Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 05 (Sept–Oct 2024); pp 1151–1155

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Diversity of Endophytic Fungi Associated with *Saccharum officinarum* L and their Potential to Produce Indole Acetic Acid and Enzymes

Arumugam Pitchaipillai[1], Kalyanaraman Rajagopal*[2], Kokila Vasanthakumar[3], Arulmathi Ramalingam[1], Muralidharan Ramachandran[4], Salem Varadharajan Rajesh[2], Prabakaran, Ramalingam[2], Kathiravan Govindarajan[2] and Meenambiga Setti Sudharsan[5]

[1] Department of Biotechnology, School of Life Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai - 600 117, Tamil Nadu, India
[2] Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Affiliated to the University of Madras, Chennai - 600 004, Tamil Nadu, India
[3] Department of Biotechnology, Gojan School of Business and Technology, Affiliated to Anna University, Chennai - 600 052, Tamil Nadu, India
[4] Department of Botany, D. G. Vaishnav College, (Autonomous), Affiliated to University of Madras, Chennai - 600 106, Tamil Nadu, India
[5] Department of Biotechnology, School of Engineering, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai - 600 117, Tamil Nadu, India

Received: 20 Jul 2024; Revised accepted: 16 Sep 2024

Abstract

Endophytic fungi live inside healthy plant tissues and inhabit almost all varieties of plants. Endophytic fungi can secrete several metabolites, which are useful in the biotechnology industry. In the present study, endophytic fungi were isolated from the leaf of *Saccharum officinarum* L, belonging to the family Poaceae. A total of sixty endophytic isolates belonging to six different fungal species were isolated from 300 leaf segments. Among six fungal species four endophytic fungi were hyphomycetes and two species belongs to coelomycetes and ascomycetes. *Chaetomium globosum* was the dominant endophyte and showed the maximum colonization frequency (CF/6.0) and the least colonization frequency shown by *Humicola* sp. (CF/1.0). The isolated endophytic fungi were tested for the production of extracellular enzymes like amylase, cellulase, pectinase, protease, and IAA.

***Key words*:** *Saccharum officinarum* (sugarcane), Leaf, Endophytic fungi, Diversity, Enzymes, IAA

Endophytic fungi survive in all parts of the plant body without damaging or producing any obvious symptoms. They are located in both intracellular and intercellular spaces and colonize seeds, leaves, stems, bark, roots, etc. [1]. Endophytic fungi produce many bioactive compounds that help in the endophytic mode of life in host plants [2]. Now it has been well established that endophytic fungi of different plant hosts are a potential and novel source of bioactive secondary metabolites, industrially important enzymes, plant growth-promoting substances, etc. [3-5]. It is considered a microbial factory for the production of various bioactive compounds [6]. Endophytic fungi are studied from various terrestrial plants in tropical, subtropical, and temperate regions of the world. Endophytic fungi from grasses (Poaceae) of both wild and agricultural origin were studied [7]. Incidences of entomopathogenic fungi as endophytes of sugarcane hosts and in the soil of sugarcane fields were reported [8]. Endophytic bacterial colonization of *Saccharum officinarum* by diazotrophs like *Shinella* sp. and *Enterobacter* sp. was studied [9]. Tam and Diep [10] reported the isolation, identification, and characterization of endophytic bacteria in *Saccharum* sp. cultivated on soils in Dong Nai province, Vietnam. Dark septate endophytic fungi associated with sugarcane plants cultivated in Brazil [11]. Fungi from the leaf litter of *Saccharum officinarum* L were reported [12]. However, scant attention has been given to the diversity of endophytic fungi from cash crops in India and their potential for producing various compounds. The paucity of research on cash crops, particularly sugarcane, prompted us to study the diversity of endophytic fungi in *Saccharum officinarum* L. and their potential to produce various compounds.

MATERIALS AND METHODS

The host plant is *Saccharum officinarum* L. (Poaceae), collected from the Tamil Nadu Agriculture University (TNAU) Agricultural Farm, Coimbatore. The surface sterilization of leaf tissue was carried out as described by Dobranic *et al*. [13].

***Correspondence to:** Kokila Vasanthakumar, E-mail: kokila.vasanth5@gmail.com; Tel: +91 9384022770

Citation: Pitchaipillai A, Rajagopal K, Vasanthakumar K, Ramalingam A, Ramachandran M, Rajesh SV, Prabakaran, Ramalingam, Govindarajan K, Sudharsan MS. 2024. Diversity of endophytic fungi associated with *Saccharum Officinarum* L and their potential to produce indole acetic acid and enzymes. *Res. Jr. Agril. Sci.* 15(5): 1151-1155.

Sporulating isolates were identified with the help of the standard manuals [14-15].

Data analysis

The colonization frequency (CF%) of each endophytic fungus was calculated by the method of Hata and Futai [16].

$$CF = x = Ncol / Nt \times 100$$

Where; Ncol and Nt are the number of segments colonized by each endophytic fungi and the total number of segments examined, respectively.

Indole acetic acid (IAA) production

The endophytic fungi were grown in liquid CDA medium for 2 weeks as a shake culture. The endophytic fungal mycelium was separated, and the culture extracts were acidified to pH 3.8 with 1 N HCl and washed in a separating funnel with an equivalent volume of ethyl acetate. The ethyl acetate fraction was extracted with an equal volume of 5% $NaHCO_3$. The alkaline solution was carefully acidified to pH 3.4 with 6 N HCl and then extracted with ethyl ether. The ethyl ether fraction was concentrated to 1 ml and spotted on the Whatman No. 1 filter paper. The chromatogram was developed with a solvent system composed of isopropanol, acetone, and water (10:1:1). The chromatogram was sprayed with Gordon and Weber's reagent (1 ml of 0.5 M $FeCl_3$ and 50 ml of 35% $HClO_4$). The control sample of authentic IAA dissolved in ethyl ether was running parallel to the crude extract of the sample. The formation of a bright red spot on the chromatogram showed the presence of Indole Acetic Acid in the crude extract [17].

Extracellular enzyme production

The enzyme production was tested using Petri plates that were inoculated with mycelial discs (5 mm diameter) of different endophytic fungi taken from the growing edge of the colony of Czapek's Dox Agar medium and incubated at 25°C±1°C. The enzyme production was determined semi-quantitatively by adding respective substrates into the medium or by reagents over a period of growth. The formation of color or clear zones was recorded in arbitrary units (++/strong activity, +/good activity, and -/no production).

Amylase enzyme

Czapek's Dox Agar medium was amended with 0.2% soluble starch. After 5 days of endophyte fungal growth, it was flooded with iodine solution. The formation of a yellow zone around the fungal colony indicated amylase activity.

Cellulase enzyme

Czapek's Dox Agar medium incorporated with 0.5% Na-carboxymethyl cellulose (Na CMC). After 5 days of endophytic fungi growth, add 0.2% aqueous Congo red solution and destained with 1 M sodium chloride solution. The appearance of yellowish zones around the colony fungi in the red substrate showed enzyme cellulase activity.

Pectinase enzyme

Czapek's Dox Agar medium amended with 0.5% of pectin (pH 5) was used. After 5 days of endophyte growth, the colonies were flooded with a 1% aqueous solution of hexadecyl trimethyl ammonium bromide ($C_{19}H_{42}BrN$), which precipitated pectin in the medium. Thus, the appearance of clear zones around the fungal colony or the formation of an opaque medium showed pectinase activity.

Protease enzyme

Czapek's Dox Agar medium containing 0.4% gelatin was prepared. After a week of incubation, the degradation of gelatin was observed as a clear zone around the edge of the fungal colonies. The Petri plates were then flooded with a saturated solution of $(NH_4)SO_4$ ammonium sulfate; thus, a white precipitate was formed, making the medium opaque and enhancing the clear zones around the colony [17-19].

RESULTS AND DISCUSSION

Different varieties of plants cultivated in various ecological conditions have been screened for endophytic fungal diversity and their biotechnological capability [20]. However, very few studies have been carried out on endophytic fungal studies on *Saccharum officinarum* L worldwide, particularly in India. Gideon [21] reported the efficacy of endophytic fungi metabolites on uropathogens isolated from *Saccharum officinarum* L. But very little attention has been given to the study of endophytic fungal communities in food crops [22]. This paucity of research on the endophytic fungal distribution of crop plants, particularly cash crops, drove us to investigate endophytic fungi from *Saccharum officinarum* L.

Table 1 Number of isolates recovered and species identified from leaf tissue of *Saccharum officinaram*

Total number of isolates	Total number of species	Fungal groups		
		Ascomycetes	Coelomycetes	Hyphomycetes
60	6	1	1	4

Table 2 Mean density of colonization of endophytic fungi from leaves of *Saccharum officinaram*

Endophyte	Mean colonization frequency %
Ascomycetes	
Chaetomium globosum	6.0
Coelomycetes	
Phyllosticta sp.	4.5
Hyphomycetes	
Acremonium sp.	3.0
Aspergillus glaucus	1.5
Bipolris sp.	3.5
Humicola sp.	1.0

To our knowledge, there is no report on the endophytic fungal diversity study and their potential to produce indole acetic acid and enzymes. In the current investigation, *Saccharum officinarum* L was colonized by a total of six endophytic fungi (60 isolates) (Table 1) that were present in three hundred segments of the *Saccharum officinarum* L leaf. Hyphomycete was the dominant group, represented by four endophytic fungi (66.6%): *Acremonium* sp., *Aspergillus glaucus, Bipolaris* sp., and *Humicola* sp. It is followed by *Chaetomium globosum,* an ascomycete, and a coelomycete, *Phyllosticta* sp. (Table 2). Among the various groups of endophytic fungi, *Chaetomium globosum* showed the highest colonization frequency (6.0%), followed by *Phyllosticta* sp. (4.5%) (Fig 1). In the hyphomycetes group, *Bipolaris* sp showed maximum CF (3.5%), *Acremonium* sp (3.0%), followed by *Aspergillus glaucus* (1,5%), and *Humicola* sp (1%) (Fig 1). Although six endophytic fungi were present in *S. officinarum,* only one, *Chaetomium globosum,* showed significant CF above

5%. These results align with Petrini [23] statement that only one or a few endophytic fungal species dominate a single host plant. Several endophytic fungi isolated in the present investigation were already reported as endophytes from other host plants [24]. Reddy [25] and Suryanarayanan [26] also reported that *Curvularia*, *Pestalotiopsis*, *Aspergillus*, *Chaetomium*, and *Phyllosticta* existed as endophytes in other hosts, which indicates these genera show a broad host range.

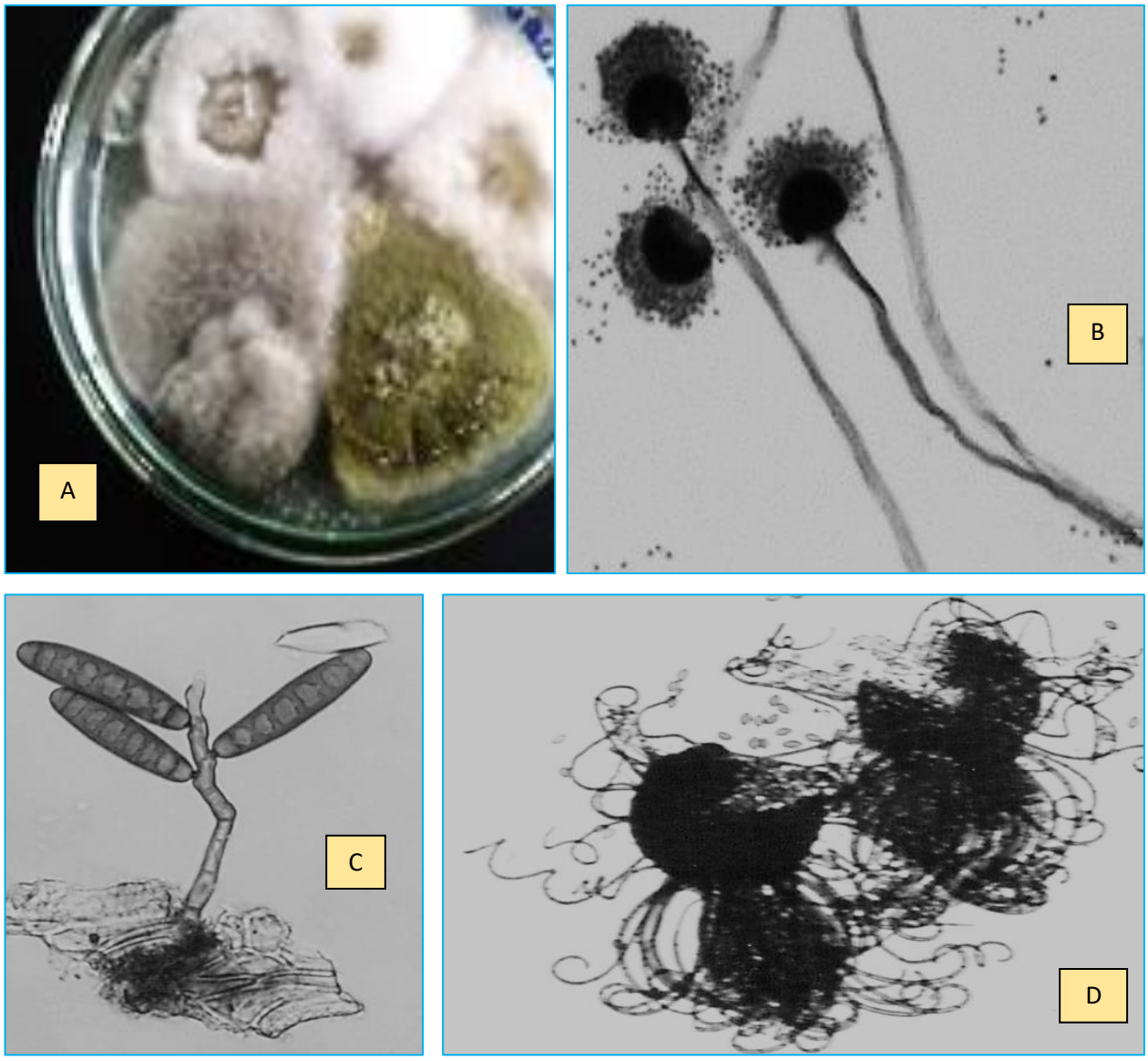

Fig 1 Endophytic fungus isolated from *Saccharum officinarum* L. **A**. Endophytic fungi emerging from leaf tissue, **B**. *Aspergillus glaucus* **C**. *Bipolris* sp, **D**. *Chaetomium globosum*

Table 3 Indoleacetic acid production by endophytic fungi isolated from the *Saccharum officinaram* L.

Endophyte	IAA production with tryptophan	IAA production without tryptophan
C. globosum	+	+
Phyllosticta sp.	+	+
Acremonium sp.	+	+
A. glaucus	+	+
Bipolris sp.	+	-
Humicola sp.	+	-

(+ IAA produced/ - IAA not produced)

There are many studies on endophytic fungi residing in the foliar region as reservoirs of novel bioactive compounds, plant growth-promoting substances, and the production of industrially important enzymes [27-28]. Endophytic fungi have been reported to produce plant growth-promoting compounds like IAA-indole acetic acid and gibberellins [29-32]. Waqas [33] isolated two endophytic fungi, *Phoma glomerata* LWL2 and *Penicillium* sp. LWL3, that produced gibberellic acid and indoleacetic acid and significantly promoted shoot growth in dwarf mutant Waito-C and Dongjin-beyo rice varieties. In the present study, all six endophytic fungi isolated from *S. officinarum* L were tested for IAA production. Since it is a preliminary study to test whether endophytic fungi of *S. officinarum* L can produce IAA or not with and without the precursor of the amino acid tryptophan. The results were reported as (present + or absent -). The amino acid tryptophan was added at a concentration of 1.0 g/l in the medium [34].

In the current research, all six endophytic fungi produced IAA in cultures amended with tryptophan, among these *Acremonium* sp., *A. glaucus*, *C. globosum*, and *Phyllosticta* sp. produced IAA even without tryptophan in the medium (Table 3). A similar observation was made *in vitro* about auxin production by *Aspergillus awamori*, an endophytic fungus of *Zea mays that* produced IAA in culture [35] and *Balansia epichloe*, a plant pathogen isolated from *Sporobolus poiretii* [36]. It is well established that the IAA phytohormone induces

cell elongation and cell division in plants. Hence, the production of IAA by some of the endophytic fungi of *S. officinarum* L may help in plant growth and development.

Endophytic fungi can produce different extracellular enzymes like pectinase, cellulase, etc., which enable the endophytic fungi to penetrate the host tissue to gain entry into the host, and those endophytes can be exploited industrially for important enzyme production [37-38]. In this study, all six endophytic fungi isolated from *S. officinarum* L were tested for extracellular enzyme production like amylase, cellulase, pectinase, and protease. The results are presented in (Table 4). The formation of a clear zone or color was recorded in arbitrary units. *C. globosum*, *Phyllosticta* sp., *Acremonium* sp. and *Bipolaris* sp. produced all four enzymes tested. *A. glaucus* and *Humicola* sp. produced amylase, cellulase, and pectinase only (Table 4). Among the six endophytic fungi, *Acremonium* sp. showed potential activity on all four enzymes tested, whereas *Phyllosticta* sp. showed highest activity on production of amylase, cellulase, and pectinase enzymes. *A. glaucus*, *Humicola* sp., and *Bipolris* sp. showed good activity against all the enzymes tested (Table 4).

The endophytic fungi produced different enzymes *in vitro* that are necessary for entering the host; however, they differed in their production. A similar trend was observed by Schulz [39], who found that the enzymes produced by endophytic fungi differed from isolate to isolate. To conclude, to our knowledge, the leaf of *S. officinarum* L. has been studied for endophytic fungi and for the production of IAA and enzymes, which are industrially important. In the present study, it was shown that some of the endophytic fungi of *S. officinarum* L were successful in producing indoleacetic acid and a few enzymes in the culture.

Table 4 Extracellular enzymes production by endophytic fungi isolated from the *Saccharum officinaram*

Endophyte	Amylase	Cellulase	Pectinase	Protease
		Ascomycetes		
Chaetomium globosum	+	++	++	+
		Coelomycetes		
Phyllosticta sp	++	++	++	+
		Hypomycetes		
Acremonium sp	++	++	++	++
Aspergillus glaucus	+	+	+	-
Bipolris sp	+	+	+	+
Humicola sp	+	+	+	-

- No production/ + Good activity/ ++ Strong activity

CONCLUSION

Endophytic fungi play a vital role in the diversity of biological resources. The present study shows that endophytic fungi from *S. officinarum* are capable to produce enzymes like amylase, cellulase, pectinase, protease and plant growth component indole acetic acid. Hence this study proves that endophytic fungi and host plants have a symbiotic bond, because these factors proves that host were protected from serious environmental conditions and also a growth inducer. Consequently, further studies are required to explore the significance and application of endophytic fungi in agricultural field.

Acknowledgement

The authors would like to thank Vels Institute of Science, Technology and Advanced Studies for providing platform to carry out the research work.

LITERATURE CITED

1. Arnold AE. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol. Rev.* 21: 51-66.
2. Strobel G. 2003. Endophytes as sources of bioactive products. *Microbes Infect*. 5: 535-544.
3. Gangwar M, Kaur N, Saini P, Kalia A. 2015. The diversity, plant growth promoting, and anti-microbial activities of endophytic actinomycetes isolated from *Emblica officinalis* Gaertn. *Int. Jr. Adv. Research* 3(4): 1062-1071.
4. Ronsberg D, Debbab A, Mandi A, Wray V, Dai HF, Kurtan T. 2013. Secondary metabolites from the endophytic fungus *Pestalotiopsis virgatula* isolated from the mangrove plant *Sonneratia caseolaris*. *Tetrahedron Letters* 54(25): 3256-3259.
5. Stierle AA, Stierle DB. 2015. Bioactive secondary metabolites produced by the fungal endophytes of conifers. *Nat. Prod. Communication* 10(10): 1671-1682.
6. Wang WX, Zheng MJ, Li J. 2019. Cytotoxic polyketides from an endophytic fungus, *Phoma bellidis* harbored in *Tricyrtis maculate*. *Phytochem. Letters* 29: 41-46.
7. Szécsi Á, Magyar D, Tóth Š, Szőke C. 2013. Poaceae: A rich source of endophytic fusaria. *Acta Phytopathologica Et Entomologica Hungarica*. 48: 19-32.
8. Kasambala Donga T, Meadow R, Meyling NV, Klingen I. 160. Natural occurrence of entomopathogenic fungi as endophytes of sugarcane (*Saccharum officinarum*) and in soil of sugarcane fields. *Insects* 12(2): 160.
9. Taulé C, Castillo AE, Villar SM, Olivares FL, Battistoni F. 2016. Endophytic colonization of sugarcane (*Saccharum officinarum*) by the novel diazotrophs *Shinella* sp. UYSO24 and *Enterobacter* sp. UYSO10. *Plant and Soil* 403: 403-418.
10. Tam HM, Diep CN. 2014. Isolation, characterization and identification of endophytic bacteria in sugarcane (*Saccharum* spp. L.) cultivated on soils of the Dong Nai Province, Southeast of Vietnam. *Am. Jr. Life Science* 2(6): 361-368.
11. Fors RO, Patreze CM, Louro Berbara RL, Carbone Carneiro MA, Saggin-Júnior OJ. 2020. Dark septate endophytic fungi associated with sugarcane plants cultivated in São Paulo, Brazil. *Diversity* 12(9): 351
12. Pandey S, Sharma TK, Dassani S. 2020. Isolation and screening of cellulolytic fungi from degrading leaf litter of *S. officinarum* L. *Plant Archives* 20(2): 7013-7020.

13. Dobranic JK, Johnson JA, Alikhan QR. 1995. Isolation of endophytic fungi from eastern larch (*Latrix laricina*) leaves from New Brunswick, Canada. *Can. Jr. Microbiology* 41: 194-198.
14. Onions AHS, Allosopp D, Eggins HOW. 1981. Smith's Introduction to Industrial Mycology. 7th Edition. Edward Arnold, London. pp 168-209.
15. Ellis MB, Ellis JP. 1988. Micro-fungi of miscellaneous substrates: an identification handbook. Timber Press, Portland, Ore. pp 100-158.
16. Hata K, Futai K. 1995. Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, *Thecodiplosis japonensis*. *Can. Jr. Botany* 73: 384-390.
17. Rajagopal K. 1999. Biology and ecology of fungal endophytes of forest trees with special reference to neem (*Azadirachta indica* A Juss). *Ph. D. Thesis*, University of Madras, Chennai, India.
18. Rohrmann S, Lorenz R, Molitoris HP. 1992. Use of natural and artificial seawater for investigation of growth, fruit body production, and enzyme activities in marine fungi. *Can. Jr. Botany* 70: 2106-2110.
19. Hankin L, Anagnostakis SL. 1975. The use of solid media for the detection of enzyme production by fungi. *Mycologia* 67: 597-607.
20. Rajagopal K, Meenashree, B, Binika D, Joshila D, Tulsi PS, Arulmathi R, Kathiravan G, Tuwar, A. 2018. Mycodiversity and biotechnological potential of endophytic fungi isolated from hydrophytes. *Current Research in Environmental and Applied Mycology* 8(2): 172-182.
21. Gideon IO, John ME, Ekenem GU. 2016. Efficacy of metabolites from *S. officinarum* Linn. endophytic fungi against some uropathogenic bacteria. *Int. Jr. Res. Med. Basic Science* 2(1): 1-9.
22. Arumugam P. 2016. Diversity of endophytic fungi in few Food crops and their bioactivity. *Ph.D. Thesis*, VELS Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India.
23. Petrini O. 1986. Taxonomy of endophytic fungi of aerial plant tissues. *In*: Microbiology of the Phyllosphere (Eds). Fokkema NJ and Van den Huevel J.) Cambridge University Press, Cambridge, UK. pp 175-187.
24. Jamith Basha W, Rajagopal K, Meenashree B, Arulmathi R, Kathiresan AK, Gayathri G, Kathiravan G, Meenambiga SS. 2019. Diversity and antimicrobial activity of endophytic fungi associated with a hydrophyte *Aponogeton natans*. *Kavaka* 53: 61-66.
25. Reddy MS, Murali TS, Suryanarayanan TS, Govinda Rajulu MB, Thirunavukkarasu N. 2016. *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. *Fungal Ecology* 24: 70-75.
26. Suryanarayanan TS, Kumaresan V, Johnson JA. 1998. Foliar fungal endophytes from two species of the mangrove Rhizophora. *Can. Jr. Microbiology* 44: 1003-1006.
27. Debbab A, Aly AH, Proksch P. 2013. Mangrove-derived fungal endophytes-a chemical and biological perception. *Fungal Divers* 61: 1-27.
28. Rajamani T, Suryanarayanan TS, Murali TS, Thirunavukkarasu N. 2018. Distribution and diversity of foliar endophytic fungi in the mangroves of Andaman Islands, India. *Fungal Ecology* 36: 109-116.
29. Petrini O. 1991. Fungal endophytes of tree leaves. *In*: Microbial ecology of the leaves (Eds) Andrews JH, Hirano SS. pp 179-197.
30. Khan AR, Ullah I, Waqas M. 2015. Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. *World. Jr. Microbiology Biotechnology* 31: 1461-1466.
31. Bilal L, Asaf S, Hamayun M. 2018. Plant growth-promoting endophytic fungi *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulate plant endogenous hormones. *Symbiosis* 76: 117-127.
32. Jagannath S, Konappa NM, Alurappa R, Chowdappa S. 2019. Production, characterization of indole acetic acid and its bioactive potential from endophytic fungi of *Cymbidium aloifolium* L. *Jr. Biol. Act. Prod. Nat.* 9(5): 387-409.
33. Waqas M, Khan AL, Kamran M, Hamayun M, Kang SM, Kim YH, Lee IJ. 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules* 17(9): 10754-10773.
34. Bacon CW. 1988. Procedure for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. *Appl. Environ. Microbiology* 54: 2615-2618.
35. Mehmood A, Hussain A, Irshad M. 2019. *In vitro* production of IAA by endophytic fungus *Aspergillus awamori* and its growth-promoting activities in *Zea mays*. *Symbiosis* 77: 225-235.
36. Porter JK, Bacon CW, Cutler HG, Arrendale RF, Robbins JD. 1985. *In vitro* auxin production by *Balansia epichloe*. *Phytochemistry* 24: 1429-1431.
37. Carroll GC, Petrini O. 1983. Patterns of substrate utilization of some endophytes from coniferous foliage. *Mycologia* 75: 53-63.
38. Maria GE, Sridhar KR, Raviraja NS. 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of the southwest coast of India. *Jr. Agri. Technology* 1: 67-80.
39. Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K. 2002. Endophytic fungi: A source of novel biologically active secondary metabolites. *Mycology Research* 106(9): 996-1004.