

Endophytic Fungal Diversity in Corms of *Amorphophallus sylvaticus* (Roxb.) Kunth

Sayyad Shahim, Mulani R. M.

Department of Botany, DST-FIST, UGC-SAP sponsored School of Life sciences, Swami Ramanand Teerth Marathwada University, Nanded-431606 (MS), India

Abstract: In present investigation *Amorphophallus sylvaticus* (Roxb.) Kunth Commonly called as Jangali suran is a monsoon perennial cormatus plant species first time studied for endophytic fungal diversity of corm. The different corm sample were collected from two different sites namely Pota and Nageli sites in Nanded district of Maharashtra. The 45 corm samples were screened for colonization of different endophytic fungi on czapek-dox agar medium. The different corm samples show different endophytic fungal genera the isolated and identified genera includes *Fusarium*, *Cladosporium*, *Alternaria*, *Helminthosporium*, *Curvularia*, *Rhizoctonia* and *Drechslera*.

Keywords: *Amorphophallus sylvaticus*, corm and endophytic fungi.

1. Introduction

Endophytic fungi are important components of plant micro-ecosystems. They spend their whole life by colonizing intra or inter-cellularly within the healthy tissues of the host plants, without showing any symptoms of disease (Zhang *et al.* 2006). Endophytes are found in a wide variety of plant tissue types such as roots, stems, leaves, tubers, buds, ovules, seeds, fruits, xylem and bark (Tan and Zou, 2001).

Study revealed that approximately, there are near to 300,000 plant species on the earth having host to one or more endophytes, and many of them may colonize different hosts. (Strobel & Daisy 2003, Huang *et al.*, 2007).

Plant endophytic fungi are novel and important for production of natural bioactive compounds with their potential use in agriculture, medicine and food industry. The various important bioactive compounds from endophytic fungi isolated which shows antimicrobial, insecticidal, cytotoxic and anticancer activities. An anticancer taxol isolated from *Colletotrichum gloeosporioides* of concentration from 0.005 – 0.05 μm induced increased cell death against human cancer cell through apoptosis (Gangadevi & Muthumary, 2008). They produces anticancer enzyme L-asparaginase (Yiing and Adeline, 2014). The production of bioactive compounds which shows antimicrobial activity against different pathogenic microbes (Jingfeng *et al.*, 2013, Tanmayee *et al.*, 2015). Endophytes having ability to produce different enzymes like amylase, cellulase, protease, lipase, and laccase. Endophytes like *Aspergillus terreus* produces lovastatin used in treatment of coronary heart diseases, renal diseases, Alzheimer's disease, bone fractures etc (Praveen *et al.*, 2014). Endophytic fungus *Pestalotiopsis microspora* from *Terminalia morobensis* to show antioxidant activity to scavenge superoxide and hydroxyl free radicals (Harper *et al.*, 2003). The endophytic *Aspergillus* sp., *Phoma* sp. produces Antidiabetic drug 2, 6-di-tert-butyl-p-cresol and Phenol, 2, 6-bis [1, 1-dimethylethyl]-4-methyl which Reduce blood glucose level (Dhankhar and Yadav 2013).

There are plenty of reports on endophytic fungal diversity of different plants like *Cymbopogon citratus*, *Murraya koenigii*, *Oldenlandia diffusa* and *Pereskia bleo* (Yiing *et al.*, 2014), Roots of *Salvia miltiorrhiza* Bunge (Jingfeng *et al.*, 2013), Stem of Rose plant and leaves of Mango tree (Tanmayee, *et al.* 2015). *Urginea indica* (Shiva *et al.*, 2015), orchids like *Anacamptis pyramidalis* (L.), *Orchis sancta* L., *Ophrys fusca* Link. and *Serapias vomeracea* subsp. *orientalis* (Greuter) from roots and tubers (Yuksel and Rengin, 2009), tuber of *Solanum tuberosum* (M. O'Callaghan *et al.* (2004). The rhizospheric fungi associated with the corms of *Amorphophallus sylvaticus* (Roxb.) Kunth were isolated (Mulani and Sayyad, 2015).

There is a no report on endophytic fungal diversity of *Amorphophallus sylvaticus* (Roxb.) Kunth, so the present investigation was undertaken.

2. Materials and Methods

2.1. Collection of corms

Corms of *Amorphophallus sylvaticus* (Roxb.) Kunth were collected from fields of village Pota, Tq. Himayatnagar and village Nageli, Tq. Mudkhed of Nanded district during winter (November, 2014). These corm samples were collected in sterile polythene bags and brought to the laboratory used for the investigation of endophytic fungi.

2.2. Isolation of Endophytic Fungi

Endophytic fungi were isolated by following methods employed by Hallman *et al.* (2007) and Selvakumar *et al.* (2014).

The collected corm samples were washed in running tap water to remove the debris and epiphytic microorganisms and soaked in 0.1 % mercury chloride subsequently surface sterilized by using ethanol. Followed by sterilizing agent, such as 2% Sodium hypochloride solution for 2-4 minutes and then by sterile distilled water. The Sterile corm were then chopped into 3-4 mm x 0.1 cm lengths and inoculated in petridishes containing potato dextrose agar (PDA) medium supplemented with streptomycin. The petridishes

were sealed and incubated at room temperature (28±2°C) for 15 days. Fungal growths growing out of the corm pieces were sub cultured on separate PDA plates and slants before use for the identification.

The colonization frequency (CF) percentages of Endophytic fungi were calculated using the method (Kumar & Hyde 2004).

2.3. Colonization Frequency

$$\text{CF\%} = \frac{\text{No of species isolated} \times 100}{\text{No of segments screened}}$$

2.4. Identification of Isolated Fungal Organisms

The identification of endophytic fungi was done by using the culture characteristics like fungal culture shape, color, pattern and microscopic characteristics like arrangement of the mycelium, conidial arrangement, types of spore etc. by consulting relevant literature. The pure cultures of isolated fungal strains were maintained on PDA slants at 28°C during the study.

3. Results & Discussion

In present investigation a total forty five small corm segments were screened for isolation of endophytic fungi on Czapek dox agar medium. The total seven endophytic

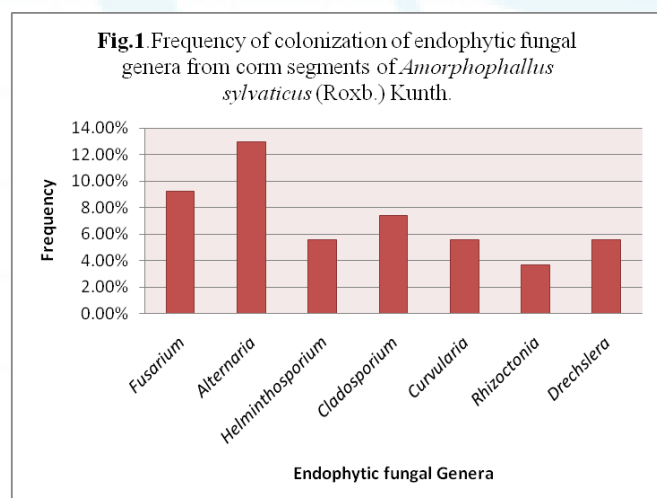
fungal genera were isolated and identified from corms of *Amorphophallus sylvaticus* (Roxb.) Kunth. The isolated genera include *Fusarium*, *Alternaria*, *Helminthosporium*, *Cladosporium*, *Curvularia*, *Rhizoctonia* and *Drechslera*.

The species colonization consist of *Fusarium* 9.25 %, *Alternaria* 12.96 %, *Helminthosporium* 5.55 %, *Cladosporium* 7.4 %, *Curvularia* 5.55 %, *Rhizoctonia* 3.7 %, and *Drechslera* 5.55 % (Table.1 and Fig.1)

Similarly different researchers have isolated endophytic fungi from different plant parts, mainly Maria *et al.* (2013) isolated *Monilia* sp., *Aureobasidium* sp., *Moniliella* sp., and *Sporothrix* sp. from tubers of *Dahlia variabilis*. Yuksel and Rengin (2009) isolated *Rhizoctonia*, *Fusarium* and *Papulaspora* from roots and tubers of orchids. Chanda *et al.*, (2013) isolated endophytes include *Aspergillus* sp., *Nigrospora* sp., *Mucor* sp., *Curvularia* sp., *Fusarium* sp., *Alternaria* sp., *Stemphyllum* sp. and *Chaetomium* sp. O'Callaghan *et al.*, (2004) isolated endophytic fungal genera include *Chaetomium*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Paecilomyces*, *Phomopsis* and *Rhizoctonia* from tuber of *Solanum tuberosum*. Tanmayee *et al.*, (2015) isolated Total, 5 fungal isolates of endophytes were obtained from Rose stem. The less endophytic fungal diversity may be due to medicinal property of plant that affect colonization due to secretion of some antifungal compounds (Rajgopal *et al.*, 2010).

Table 1: Frequency colonization of endophytic fungal genera from corm segments of *Amorphophallus sylvaticus* (Roxb.) Kunth.

Sr. No	Name of fungal Endophytic genera	Number of isolates	Frequency of colonization
1	<i>Fusarium</i>	5	9.25 %
2	<i>Alternaria</i>	7	12.96 %
3	<i>Helminthosporium</i>	3	5.55 %
4	<i>Cladosporium</i>	4	7.4 %
5	<i>Curvularia</i>	3	5.55 %
6	<i>Rhizoctonia</i>	2	3.7 %
7	<i>Drechslera</i>	3	5.55 %



4. Conclusion

In present investigation the total seven endophytic fungal genera were isolated and identified from different corms of

Amorphophallus sylvaticus which will be further used for production of some bioactive compounds which is essentially used in medicine, agriculture and food industry.

Acknowledgement

The acknowledgements are due to the authorities of Swami Ramanand Teerth Marathwada University and The Director, DST-FIST, UGC-SAP sponsored School of Life Sciences, and SRTM University Nanded for providing necessary facilities for carried out present investigation. The author Sayyad Shahim is grateful to the Maulana Azad National Fellowship (MANF), New Delhi for their financial assistance to conduct this research work.

References

- [1] Chanda, P., Sulekha, Y., Sonu, R. and Anuradaha, D. (2013). Studies on Biodiversity of Fungal Endophytes of Indigenous Monocotaceous and

- Dicotaceous Plants and Evaluation of their Enzymatic Potentialities. *International Journal of Scientific and Research Publications*. 3 (7):1-5.
- [2] **Dhankhar, S. and Yadav, J.P. (2013)**. Investigations towards New Antidiabetic Drugs from Fungal Endophytes Associated with *Salvadora oleoides* Decne, *Medicinal Chemistry*, 9(4):624-632.
- [3] **Gangadevi, V. and Muthumary, J. (2008)**. Isolation of *Colletotrichum gloeosporioides*, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, *Justicia gendarussa*. *Mycologia Balcanica*, 5:1-4.
- [4] **Hallmann, J.B.G. and Schulz, B. (2007)**. Isolation procedures for endophytic microorganisms, Springer, Berlin Heidelberg, p. 299 Khare C.P. 2004. Encyclopedia of Indian Medicinal Plants. Springer- Verlag Berlin Heidelberg, New York ISBN 3-540-20033-9:464-465.
- [5] **Harper, J.K., Arif, A.M., Ford, E.J., Strobel, G.A., Tomer, D.P., Grant, D.M., Porco, J. and Oneill, K. (2003)**. Pestacin: a 1, 3- dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron*, 59(14):2471-2476.
- [6] **Huang, W.Y., Cai, Y.Z., Xing, J., Corke, H. and Sun, M. (2007)**. Potential antioxidant resource: endophytic fungi isolated from traditional Chinese medicinal plants. *Economic Botany* 61, 14-30.
- [7] **Jingfeng, L., Linyun, F., Ruiya, L., Xiaohan, W., Haiyu, L. and Ligang, Z. (2013)**. Endophytic fungi from medicinal herb *Salvia miltiorrhiza* Bunge and their antimicrobial activity. *African Journal of Microbiology Research*. Vol. 7(47):5343-5349.
- [8] **Kumar, D.S.S. and Hyde, K.D. (2004)**. Biodiversity and tissue recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Diversity* 17: 69-90.
- [9] **Maria, L., Yuli, H., Fifi, P., Didit, T. and Saryono, S. (2013)**. Screening of endophytic fungi from tubers of *Dahlia variabilis*. *Journal of Agricultural Technology*. 9(3): 565-570.
- [10] **Mulani, R.M. and Sayyad, S. (2015)**. Diversity of rhizosphere fungi and soil nutrient properties of *Amorphophallus sylvaticus* (Roxb.) Kunth. *International Journal of science and research*, 4(8):1958-1966.
- [11] **O'Callaghan, M., Gerard, E. M., Waipara, N.W., Young, S. D., Glare, T. R., Barrell, P. J. and Conner, A. J. (2004)**. Microbial communities of *Solanum tuberosum* and magainin-producing transgenic lines. *Plant and Soil* 266: 47-56.
- [12] **Praveen, V. K., Bhargavi, S. D. and Savitha, J. (2014)**. Endophytic Fungi: A Poor Candidate for the Production of Lovastatin. *British Microbiology Research Journal*. 4(12):1511-1520.
- [13] **Rajagopal S, Kumar RA, Deevi DS, Satyanarayana C, Rajagopalan R (2010)**. A potential cancer therapeutic agent isolated from *Andrographis paniculata*. *Journal of Experimental therapeutics and Oncology*, 3:147-158.
- [14] **Selvakumar, V., Panneerselvam, A., Vijayakumar, N., Mohan, A. and Thajuddin, N. (2014)**. Diversity of Endophytic and Rhizosphere Soil Fungi of *Avicennia marina* in Maravakadu Mangrove Forest. *IOSR Journal of Pharmacy and Biological Sciences*. 9 (2):24-28.
- [15] **Shiva, K.M.N., Mohana, B. and Thara, S.K.J. (2015)**. Isolation and identification of endophytic Fungi from *Urginea indica*, a medicinal Plant from diverse regions of south India. *International Journal of Latest Research in Science and Technology*. 4 (1):75-80.
- [16] **Strobel, G. and Daisy, B. (2003)** – Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67, 491-502.
- [17] **Tan, R.X. and Zou, W.X. (2001)**. Endophytes: a rich source of functional metabolites. *Nat Prod Rep*. 18: 448-59.
- [18] **Tanmayee, R. D., Suchita, M. I. and Kshitija, V. P. (2015)**. Study on Production of Bioactive Compounds and Plant Promoting Ability of Endophytes Isolated from *Rosa sp.* and *Mangifera indica*. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-2: 136-143.
- [19] **YiingYng, C., Adeline, S.Y. and Ting. (2014)**. Endophytic L-asparaginase-producing fungi from plants associated with anticancer properties. *Journal of Advanced Research* .xxx, xxx-xxx .<http://dx.doi.org/10.1016/j.jare.2014.07.005>.
- [20] **Yuksel, G. and Rengin, E. (2009)**. Diversity of endophytic fungi from various Aegean and Mediterranean orchids (saleps). *Turk J Bot.* 33:439-445.
- [21] **Zhang, H.W., Song, Y.C. and Tan, R.X. (2006)**. Biology and chemistry of endophytes. *Natural Product Reports*. 23: 753-771.

Authors Profile



Dr. R. M. Mulani received the M.Sc. in Botany from Shivaji University, Kolhapur in 1985 and received Dr. G.V. Joshi Gold Medal and Ph.D. degree from Mumbai University 1989. Presently working as Associate Professor in Botany at School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. He has 54 National and International Publication to his credit and he is a member of RRC for Botany to Mumbai University.



Miss. Sayyad Shahim Fatima Karim received the M.Sc. in Botany from NES Science College, Nanded in 2012 and now she is working as Research Scholar pursuing M.Phil. Botany in the field of Mycology and Plant Pathology at School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. She has published one research paper in International Journal.