

RESEARCH ARTICLE

MORPHOLOGICAL VARIATION OF FUNGAL ENDOPHYTES OF *Terminalia* spp. IMPACT OF DIFFERENT NUTRIENT MEDIA

Debajani Samantaray, Nibha Gupta*

Regional Plant Resource Centre, Bhubaneswar- 751015, Odisha, India

*Corresponding author E-mail: nguc2003@yahoo.co.in

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ABSTRACT

The growth rate of the mycelium, sporulation, and colony characters of 29 fungal endophytes from four different *Terminalia* spp. grown on five different media were observed after the incubation period of 6 days. The texture, margin, culture characteristics, front colouration, reverse colouration, sporulation was affected by the type of nutrient medium used. Sabouraud dextrose agar medium showed comparatively precise growth in 23 fungal endophytes whereas 7 fungal endophytes exhibited heavy sporulation on this medium. *Aspergillus* sp., *Penicillium* sp., and *Nectria* sp. expressed highest growth in sabouraud dextrose agar media and potato dextrose agar media while *Fusarium* sp., *Trichoderma* sp., *Colleotrichum* sp. exhibited highest growth in Potato dextrose agar media and Czapek dox agar media. The result of our study will be effective for advance mycological research.

KEYWORDS

Culture media, Growth, *Terminalia* spp., Fungal endophytes

1. INTRODUCTION

Every microbes required energy source, environmental conditions especially nutrients for their development and reproduction (Ravimannan et al., 2014). In Invitro condition, culture media used for the cultivation of microorganisms (Uthayasooryan et al., 2016). Czapek dox agar media, Malt extract agar media, Oat meal agar media, Sabouraud dextrose agar media and mostly Potato dextrose agar medium, are the used media in laboratory for the growth of fungi. These are the basic media composed of carbon source and nitrogen source and these components are the main requirement for the development of microorganisms.

In microbial world, fungi are the essential part having tubular filaments enclosing stiff cell wall and perform various bioactivity (Gupta, 2020). Fungi are the eukaryotic microbes which reproduces both asexually and sexually. Fungi grow on various habitats and the distribution is worldwide which require several elements for their growth and reproduction. Isolation of fungi in different culture media held for various parameters like preservation, microscopic research, taxonomy, and various biological activity test. Now-a-days various group of media are taken for the isolation which have the impact on colony characters, texture, sporulation, pigmentation depending upon the specificity of culture medium (Kumara and Rawal., 2008).

Fungal endophytes reside in the host tissue and mimic the similar metabolism as the host does without causing any disease to the host and these are a "gold mine" of bioactive chemicals with potential applications in agriculture, medicine, and the food business. These microbial chemicals have antibacterial, insecticidal, cytotoxic, and anticancer activities (Samantaray and Gupta., 2024). The present study was focused on the observation of the impact of five different nutrient media on colony morphology, mycelia growth and sporulation of 29 endophytic fungi isolated from *Terminalia* arjuna, *Terminalia* bellerica, *Terminalia* chebula, and *Terminalia* catappa.

2. METHODOLOGY

Samples like leaves and barks of *Terminalia* arjuna, *Terminalia* bellerica, *Terminalia* chebula, and *Terminalia* catappa were collected from

the campus of Regional Plant Resource Centre, Nayapalli, Bhubaneswar, Odisha. The samples were taken to the laboratory, rinsed to eliminate any dirt, and air-dried. Leaf and bark samples were chopped into with sterile scalpels and surface sterilized and approximately surface sterilized 2-3 segments were put in SD agar media and incubated (Tejasvi et al., 2005). Daily observations were made until endophytic fungi began to proliferate (Basha et al., 2012). Following incubation, fungal colonies were collected, streaked on agar plates, and incubated. The processes like streak plate and central inoculation were repeated 3-4 times until we got purified pathogens.

Sabouraud dextrose medium, Czapek-dox medium, Malt extract agar medium, Potato dextrose agar medium and oat meal agar medium were prepared. All segregated isolates were inoculated in five different medium and incubated for 6 days. The size, colour, texture, margin, sporulation, pigmentation of endophytic fungi were observed, and photographed

3. RESULTS AND DISCUSSIONS

A total of 29 nos. of fungi have been isolated from leaf and bark samples collected from four different species of *Terminalia* (*Terminalia* arjuna, *Terminalia* bellerica, *Terminalia* chebula, and *Terminalia* catappa). In all 29 different fungi belonging to *Aspergillus* sp. (15nos.), *Penicillium* sp. (4nos.), *Trichoderma* sp. (3nos.), *Fusarium* sp. (2nos), *Myceloid* (2nos.), *Nectria* sp. (1no) *Acladium* sp. (1no) *Colleotrichum* sp.(1no) were isolated, denoted in Table 1 and the plate growth in five different media of isolated fungi are shown in Figure 1. All five culture media supported the growth of fungi. Out of them, SD, PDA, CZA showed maximum and clear mycelial growth and heavy sporulation exhibited in OMA, MEA media. To the best of our knowledge, one report was on the influence of three different media on endolichenic fungi and the result showed highest number of fungal isolates fully grown in MYA (Malt yeast extract agar medium) (Vinayaka et al., 2016).

Aspergillus sp., *Penicillium* sp., and *Nectria* sp. exhibited highest growth in sabouraud dextrose agar media and potato dextrose agar media while *Fusarium* sp., *Trichoderma* sp., *Colleotrichum* sp. exhibited highest growth in Potato dextrose agar media and Czapek dox agar media. *Nectria* sp. exhibited complete different colour and texture in sabouraud dextrose

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media whereas very much similar in all rest four culture media. There is a variation of colony character, Mycelia growth, and sporulation pattern of the 29 isolates of endophytic fungi varied. *Aspergillus* sp. 3, *Aspergillus* sp. 5, *Aspergillus* sp. 9, *Penicillium* sp. 2 exhibited different texture in all five different media.

The colouration of almost all isolates are very light in czapek dox agar

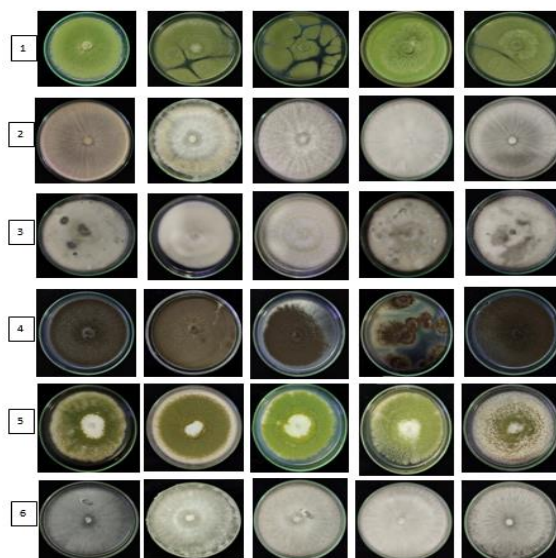
media but the growth is comparatively similar with rest media. The culture media composition was a very important aspect for proper growth of microorganisms (Shareef, 2019). In our study, composition of sabouraud dextrose media and potato dextrose media contains peptone and dextrose while czapek dox media contains various components like sucrose, sodium nitrate,

Table 1: List of endophytic fungi isolated from four different *Terminalia* sp.

Sl no	Fungal isolates	Plant parts	Occurance
1	<i>Aspergillus</i> sp. 1	Bark	<i>Terminalia</i> arjuna
2	<i>Trichoderma</i> sp. 1	Leaf	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa
3	Myceloid 1	Leaf	<i>Terminalia</i> arjuna, <i>Terminalia</i> chebula
4	<i>Aspergillus</i> sp. 2	Leaf, Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> arjuna
5	<i>Aspergillus</i> sp. 3	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa
6	<i>Trichoderma</i> sp. 2	Leaf	<i>Terminalia</i> arjuna, <i>Terminalia</i> belerica
7	<i>Penicilium</i> sp. 1	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> chebula
8	<i>Aspergillus</i> sp. 4	Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa
9	<i>Aspergillus</i> sp. 5	Leaf	<i>Terminalia</i> belerica, <i>Terminalia</i> catappa
10	<i>Aspergillus</i> sp. 6	Leaf, Bark	<i>Terminalia</i> belerica, <i>Terminalia</i> arjuna
11	<i>Aspergillus</i> sp. 7	Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> belerica
12	<i>Aspergillus</i> sp. 8	Leaf, Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> belerica
13	<i>Aspergillus</i> sp. 9	Leaf, Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> belerica
14	<i>Aspergillus</i> sp. 10	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> belerica
15	Myceloid 2	Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> chebula
16	<i>Acladium</i> sp.	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> chebula
17	<i>Fusarium</i> sp. 1	Leaf, Bark	<i>Terminalia</i> chebula, <i>Terminalia</i> catappa
18	<i>Aspergillus</i> sp. 11	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> chebula
19	<i>Penicilium</i> sp. 2	Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> chebula
20	<i>Penicilium</i> sp. 3	Leaf, Bark	<i>Terminalia</i> chebula, <i>Terminalia</i> catappa
21	<i>Trichoderma</i> sp. 3	Leaf	<i>Terminalia</i> chebula, <i>Terminalia</i> catappa, <i>Terminalia</i> belerica
22	<i>Aspergillus</i> sp. 12	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> chebula
23	<i>Aspergillus</i> sp. 13	Leaf,	<i>Terminalia</i> catappa, <i>Terminalia</i> belerica
24	<i>Aspergillus</i> sp. 14	Leaf, Bark	<i>Terminalia</i> catappa, <i>Terminalia</i> arjuna
25	<i>Colletotrichum</i> sp.	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa
26	<i>Nectria</i> sp.	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa
27	<i>Aspergillus</i> sp. 15	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa, <i>Terminalia</i> belerica
28	<i>Penicilium</i> sp. 4	Leaf, Bark	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa, <i>Terminalia</i> belerica
29	<i>Fusarium</i> sp. 2	Leaf	<i>Terminalia</i> arjuna, <i>Terminalia</i> catappa

Dipotassium hydrogen phosphate, magnesium sulphate, potassium chloride, ferrous sulphate which is required for the growth of fungi. In oat meal media, heavy sporulation occurred in almost all fungal endophytes except *Aspergillus* sp. 14. One investigation exhibited least sporulation and minimal growth of mycelia of *Myrothecium* sp. due to availability of chloride in czapek dox agar medium (Okunowo et al., 2010). The simple formulation of PDA (potato infusion from dextrose) makes the media very much important of mycelia growth increase. This media is rich in nutrients and effective elements.

In case of our research other than PDA, Sabouraud dextrose media, czapek dox media, malt extract media is effective for growth of fungi. Our study revealed that the different media influence the mycelia development, morphology of colony, sporulation of test fungi. Sabouraud dextrose agar media was found to be the most suitable for sporulation while Potato dextrose agar media shown the most visible colony characters. The systematics of fungi is now based on the morphological study, so the fungal recognition and identification was based on the phenotypes. Through present study, there is an extended vital clue towards the recognition and segregation of fungal endophytes using different nutrient media. For detailed research, identification and bioactivity is needed.



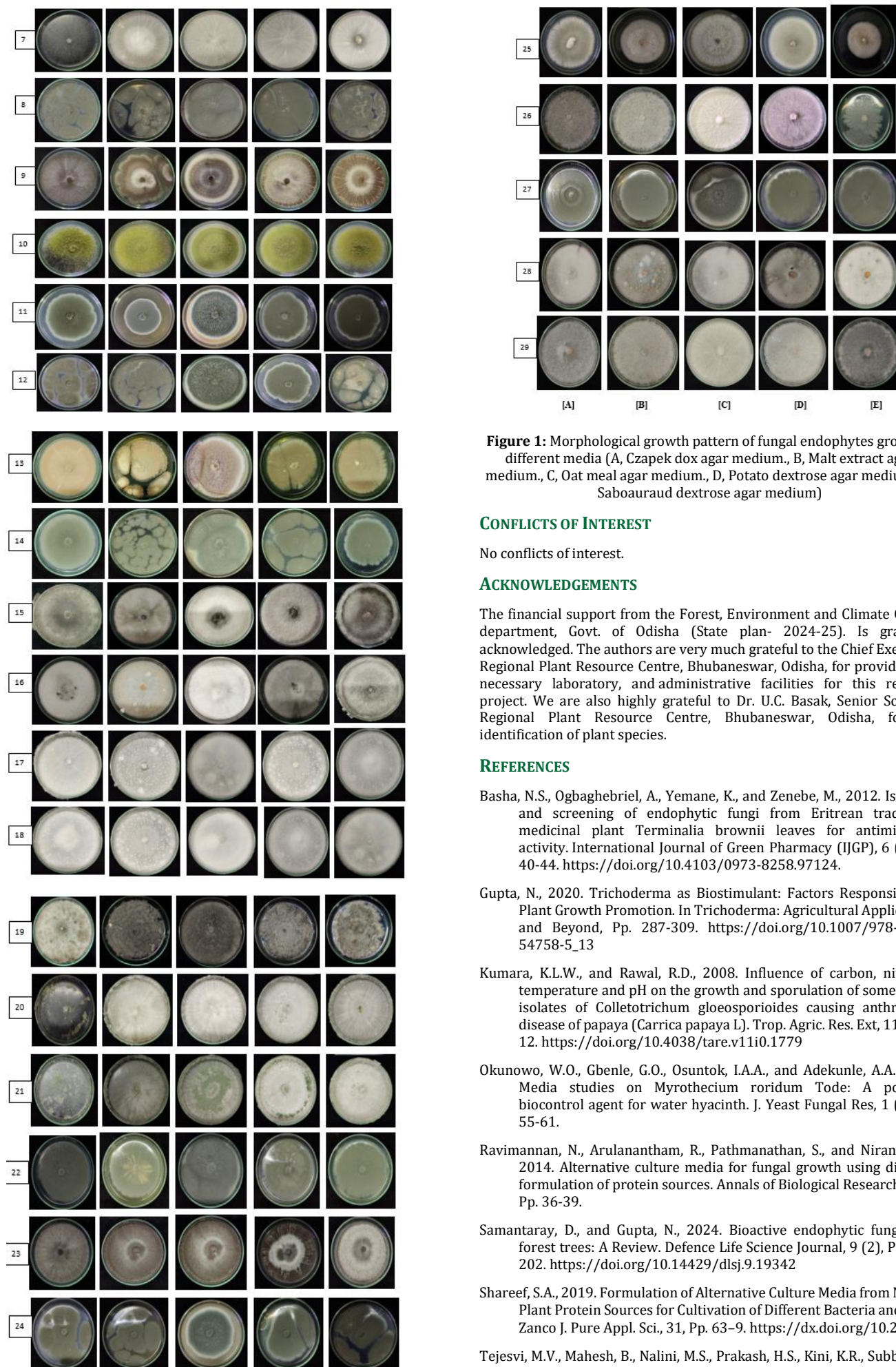


Figure 1: Morphological growth pattern of fungal endophytes grown in different media (A, Czapek dox agar medium, B, Malt extract agar medium, C, Oat meal agar medium, D, Potato dextrose agar medium, E, Sabouraud dextrose agar medium)

CONFLICTS OF INTEREST

No conflicts of interest.

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