International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Enumeration of endophytic fungi isolated from *Tinospora cordifolia* found in Ranchi district of Jharkhand and screening of their extracellular enzyme activity

Rakhi Kumari Singh * and Ishwari Prasad Gupta

University department of botany, Dr Shyama Prasad Mukherjee University, Ranchi, Jharkhand, 834008.

International Journal of Science and Research Archive, 2026, 18(03), 1250-1259

Publication history: Received on 10 February 2026; revised on 17 March 2026; accepted on 20 March 2026

Article DOI: https://doi.org/10.30574/ijsra.2026.18.3.0566

Abstract

Endophytes are microorganisms, primarily fungus and bacteria, that colonize the intracellular and intercellular spaces of healthy plant tissues at specific times. These germs are asymptomatic and unobtrusive. It is thought that fungal endophytes are a wealth of physiologically and structurally active substances. The presence of endophytes in the host plant and the kinds of secondary metabolites that endophytes produce can occasionally provide plants their therapeutic qualities. The goal of the current work was to extract and identify endophytic fungus from the leaves of *Tinospora cordifolia*, or the common medicinal herb Giloy, using a culture-dependent method. Three endophytic fungal isolates were collected for this investigation. Following a single spore isolation procedure in which a distinct culture of each individual colony was prepared and phyto-genetically analyzed by molecular studies, all isolates were predominantly identified by morphological traits. The agar was turned red, grey, or pink by the endophytic fungi, which were velvety reddish white or grey, cottony and hairy growth with spores brushing the lid. Endophytic fungal isolates' growth features in potato dextrose broth included mycelia development at the broth's surface and broth coloration on the agar.

Keywords: Pathogenicity; Enzyme assay; Antimicrobials; Fungal endophyte; *Tinospora cordifolia*

1. Introduction

Antimicrobial resistance develops as a result of the careless and improper usage of antibiotics. As a result, the medications lose their effectiveness and infections continue to exist in the body, raising the possibility that resistance will spread to other people (Ruiz 2003). To treat these infections, new antimicrobials must be developed (Martinez-Klimova et al. 2017). When medicinal plants are used in place of antimicrobial medications, the environment is degraded, biodiversity is lost, land and water are spoiled, and they are also costly. According to Tejesvi et al. (2007), these plants have a unique invading microbiome that may create distinctive and varied bioactive chemicals. As a result, microorganisms found within medicinal plants make good substitutes during the drug discovery process (Porras-Alfaro and Bayman 2011).

The Greek words endon, which means inside, and phyte, which means plant, are the source of the word "endophyte." De Bary initially presented it in 1866 (Arnold 2008). According to Schulz and Boyle (2006), endophytes are microorganisms, primarily fungus and bacteria, that colonize the intracellular and intercellular spaces of healthy plant tissues at specific times. These germs are asymptomatic and unobtrusive. The understudied class of microorganisms known as endophytic fungus is thought to have a wealth of structurally and physiologically active substances (Tan and Zou 2001). The presence of endophytes in the host plant and the kinds of physiologically active secondary metabolites that endophytes produce can occasionally provide plants their therapeutic qualities (Schulz and Boyle 2006).The host plant's ability to respond to both biotic and abiotic stressors is aided by the colonization of endophytic fungus. According to Redman et al. (2011), beneficial endophytes aid in the growth of the host plant, improve nutrient intake, prevent pathogenic growth inside the host plant, and lessen the severity of illness.In most Indian languages, the medicinal herb

* Corresponding author: Rakhi kumari singh

Tinospora cordifolia is referred to by names like guduchi, giloy, or amrita (heavenly elixir). It is dioecious, climbs, rarely stands upright, spreads swiftly, and typically grows on big trees (Nayak 2018). In the pharmaceutical industry, it is the most widely used plant for commercial purposes. An Ayurvedic herb with well-established anti-rheumatic, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, antiallergic, immunomodulatory, and anti-diabetic qualities (Kapoor and Saxena 2018).According to Singh and Singh (2012), the plant's stem and leaves have been shown to have antibacterial action against a variety of bacterial pathogens, including Salmonella Typhi, Staphylococcus aureus, Pseudomonas aeruginosa, and Shigella dysentria.The current investigation used a culture-dependent methodology to separate fungal endophytes from *Tinospora cordifolia* plant leaves that were gathered from three distinct locations. Molecular and morphological techniques were used to identify endophytic fungus.

2. Materials and methods

Collection of *Tinospora cordifolia* leaf: The *Tinospora cordifolia* plant's healthy leaves were gathered. Samples of mature, healthy plant leaves from two distinct plants were collected from each region, transported in bags to the lab, and processed further to isolate endophytic fungus.

Inoculation of leaves and isolation of endophytic fungi: According to Madhu Priya and Theoder (2018), samples of *Tinospora cordifolia* leaves were sterilized by first washing them under running water and then submerging them in 0.1% sodium hypochlorite for five minutes. After being submerged in 0.01 percent bavistin, they were treated with double-distilled water for five minutes before being exposed to 0.05 percent streptomycin. They were kept in double-distilled water for five minutes after being exposed to 70% ethanol once more.The sterility check test was carried out by adding 50 µl of the distilled water used in the final rinse of the surface sterilization procedure of the stem samples to nutrient agar medium (NA) and incubating at 37°C for 24 hours in order to verify and confirm the effectiveness of the surface sterilization procedure (De Souza Ferriera et al. 2017).Sterile petri plates were filled with potato dextrose agar (PDA) medium treated with antimicrobial chloramphenicol (100 µg.ml−1) to inhibit bacterial growth (Prasher and Dhanda, 2017). Following drying, the sterilized stem samples were divided in half and inoculated on PDA plates with inner tissue facing agar. After being covered with parafilm, the plates were incubated for five to seven days at 25°C.Fresh potato dextrose agar was filled with the hypheal ends of a 1 cm2 primary culture of endophytic fungus that had grown out of the plant tissue. Colony morphology was used to assess the cultures' purity after they were incubated at 25°C for seven to fourteen days. Endophytic fungi were periodically subcultured, and the cultures were kept as stock.

Pathogenicity of endophytic fungi: In healthy, immune-competent hosts, fungi typically do not cause illness. Disease arises when fungi unintentionally breach host barriers or when immunologic deficiencies or other crippling conditions exist that promote fungal entry and proliferation. In order to multiply within the host, fungi frequently acquire both morphologic forms (such as yeasts, hyphae, spherules, and sclerotic bodies) and virulence mechanisms (such as capsules and the capacity to grow at 37°C). To assess the fungi's pathogenicity, the endophytic fungi that emerged from the *Tinospora cordifolia* plant's stem tissue were cultivated at 37°C. Fungal pathogenesis, which entails a complex interaction of numerous fungal and host variables, is influenced by the existence of capsules, enzymes like keratinase, the capacity to grow at 37°C, and dimorphism (Kobayashi 1996).

Morphological based identification of fungi: Endophytic fungi were detected using the wet mount method, slide culture technique, and morphological traits like colony morphology (Markey et al. 2013).

2.1. Morphological characteristics of endophytic fungi isolated from *Tinospora cordifolia*

The absence of microorganisms on the culture media using the most recent washing water confirmed that the surface sterilization was successful in eliminating and eliminating epiphytes. Fig. displays primary endophytic fungal growths. Three isolates, designated TN-1 to TN-3, were discovered and purified from *Tinospora cordifolia* plant leaves (pure cultures are shown in Fig. They were identified as Botrytis spp., endophytic fungus. Aspergillus and Cladosporium species, as shown in Table 01.

Table 1 Growth of endophytic fungi isolated from leaf of *Tinospora cordifolia*

Isolate name	Macroscopic characteristics		Microscopic characteristics	Fungal type
	Obverse	Reverse		
TC_1	Wolly,cottony, velvety	White at first, later brown to dark brown or nearly black	Erect,septate,hyaline to light brown conidiophores that resembles a tree or grape bunch. Unicellular,smooth,hyaline to greyish,ovoid to globose conidia on short denticles	Botrytis spp
TC_2	Olivaceous green to olivaceous brown, grey green, or grayish brown	Typically dark, olivaceous-black,greenish-black,or deep black	Septate, olive-brown hyphae and distinct branching,acropetal chains of conidia, 1-4 celled conidia,smooth to verrucose walls,and specialized geniculate conidiophores with dark,conspicuous scars where conidia disarticulate	Cladosporium spp
TC_3	Starts white,rapidly becomes black, granular/wolly,often with a white edge	Typically yellow to pale yellow	Septate,transparent hyphae with dichotomous branching,conidiophore arriving from a foot cell,flask shaped phialides that produce chains of globose,pigmented conidia	Aspergillus spp

2.2. Screening of extracellular enzymatic activities of fungal Endophytes

By cultivating fungal endophytes on Yeast-Malt (YM) agar media (YM: 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract, 1.5% agar, pH 6.7) (Molina et al., 2012) and adding 5 mm fungal plugs to the YM agar media supplemented with dissolved and particular indicative substrates. The formation of a clear zone encircling the fungal colony was assessed after applying a specific reagent and utilized as an indicator for extracellular enzymatic activities after three to five days of incubation, depending on the growth rates of fungal endophytes at 28 °C. All the assays were perfomed in triplicate.

- Amylase activity: The fungal isolates were grown on YM agar medium supplemented with 1% soluble starch in order to measure amylase activity. The plates were soaked with 1% iodine following incubation. The amylolytic activity was assessed by measuring the presence of clear zones surrounding fungal growth.
- Protease activity: The fungal protease enzyme activity was measured using the YM agar medium with 1% gelatine. Using acidic mercuric chloride as an indicator, the gelatin degradation was visible as a distinct zone surrounding the colonies following incubation.
- Cellulase activity: After adding iodine solution as an indicator, the emergence of a clear zone surrounding the fungal colony cultivated on YM medium supplemented with 1% cellulose or carboxymethylcellulose (CMC) was assessed to evaluate the fungal cellulolytic activity.
- Lipase activity: The fungal isolates were grown on peptone agar medium supplemented with Tween 20. The lipase activity was assessed by measuring the presence of clear zone surrounding the fungal growth.

2.3. Physicochemical Parameter's effect on the Fungal Growth and Enzyme Production

The growth and enzyme production of screened fungal isolates was optimized by the evaluation of their response to different physicochemical parameters, including temperature, pH and salt concentration (Sharma et al., 2014).

3. Result and discussion

The present study was carried to isolate and identify endophytic fungi from various places of Ranchi district, Jharkhand. In the study, a total of three fungal colonies were isolated from different segments of the plant leaf. Among these endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of Botrytis spp, Cladosporium spp and Aspergillus spp. The enzyme production by the fungal isolates are mentioned in the table below.

Table 2 Screening of extracellular enzymes produced by different fungal isolates

SN	ENZYMES	IL 1	IL 2	IL 3
1.	Amylase	+	+	_
2.	Protease	+	_	+
3.	Cellulase	_	_	_
4.	Lipase	+	_	+

3.1. Amylase test photographs

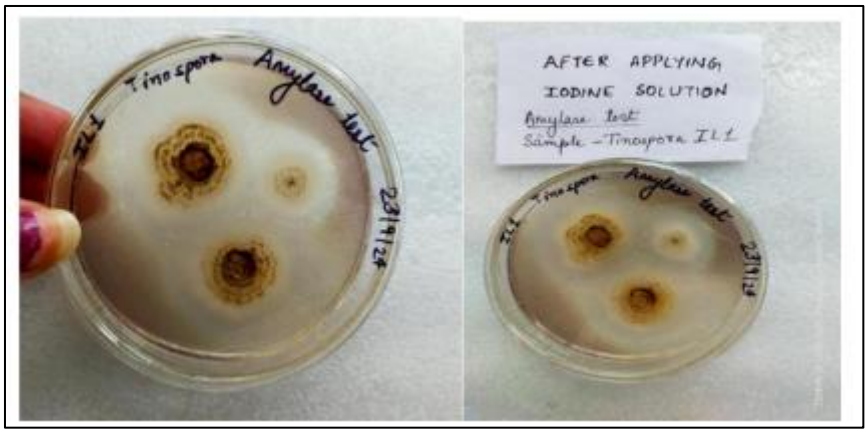

Figure 1 Amylase test showing clear zones around fungal isolate IL 1 after applying iodine solution

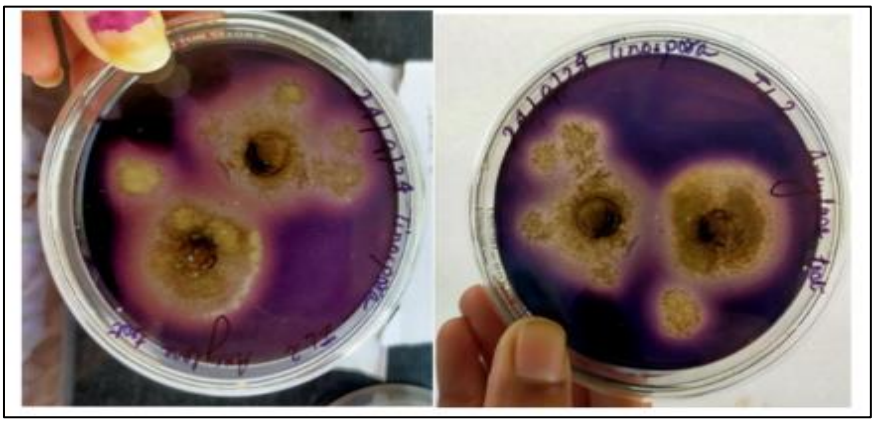

Figure 2 Amylase test showing clear zones around fungal isolate IL 2 after applying iodine solution

Figure 3 Amylase test showing no clear zones around fungal isolate IL 3 after applying iodine solution

3.2. Protease test photographs

Figure 4 Protease test showing clear zone around fungal isolate IL 1 after incubation

Figure 5 Protease test showing clear zone around fungal isolate IL 2 after incubation

Figure 6 Protease test showing clear zone around fungal isolate IL 3 after incubation period

3.3. Cellulase test photographs

Figure 7 Cellulase test showing no zone around the fungal isolate IL 1 after applying 1M NaCl solution for 15 minutes indicating negative result

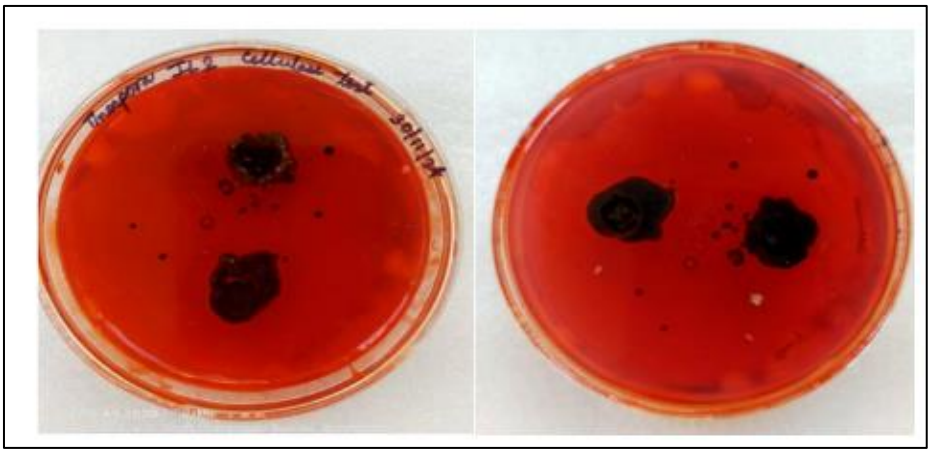

Figure 8 Cellulase test showing no zone around the fungal isolate IL 2 after applying 1M NaCl solution for 15 minutes indicating negative result

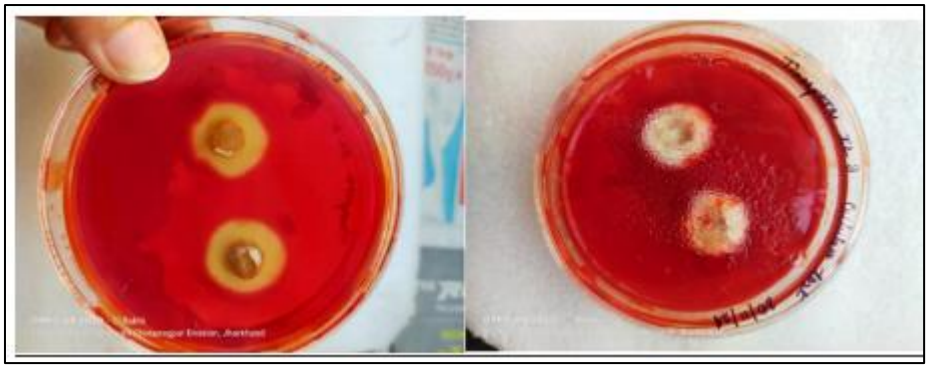

Figure 9 Cellulase test showing no zone around the fungal isolate IL 1 after applying 1M NaCl solution for 15 minutes indicating negative result

3.4. Lipase test photographs

Figure 10 Lipase test showing clear zone around the fungal isolate IL 1 indicating positive result

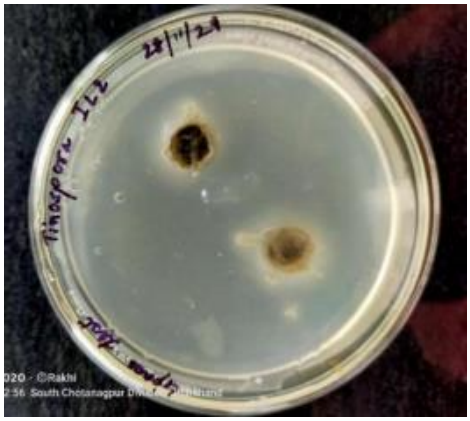

Figure 11 Lipase test showing no clear zone around the fungal isolate IL 2 indicating negative result

Figure 12 Lipase test showing clear zone around the fungal isolate IL 3 indicating positive result

4. Conclusion

The three strains of endophytic fungi tested were able to produce one or the other extracellular enzymes(Table 1). In the present study, none of the strain was able to produce all the four enzymes tested. The production of these four enzymes also varied depending on 3-7 days of incubation. The duration and degree of enzyme production variations by the endophytic fungi isolated from medicinal plants are the most important conclusions drawn from this investigation. This suggests that the production of enzymes varies among fungi and frequently reflects the needs of their environment. The biology of the fungi may be impacted by environmental elements like climate and geographic location, as well as the host's many shifting aspects connected to age.Nonetheless, choosing organisms most appropriate for industrial needs would benefit from an understanding of the kinds, quantities, and traits of enzymes produced by the endophytic fungi mentioned above. Quantitative research is being done on the potential endophytic fungi to produce extracellular enzymes in liquid conditions.

Endophytes are a poorly understood class of organisms that produce a variety of bioactive compounds. In order to draw the attention of the research community to this developing field and the potential exploitation of the available sources for their therapeutic uses in various fields, such as the medical, pharmaceutical, food, and cosmetic industries, it is essential to evaluate and highlight the prior accomplishments, ongoing research, and recent advancements in research related to endophytic microorganisms.In this study, we isolated endophytes from *Tinospora cordifolia* leaves and screened their extracellular enzyme activity. Further research is required for the scaling up of pure endophytic cultures, activity-guided fractionation, and isolation of pure active compounds because several of the crude extracts showed encouraging activity.

Compliance with ethical standards

Acknowledgments

I am thankful to the University department of Botany, Dr Shyama Prasad Mukherjee University, Ranchi, Jharkhand and Sharma Global Biotech Laboratory, Bariatu, Ranchi for providing necessary facilities to carry out the research work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Disclosure of conflict of interest

The authors do not have any conflict of interest.

References

[1] Amirita A, Sindhu P, Swetha J, Vasanthi NS, Kannan KP (2012) Enumeration of endophytic fungi from medicinal plants and screeening of extracellular enzymes. World Journal of Science and Technology 2(2):13-19

[2] Khan R, Shahzad S, Choudhary MI, Khan SA, Ahmad A (2007) Biodiversity of the endophytic fungi isolated from Calotropis procera (Ait.) R. Br. Pakistan Journal of Botany 39(6):2233-2239

[3] Martinez-Klimova E, Rodriguez-Pena K, Sanchez S (2017). Endophytes as sources of antibiotics.Biochemical Pharmacology 134:1-7

[4] Yadav V, Singh A, Mathur N, Yadav R (2020) Isolation and characterization of alternaria GFAV15, an endophytic fungus from green fruit of *Tinospora cordifolia* (Willd.) Miers from semi-arid region. South African Journal of Botany 134:343-348

[5] Uzma F, Konappa NM, Chowdappa S (2016) Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. Egyptian Journal of Basic and Applied Sciences 3(4):335-342

[6] Asthana, A., & Hawker, R. (2016). Screening of enzyme-producing fungi: Methods and applications. Journal of Industrial Microbiology, 52(6), 753-764.

[7] Chadha, A., Verma, M., & Malik, A. (2015). Enzyme production by fungal strains and their potential applications in industrial processes. Journal of Applied Microbiology, 118(3), 603-615.

[8] Pathak, V., & Mehta, P. (2016). Isolation and screening of enzyme-producing fungi from agricultural waste. Biotechnological Research Journal, 15(2), 108-115.

[9] Sahu, P.K., Tilgam, J., Mishra, S., & Hamid, S. (2022). Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. Journal of Basic Microbiology,10

[10] Li, G., Cao, B., Liu, W., Ren, R., Feng, J., & Lv, D. (2020). Isolation and Identification of Endophytic Fungi in Kernels of Coix lachrymal-jobi L. Cultivars.

[11] Akello, J. Biodiversity of Fungal Endophytes Associated with Maize, Sorghum and Napier Grass and the Influence of Biopriming on Resistance to Leaf Mining, Stem Boring and Sap Sucking Insect Pests; University of Bonn: Bonn,Germany, 2012.

[12] Rajagopal, K. and Suryanarayanan, T.S., 2000. Isolation of endophytic fungi from the leaves of neem (Azadirachta indica A.Juss). Current Science. 78: 1375-1378

[13] Rajagopal, K., 2004. Endophytic fungi of a plam, Borassus flabellifer L. – As first report from India. AJMBES. 6: 541-544.

[14] Kumar, D.S.S. and Hyde, K.D., 2004. Biodiversity and tissue recurrence of endophytic fungi from Tripterygium wilfordii. Fungal Diversity. 17: 69–90.

[15] Devi NN, Prabaka JJ, Wahab F. Phytochemical analysis and enzyme analysis of endophytic fungi from Centella asiatica. AsianPac J Trop Biomed. 2012; 2:1280–1284. doi: 10.1016/S2221-1691(12)60400-6. [CrossRef] [Google Scholar]

[16] Selim KA, El-Beih AA, AbdEl-Rahman TM, El-Diwany AI. 2012 - Biology of Endophytic Fungi. Current Research in Environmental & Applied Mycology 2(1), 31- 82, Doi 10.5943/cream/2/1/3.

[17] Zhang HW, Song YC, Tan RX. 2006 - Biology and chemistry of endophytes. Natural Product Reports. 23, 753–771.

[18] Strobel G, Daisy B. 2003-Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews. 67(4), 491-502.

[19] Petrini O. 1991 - Fungal endophytes of tree leaves. In: Andrews, J.H., Hirano, S.S. (Eds.), Microbial Ecology of Leaves. Springer-Verlag, New York, USA, pp. 179–197.

[20] Maria, G.L., K.R. Sridhar and N.S. Raviraja, 2005.Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. J. Agricultural Technol., 1: 67-80.

[21] Choi, Y.W., I.J. Hodgkiss and K.D. Hyde, 2005. Enzyme production by endophytes of Brucea javanica. J. Agricultural Technol., 1: 55-66.

[22] T.P. Napitupulu, A. Kanti, I.M. Sudiana, Evaluation of the environmental factors modulating indole-3-acetic acid (IAA) production by Trichoderma harzianum InaCC F88. InIOP conference series: earth and environmental science, IOP Publishing 308 (1) (2019) 012060.

[23] P. Tiwari, M. Bajpai, L.K. Singh, S. Mishra, A.N. Yadav, Phytohormones producing fungal communities: metabolic engineering for abiotic stress tolerance in crops, in: Agriculturally Important Fungi for Sustainable Agriculture, Springer, 2020, pp. 171–197.

[24] N.C. Baron, E.C. Rigobelo, Endophytic fungi: a tool for plant growth promotion and sustainable agriculture, Mycology 13 (1) (2022) 39–55.

[25] P. Bose, S.U. Gowrie, G. Chathurdevi, Optimization of culture conditions for growth and production of bioactive metabolites by endophytic fungus-Aspergillus tamarii, Int. J. Pharm. Biol. Sci. 9 (2019) 469–478.

[26] S. Kusari, S. Singh, C. Jayabaskaran, Biotechnological potential of plant- associated endophytic fungi: hope versus hype, Trends Biotechnol. 32 (2014) 297–303.

[27] P. Paramanantham, S. Pattnaik, B. Siddhardha, Natural products from endophytic fungi: synthesis and applications, in: B. Singh (Ed.), Advances in Endophytic Fungal Research. Fungal Biology, Springer, Cham, 2019.

[28] S. Kaul, S. Gupta, M. Ahmed, M.K. Dhar, Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites, Phytochem. Rev. 11 (2012) 487–505.

[29] A. Mishra, S. Kumar, A. Bhatnagar, Potential of fungal laccase in decolorization of synthetic dyes, Microbial Wastewater Treatment 7 (2019) 127–151.

[30] B. Schulz, U. Wanke, S. Draeger, H.J. Aust, Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods, Mycol. Res. 97 (1993)1447–1450.

[31] G.L. Maria, K.R. Sridhar, N.S. Raviraja, Antimicrobial and enzyme activity ofmangrove endophytic fungi of southwest coast of India, J. Agric. Technol. 1 (2005) 67–80.

[32] M.M. Saad, R.Y. Gharee, A.A. Saeed, The potential of endophytic fungi as biocontrol agents against the cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), Egypt J. Biol. Pest Cont. 29 (2019) 7–10

[33] Tejesvi, M.V., Mahesh, B., Nalini M.S., Prakash, H.S., Kini, K.R., Ven Subbiah and Shetty, H.S. (2005). Endophytic fungal assemblages from inner bark and twig of Terminalia arjuna W & A. (Combretaceae). World Journal of Microbiology and Biotechnology 21:1535–1540.

[34] Krishnamurthy, Y.L., Naik, S.B., and Jayaram, S., 2008.'Fungal Communities in herbaceous medicinal plants from the Malnad Region', Southern India. Microbes Environ., 23;24-28.

[35] Rajagopal K, Kalavathy S, Kokila S, Kathiravan G, Prasad R, Balasubramanian P (2010). Diversity of fungal endophytes in few medicinal herbs of South India. Asian J. Exp. Biol., 1(2): 415-418.

[36] Sunitha VH, Devi ND, Srinivas C. Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. World Journal of Agricultural Science 2013; 9:1-9.