical. Biological control is an additional strategy for controlling diseases in a more harmonious way, and the hunt for antagonistic microorganisms has produced a number of very active antagonistic fungi and bacteria (Saravanan et al., 2004 and Getha et al., *2005*). Endophytic fungi can protect their hosts from various biotic and abiotic factors like attacks from insect pest, plant pathogens and herbivores (Bamisile et al., 2018). Plants and endophytic fungi interactions results in production of several interesting biotechnological substances. Endophytic fungi are known to produce secondary metabolites, they are considered to be rich source of bioactive compounds and enzymes (Pilnik et al., 1985). Fungal endophytes are also known to be rich source of novel antimicrobial substances that provides protection to host plants against plant diseases (Panka et al., 2013). There are relatively only few studies that have been focussed on the study of fungal endophytes associated with banana in India and no studies have been done on the fungal endophytes of banana plants from Nagaland, a northeastern state of India. Hence, the present investigation was undertaken to study the fungal endophytes collected from the leaves and roots of wild and locally cultivated banana species of Chumoukedima district, Nagaland, India.

1. **MATERIALS AND METHODS**

**2.1 Sample collection**

Collection of samples was carried out for isolation of fungal endophytes from the leaves and roots of wild and locally cultivated banana plants from four location sites of Chumoukedima district, Nagaland, India. Twelve samples each from the healthy leaves and roots of wild banana and one sample each from the healthy leaves and roots of cultivated banana was collected.

**2.2 Isolation of fungal endophytes from leaves and roots**

Isolation of fungal endophytes from the healthy leaf samples was done as per the method given by Zakaria and Aziz (2018). The samples were wash with distilled water and air dried, 1 cm of the healthy leaf segment was cut with sterilized blade and the cut bits were sterilized with 2% sodium hypochlorite solution for 3 minutes, then rinsing it with sterilized distilled water (1 min), blot drying with filter paper to remove excess water. The blot dried leaf bits were placed in Potato Dextrose Agar (PDA) plates and incubated at 25±1°C. Four leaf bits were placed on each plate and incubated for 1-4 days for the mycelium to grow. The plates were observed periodically and the mycelium arising from the leaf bits were sub cultured onto new PDA plates. The plates were maintained for further investigation.

Isolation of fungal endophytes from the healthy root samples was carried out as per the method given by Zakaria et al. (2016). The roots were collected and placed in plastic bags and cleaned with running water to remove the soil that were attached to them. The roots were cut into tiny pieces and surface sterilized with 70% ethanol for 30 seconds, 1% sodium hypochlorite for 3 minutes, and 95% ethanol for 5 minutes. The roots were then washed with sterilized distil water for 3 times. The washed roots were dried in sterile filter paper and the root fragments were cut into much tiny pieces and placed onto PDA plates. The plates were incubated at 27±1°C to monitor for any fungal growth from the fragmented roots. On fresh PDA plates, mycelial growths from the roots were sub-cultured.

**2.3 Morphological characterization of the fungal endophytes**

The isolated fungal endophytes were identified based on their morphological and cultural characteristics and the result was compared with published literatures. Photomicrographs and measurements of all the isolated fungal endophytes were also recorded. The fungal endophyte isolates were grown on PDA medium for up to 10 days and colony description of each isolate were recorded. Colony characters such as color, texture, presence or absence of spores were recorded (Wattanabe, 2012). An average of 100 spores/conidia was taken.

1. **RESULTS AND DISCUSSION**

Healthy leaf and root samples from the banana growing regions of Chumoukedima district, Nagaland was collected and isolated on PDA medium to study their morphological characteristics. Thirteen samples were collected and out of these, 12 samples were from the wild banana plants and 1 sample from the cultivated banana plant. A total of 58 fungal endophytes were isolated from the leaves and roots of banana plants. Out of these, 50 isolates were from the wild banana plants (38 isolates from the leaf samples and 12 from the root samples) and 8 isolates from the cultivated banana plants (5 from the leaf samples and 3 from the root samples).

Table 1 shows the morphological characterization of fungal endophytes isolated from the leaves and roots of wild and locally cultivated banana plants. Basic characters like texture, colony color, microscopic characters like presence or absence of spores, color, size, shape were observed and analysed for species identification (Wattanabe, 2012). Based on morphological characteristics, seven isolates were identified as *Penicillium* sp. (FE1, FE8, FE10, FE11, FE12, FE14 and FE56), five isolates as *Trichoderma* sp. (FE5, FE6, FE44, FE46 and FE47), five isolates as *Diaporthe* sp. (FE20, FE27, FE29, FE30 and FE32), four isolates as *Fusarium* sp. (FE3, FE9, FE26 and FE34), three isolates as *Aspergillus* sp. (FE23, FE49 and FE51), three as *Colletotrichum* sp. (FE36, FE39 and FE43) and one each of *Apiospora* sp. (FE24) and *Botrytis* sp. (FE58). A total of 20 isolates were identified as Mycelia sterilia (FE2, FE7, FE15, FE16, FE17, FE18, FE19, FE21, FE22, FE25, FE31, FE35, FE37, FE38, FE40, FE42, FE48, FE52, FE54 and FE57). Out of the seven identified *Penicillium* sp., six species were isolated from the leaves of wild banana and one from the roots of wild banana. For *Trichoderma* sp., two species were isolated from the leaves and two species from the roots of wild banana. All the

**Table 1. Morphological characteristics of the isolated fungal endophytes**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Isolate No.** | **Tissue part** | **Wild/ Cultivated** | **Colony color** | **Texture** | **Spore/ Conidia** | **Organism** |
| **Present/ Absent** | **Size** | **Color** |
| 1. | FE1 | Leaves | Wild | Light pink | Powdery | Present | 10-12 μm | Bluish green | *Penicillium* sp. |
| 2. | FE2 | Leaves | Wild | White | Cottony | Absent | Absent | Absent | Mycelia sterilia |
| 3. | FE3 | Leaves | Wild | White | Light cottony | Present | 67.07 x 24.09 μm | Hyaline | *Fusarium* sp. |
| 4. | FE4 | Leaves | Wild | Dark grey | Velvety | Present | 46.05 x 19.00 μm | Brown | Unidentified |
| 5. | FE5 | Leaves | Wild | Green | Light cottony | Present | 16 x 12 μm | Greenish | *Trichoderma* sp. |
| 6. | FE6 | Leaves | Wild | Dark green | Cottony | Present | 10.72 – 12.17 μm | Yellowish green | *Trichoderma* sp. |
| 7. | FE7 | Leaves | Wild | White | Cottony | Absent | Absent | Absent | Mycelia sterilia |
| 8. | FE8 | Leaves | Wild | Light pink | Powdery | Present | 10 – 12 μm | Bluish green | *Penicillium* sp. |
| 9. | FE9 | Leaves | Wild | White | Cottony | Present | 67.07 x 24.09 μm | Hyaline | *Fusarium* sp. |
| 10. | FE10 | Leaves | Wild | Grey | Powdery | Present | 10.14 -12.07 μm | Bluish to olive green | *Penicillium* sp. |
| 11. | FE11 | Leaves | Wild | Greyish | Powdery | Present | 20.08 – 24.04 μm | Bluish to Olive green | *Penicillium* sp. |
| 12. | FE12 | Leaves | Wild | Olive green | Powdery | Present | 15 – 20 μm | Bluish to olive green | *Penicillium* sp. |
| 13. | FE13 | Leaves | Wild | Off white | Fluffy | Present | 37.35 μm | Hyaline | Unidentified |
| 14. | FE14 | Leaves | Wild | Light pink | Powdery | Present | 10 – 12 μm | Bluish green | *Penicillium* sp. |
| 15. | FE15 | Leaves | Wild | Off white | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 16. | FE16 | Leaves | Wild | Off white | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 17. | FE17 | Leaves | Wild | White | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 18. | FE18 | Leaves | Wild | Light brownish to off white | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 19. | FE19 | Leaves | Wild | Light brown to white | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 20. | FE20 | Leaves | Wild | Brownish center with white margin | Light cottony | Absent | - | - | *Diaporthe* sp. |
| 21. | FE21 | Leaves | Wild | Dark bluish grey | Velvety | Absent | Absent | Absent | Mycelia sterilia |
| 22. | FE22 | Leaves | Wild | White | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 23. | FE23 | Leaves | Wild | Bluish green | Powdery | Present | 10 – 12 μm | Greenish | *Aspergillus* sp. |
| 24. | FE24 | Leaves | Wild | White | Light cottony | Present | 58.61 μm | Olive to dark brown | *Apiospora* sp. |
| 25. | FE25 | Leaves | Wild | Grey | Cottony | Absent | - | - | Mycelia sterilia |
| 26. | FE26 | Leaves | Wild | Light yellow | Cottony | Present | 47.39 x 23.20 μm | Hyaline | *Fusarium* sp. |
| 27. | FE27 | Leaves | Wild | White | Light cottony | Absent | - | - | *Diaporthe* sp. |
| 28. | FE28 | Leaves | Wild | Dark grey | Light cottony | Absent | Absent | Absent | Unidentified |
| 29. | FE29 | Leaves | Wild | Off white | Light cottony | Absent | - | - | *Diaporthe* sp. |
| 30. | FE30 | Leaves | Wild | White | Light cottony | Absent | - | - | *Diaporthe* sp. |
| 31. | FE31 | Leaves | Wild | White | Velvety | Absent | Absent | Absent | Mycelia sterilia |
| 32. | FE32 | Leaves | Wild | Whitish black with brown margin | Light cottony | Absent | - | - | *Diaporthe* sp. |
| 33. | FE33 | Leaves | Wild | Grey | Cottony | Present | 180.42 x 50.57 μm | Hyaline | Unidentified |
| 34. | FE34 | Leaves | Wild | Initially white to beige color | Cottony | Present | 53.35 x 6.14 μm | Hyaline | *Fusarium* sp. |
| 35. | FE35 | Leaves | Wild | Off white to light grey | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 36. | FE36 | Leaves | Wild | Blackish off white | Dense cottony | Present | 114.64 x 35.16 μm | Hyaline | *Colletotrichum* sp. |
| 37. | FE37 | Leaves | Wild | Blackish off white | Fluffy | Absent | Absent | Absent | Mycelia sterilia |
| 38. | FE38 | Leaves | Wild | Greyish black | Velvety | Absent | Absent | Absent | Mycelia sterilia |
| 39. | FE39 | Leaves | Cultivated | Off white with orange color acervuli | Light cottony | Present | 8510 x 35.86 μm | Hyaline | *Colletotrichum* sp. |
| 40. | FE40 | Leaves | Cultivated | Brownish white | Velvety | Absent | Absent | Absent | Mycelia sterilia |
| 41. | FE41 | Leaves | Cultivated | Off white | Light cottony | Present | 115.39 μm | Dark brown | Unidentified |
| 42. | FE42 | Leaves | Cultivated | Off whitish to light grey | Velvety | Absent | Absent | Absent | Mycelia sterilia |
| 43. | FE43 | Leaves | Cultivated | Grey | Light cottony | Present | 60.45 x 32.41 μm | Hyaline | *Colletotrichum* sp. |
| 44. | FE44 | Roots | Wild | Green | Light powdery | Present | 18 μm | Light green | *Trichoderma* sp. |
| 45. | FE45 | Roots | Wild | White | Light cottony | Present | 117.42 μm | Hyaline | Unidentified |
| 46. | FE46 | Roots | Wild | Whitish dark green | Cottony | Present | 10 -15.20 μm | Bluish green | *Trichoderma* sp. |
| 47. | FE47 | Roots | Wild | Whitish dark green | Cottony | Present | 10 – 16.30 μm | Bluish green | *Trichoderma* sp. |
| 48. | FE48 | Roots | Wild | Yellow | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 49. | FE49 | Roots | Wild | Black | Cottony | Present | 16 – 20 μm | Dark brown | *Aspergillus* sp. |
| 50. | FE50 | Roots | Wild | White | Velvety | Present | 8 – 10 μm | Hyaline | Unidentified |
| 51. | FE51 | Roots | Wild | Bluish-grey green | Velvety | Present | 12-18 x 10-18 μm | Hyaline | *Aspergillus* sp. |
| 52. | FE52 | Roots | Wild | Dark grey | Velvety | Absent | - | - | Mycelia sterilia |
| 53. | FE53 | Roots | Wild | Off white | Cottony | Present | 15.21 μm | Hyaline | Unidentified |
| 54. | FE54 | Roots | Wild | Dark grey | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 55. | FE55 | Roots | Wild | Dark grey | Light cottony | Present | 40.14 x 21.02 μm | Hyaline | Unidentified |
| 56. | FE56 | Roots | Cultivated | Greyish green | Powdery | Present | 8 -15 μm | Bluish green | *Penicillium* sp. |
| 57. | FE57 | Roots | Cultivated | Off white | Light cottony | Absent | Absent | Absent | Mycelia sterilia |
| 58. | FE58 | Roots | Cultivated | Grey to brown | Velvety | Present | 30.70 μm | Light brown | *Botrytis* sp. |

\*FE- Fungal endophyte; \*All the spores/conidia were observed and measured under 40x and average of 100 spores/conidia were taken.



Fig. 1. Some fungal endophytes in plates and as observed under microscope (40x)

*1.Penicillium* sp. 2. *Trichoderma* sp. 3. *Fusarium* sp. 4. *Aspergillus* sp. 5. *Colletotrichum* sp. 6. *Apiospora* sp. and 7. *Botrytis* sp.

identified *Diaporthe* sp. and *Fusarium* sp. were isolated from the leaves of wild banana plant. For *Aspergillus* sp., one species was isolated from the leaves and two from the roots of wild banana plant. For *Colletotrichum* sp., one species was isolated from the leaves of wild banana plant and two from the leaves of cultivated banana plant. *Apiospora* sp. was isolated from the leaves of wild banana plant and finally, *Botrytis* sp. was isolated from the roots of cultivated banana plant.

Photita et al. (2001) isolated fungal endophytes from 7500 samples of wild *Musa acuminata* from 5 locations and stated that *Colletotrichum gloeosporioides, Colletotrichum musae* and sterile mycelia were found to be common fungal endophytes isolated from *M. acuminata* from all the locations. Xia et al. (2011) explored the dispersal of diverse species of endophytic and epiphytic *Trichoderma* corresponding with the banana roots and isolated 189 endophytic and epiphytic *Trichoderma*. Largest group comprised of *T. asperellum, T. virens* and *Hypocrea lixii*, isolated from both the outside and inside of banana roots. Zakaria and Rahman (2011) reported the isolation of endophytic *Fusarium* species from the roots of *Musa acuminata* (wild banana) that were randomly collected from different locations in Penang Island, Malaysia and isolated 54 *Fusarium* species 100 fragments of roots and the most commonly found species were *F. oxysporum*, *F. solani* and *F. semitectum*. Zakaria and Aziz (2018) isolated fungal endophytes from banana leaves and identified 17 species belonging to 10 genera, some of which are *Colletotrichum gloeosporioides, Colletotrichum siamense, Fusarium equiseti, Fusarium chlamydosporum, Penicillium steckii, Penicillium purpurogenum* and *Aspergillus niger*. Malubag et al. (2021) isolated and identified fungal endophytes from *Musa paradisiaca* (plantain banana) and 9 fungal endophytes were identified belonging to genus *Cladosporium, Fusarium, Geotrichum, Nigrospora* and *Schizophyllum*. Thus, our study is supported by the works carried out by previous researchers, with respect to several fungal endophytes.

The identified *Penicillium* sp. were characterized by their powdery colony growth, and the colony color ranged from light pink, grey, olive green to greyish green, with the conidia observed as minute, globose or elliptical in shape, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were observed to be branched. The observed conidiophores were branched or unbranched, long or thick and hyaline. They play a crucial role in secreting bioactive compounds and they have anticancer, antibacterial and cytotoxic effects (Yadav et al., 2019). They also play an important role in food rot in industries and are well known decomposers and source of important drugs (Visagie et al., 2014).

The *Trichoderma* sp. were green to dark green or whitish dark green in color and light cottony in texture. The conidia were observed to be ellipsoidal, globose or subglobose, smooth, green in color. *Trichoderma* species are known to produce antifungal compounds and other secondary metabolites (Thangavelu and Gopi, 2015; Nagamani *et al*., 2017) which may show growth inhibiting properties and act as a defense mechanism against fungal pathogens.

The genus *Diaporthe* was identified based on their characteristic growth on the PDA plates. In general, the isolates produced brownish, whitish, grayish, blackish growth on PDA medium. *Diaporthe* genus as endophyte is known to produce secondary metabolites, and have been significantly inspected for their important compound production with various bioactivities (Abramczyk *et al*., 2022).

For *Fusarium* sp., they were mostly white in color on PDA medium with light cottony growth. Microconidia were observed in all the isolates. The microconidia were single celled, hyaline, cylindrical or oblong, some slightly curved or straight. Macroconidia were fusiform or sickled shaped and hyaline. Chlamydospores were also observed in all the isolates and they were found to be abundant, globose to ellipsoidal, thick walled, singly or in pairs. Endophytic *Fusarium* sp. are known for their antimicrobial properties and bioactive secondary metabolites and protects the plants against pest and diseases (Rana et al., 2019).

For *Aspergillus* sp., the plate color was observed to be bluish green to black. The conidia were single celled, globose to subglobose, hyaline to greenish in color for FE23 and FE51 and for FE49, the conidia were globose to subglobose, dark brown in color, rough texture, the conidiophore were observed to be smooth and hyaline. *Aspergillus* sp. is known to secrete bioactive compounds that inhibit the fungal mycelia growth through lysis of cell wall of fungi (Gomathi and Ambikapathy, 2011). Many have accounted that the *Aspergillus* genus can make lytic enzymes like glucanase (Gao *et al*., 2008) and proteases (Sethi *et al*., 2016). Additionally, they are known to produce bioactive compounds (Tiwari *et al*., 2011 and Goutam *et al*., 2017).

The *Colletotrichum* sp. were observed to be blackish off-white to grey in color in PDA plates. The conidia were one celled, ovoid to oblong, hyaline, with the presence of oil globule at the center of the conidia.The *Apiospora* sp. conidia was observed to be globose to subglobose, olive to dark brown, found in clusters under the microscope. Endophytic *Colletotrichum* sp. and *Apiospora* sp. are known to produce volatile metabolites that helps in inhibiting the growth of several plant pathogens (Rabha et al., 2014). The *Botrytis* sp. conidia was observed to be globose, light brownish, formed in clusters at the tip of the conidiophore. The conidiophore was observed to be erect, branched, septate and brownish in color.

A total of eight genera was identified in this present study and they all belong to the Phylum Ascomycota. Out of these eight genera, six genera viz., *Penicillium, Trichoderma, Diaporthe, Fusarium, Aspergillus* and *Apiospora* were only found in the isolates that were isolated from the wild banana plant. The genus *Colletotrichum* was found in both the wild and cultivated banana plant and the genus *Botrytis* was found only from the cultivated banana plant.

**CONCLUSIONS**

In the present study eight genera *viz*., *Penicillium*, *Trichoderma*, *Fusarium,* *Diaporthe*, *Aspergillus*, *Colletotrichum*, *Apiospora* and *Botrytis* were morphologically identified which shows that there are diverse group of fungal endophytes present in banana plants. These endophytes may show an ideal symbiotic relationship with the host plant in terms of protection against diseases or in their plant growth promotion. Further investigation is required to test the efficacy of these isolated fungal endophytes for their antagonistic, metabolic or other essential properties.

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Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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