



ENDOPHYTIC FUNGI FROM *ALTERNANTHERA PUNGENS* KUNTH

Harshdeep B. Sartape and Ashfaque M. Khan*

Department of Botany, Maulana Azad College of Arts, Science and Commerce,
Dr. Rafiq Zakaria Campus, P.O. Box No-27, Aurangabad (Maharashtra), India.

Abstract

Endophytes are unknown symbionts that live in plant tissues without indicating their presence. The association of endophytes with plants is well established. They not only help in water and mineral uptake but also play an important role in metabolic activities of certain plants. Other nature of endophytes is to ensure defense mechanism in plants from external forces such as mammals and birds. In present investigation about endophytes in plants, *Alternanthera pungens* Kunth a prostrate perennial herb commonly available along the road side forming mat like appearance was selected. It belongs to family Amaranthaceae of angiosperm. Traditionally the herb is used to cure measles, headache and abdominal pain. Isolates from root, stem and leaves showed the presence of endophytes. Fungal strains like *Aspergillus spp*, *Colletorichum spp*, *Alternaria spp*, *Fusarium spp* was isolated from leaf and stem region of the herb.

Key words: *Alternanthera pungens*, endophytes, fungi, perennial herb.

Introduction

More than 20,000 bioactive metabolites are of microbial origin (Bérdy, 2005). It has been estimated that there may be 1.5 million fungal species, while only about 100,000 species are presently known (Hawksworth, 2004). The horizontally transmitted endophytes have been reported from all major groups of plants including algae (Zuccaro *et al.*, 2008; Suryanarayanan *et al.*, 2010), lichens (Suryanarayanan *et al.*, 2005) mosses and ferns (Petrini, 1986), conifers (Giordano *et al.*, 2009) and angiosperms (Saikkonen, 2007; Tejesvi *et al.*, 2010) and may persist even in aseptically cultured plants (Lucero *et al.*, 2008). Endophytes are the microbes that colonize living internal tissues of plants without causing any immediate overt symptoms (Petrini, 1986). They are found in almost all plants studied, including liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns and seed plants from arctic to the most biologically diverse tropical forests (Bacon and White, 2000). Plant-associated microbes have also been recognized for their ecological roles influencing host populations, plant communities (Clay and Hollah, 1999; Rudgers and Clay, 2007), biosynthesis, biotransformation and biodegradation (Koide *et al.*, 2005; Yu and Dai 2011). Individual plants can harbor dozens of endophytic fungal species (Arnold and Lutzoni, 2007) and

these endophytes contribute to the hyper diversity of fungi (Hawksworth, 2001). Medicinal herbs are an important group of hosts for endophytic fungi (Huang *et al.*, 2008). Endophytes from Chinese medicinal plants show efficacy as pharmaceutical and agricultural compounds (Shentu *et al.*, 2007; Kusari *et al.*, 2008). The various natural products produced by endophytic fungi possess unique structures and great bioactivities, representing a huge reservoir which offers an enormous potential for exploitation for medicinal, agricultural and industrial uses (Tan and Zou, 2001; Zhang *et al.*, 2006). Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications (Stadler and Keller, 2008). In present investigation the herb *Alternanthera pungens* was collected from different locations in and around Aurangabad city of Maharashtra state to discourse number of endophytes present in it. In the preliminary investigation endophytic fungi were evident that has been isolated, screened and identified.

Material and Methods

The entire experimental plant which is perennial herb was collected in monsoon in between July to October 2017 and brought to research lab in sterile polythene bags. Plant parts such as root, stem and leaves were washed

*Author for correspondence : E-mail : ashfaqamk@gmail.com

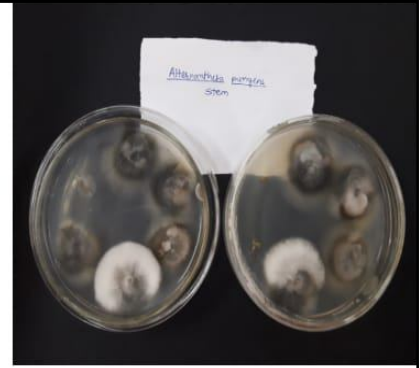
Plate-1: Endophytic Fungi from *different plant parts of Alternanthera pungens* Kunth



A. pungens root,



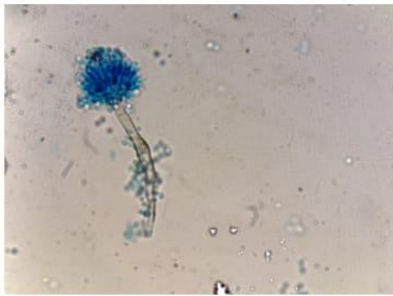
A. pungens leaf,



A. pungens stem



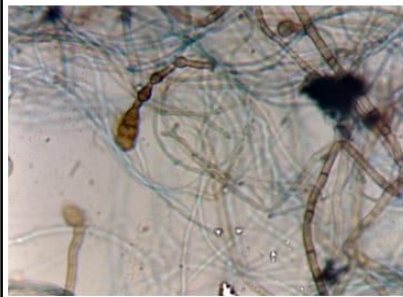
Curvularia sp



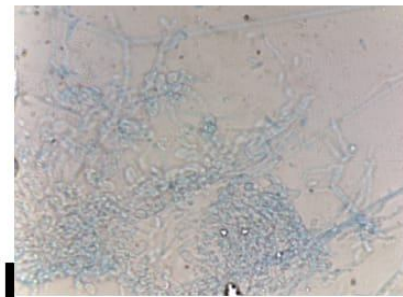
Aspergillus fumigates



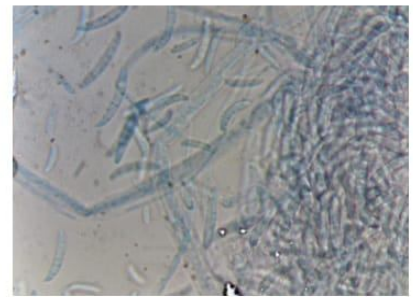
Colletotrichum truncatum



Alternaria alternate



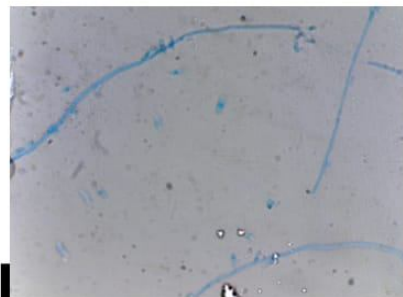
Cladosporium sp



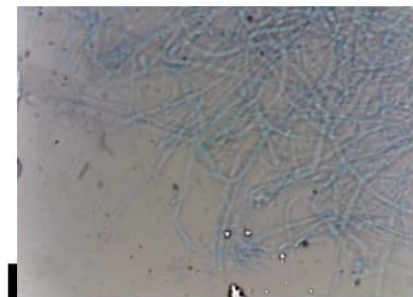
Fusarium oxysporum



Mycelia sterilia



Fusarium sp



Mycelia sterilia

Table 1: Endophytic Fungi from *different plant parts of Alternanthera pungens* Kunth

Sr. No.	Endophytic fungi	<i>Alternanthera pungens</i>		
		Root	Stem	Leaf
1	<i>Aspergillus niger</i>	—	++	++
2	<i>Aspergillus flavus</i>	—	++	++
3	<i>Aspergillus fumigates</i>	—	++	++
4	<i>Alternaria alternata</i>	—	—	++
5	<i>Cladosporium sp</i>	—	++	++
6	<i>Colletotrichum truncatum</i>	—	++	—
7	<i>Curvularia sp</i>	—	++	++
8	<i>Fusarium oxysporum</i>	++	—	—
9	<i>Fusarium sp.</i>	++	—	—
10	Mycelia sterilia	++	—	—

thoroughly in distilled water, blot dried, and first immersed in 70% ethanol (v/v) for one min followed by second immersion in sodium hypochlorite (3.5%, v/v) for three minutes. They were rinsed three times in changes of sterile distilled water and dried on sterile blotters under the airflow to ensure complete drying. 0.5cm × 0.5 cm size bits were excised with the help of a sterile blade and placed on PDA plates. Periodically the bits were examined for the appearance of fungal colony and each colony that emerged from segments was transferred to another plate of PDA for further identification. The morphological identification of the isolates was done based on the fungal colony morphology and characteristics of the reproductive structures and spores (Barnett and Hunter, 1998; Domsch *et al.*, 2003; Leslie and Summerell, 2006). All fungal mounts were made on microscopic glass slides in lactophenol-cotton blue and cultures which failed to sporulate were grouped as mycelia sterilia.

Results and Discussion

A total of 39 isolates were obtained from 54 tissue fragments from *A. pungens* plant. The extend of endophytes colonization varied in plant parts with stem and leaf fragments recording more endophytes than that of root fragments. Leaf and stem endophytes (Table 1) showed greater number of *Aspergillus sp.* *Aspergillus niger* was seem to be more dominant among *Aspergillus flavus*, and *Aspergillus fumigates*. Other endophytes isolated and identified were *Alternaria alternata*, *Cladosporium sp*, *Colletotrichum truncatum*, *Curvularia sp*, *Fusarium oxysporum* and *Fusarium sp.* Root fragments were rich in mycelia sterilia as they didn't show any kind of sporulation.

Efforts are on to isolate novel bioactive compounds from various endophytes that are living in the internal tissue of plants. Current investigation highlights preliminary examination in these regard and focuses on

endophyte diversity in *Alternanthera pungens* during monsoon season. Further study will be elaborative which will be based on increased sample area from all over Marathwada region of Maharashtra and explore more parameters. Effect of seasonal variations will be considered on the nature of endophytes plant holds. Isolates will be subjected to determine presence of bioactive compounds to obtain newer antioxidants with therapeutic applications if any.

Acknowledgement

Authors are thankful to the CSIR-HRDG, New Delhi for providing fellowship for research work.

References

- Arnold, A.E. and F. Lutzoni (2007). Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology*, **88**: 541–549.
- Bacon, C.W. and J.F. White (2000). *Microbial Endophytes*. New York: Marcel Decker INC, pp. 237–261.
- Barnett, H. and B. Hunter (1998). *Illustrated Genera of Imperfect Fungi*, Burgess Publishing, Minneapolis, Minn, USA.
- Bérdy, J. (2005). Bioactive microbial metabolites: a personal view. *Journal of Antibiotics*, **58**: 1–26.
- Clay, K. and J. Holah (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, **285**: 1742–1744.
- Domsch, K.H., W. Gams and T. Anderson (2003). *Compendium of Soil Fungi*, Academic Press, New York, NY, USA.
- Giordano, L., P. Gonthier, G.C. Varese, L. Miserere and G. Nicolotti (2009). Mycobiota inhabiting sapwood of healthy and declining Scots pine (*Pinus sylvestris* L.) trees in the Alps. *Fungal Divers*, **38**: 69–8.
- Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, **105**: 1422–1432.
- Hawksworth, D.L. (2004). Fungal diversity and its implications for genetic resource collections. *Studies in Mycology*, **50**: 9–18.
- Huang, W.Y., Y.Z. Cai, K.D. Hyde, H. Corke and M. Sun (2008). Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity*, **33**: 61–75.
- Leslie, J.F. and B.A. Summerell (2006). *The Fusarium Laboratory Manual*, Blackwell Publishing, London, UK.
- Lucero, M.E., J.R. Barrow, P. Osuna, I. Reyes and S.E. Duke (2008). Enhancing native grass productivity by co-cultivating with endophyte-laden calli. *Rangeland Ecol Manag*, **61**: 124–130.
- Koide, K., T. Osono and H. Takeda (2005). Colonization and lignin decomposition of *Camellia japonica* leaf litter by endophytic fungi. *Mycoscience*, **46**: 280–286.

- Kusari, S., M. Lamshöft, S. Zühlke and M. Spiteller (2008). An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J. Nat. Prod.*, **71**: 159–162.
- Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. *Microbiology of Plant Microbe Interactions*, **16**: 580–587.
- Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. In: N.J. Fokkema, van den Heuvel (eds) *Microbiology of the phyllosphere*. Cambridge University press, Cambridge, pp 75–187.
- Rudgers, J.A. and K. Clay (2007). Endophyte symbiosis with tall fescue: how strong are the impacts on communities and ecosystems? *Fungal Biology Reviews*, **21**: 107–124.
- Shentu, X.P., L.Z. Chen and X.P. Yu (2007). Anti-fungi activities and cultural characteristics of ginkgo endophytic fungus No. 1028. *Acta Phytophyl Sin*, **34**: 147–152.
- Saikkonen, K. (2007). Forest structure and fungal endophytes. *Fungal Biol. Rev.*, **21**: 67–74.
- Stadler, M. and N.P. Keller (2008). Paradigm shifts in fungal secondary metabolite research. *Mycological Research*, **112**: 127–130.
- Suryanarayanan, T.S., N. Thirunavukkarasu, G.N. Hariharan and P. Balaji (2005). Occurrence of non-obligate microfungi inside lichen thalli. *Sydowia*, **57**: 120–130.
- Suryanarayanan, T.S., A. Venkatachalam, N. Thirunavukkarasu, J.P. Ravishankar, M. Doble and V. Geetha (2010). Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and bio-technological potential. *Bot. Mar.*, **53**: 457–468.
- Tan, R.X. and W.X. Zou (2001). Endophytes: a rich source of functional metabolites. *Natural Product Reports*, **18**: 448–459.
- Tejesvi, M.V., A.L. Ruotsalainen, A.M. Markkola and A.M. Pirttila (2010). Root endophytes along a primary succession gradient in northern Finland. *Fungal Divers*, **41**: 125–134.
- Yu, W. and C.C. Dai (2011). Endophytes: a potential resource for biosynthesis, biotransformation and biodegradation. *Annals of Microbiology*, **61**: 207–215.
- Zhang, H.W., Y.C. Song and R.X. Tan (2006). Biology and chemistry of endophytes. *Natural Product Reports*, **23**: 753–771.
- Zuccaro, A., C.L. Schoch, J.W. Spatafora, J. Kohlmeyer, S. Draeger and J. Mitchell (2008). Detection and identification of fungi associated with the brown seaweed *Fucus serratus*. *Appl. Environ. Microbiol.*, **74**: 931–941.