

MORPHOLOGICAL EXPLORATION OF LIGNICOLOUS MACROFUNGI: XYLARIA

Abstract

Xylaria is a large and first described genus of *Xylariaceae* which is generally recognized as one of the most diverse and relatively well known largest family of Ascomycota. The fruiting bodies of *Xylaria* species were collected from Navsari, Gujarat during rainy season. All the specimens were examined for their morphological characters based on shape, size and colour of stromata followed by detailed microscopic examination of perithecia and ascospores with light microscope and were identified. From the total collection seven species were designated based on detailed macro- and micro-morphological characteristics. Among the different collected samples five were identified upto species level and two upto genus level. These were *Xylaria hypoxylon*, *X. longipes*, *X. polymorpha*, *X. filiformis* and *X. carpophila*. The diversity of *Xylaria* species in this region invokes and unveils opportunities for exploring rich source of bioactive compounds such as alkaloids, terpenoids, cytochalasins, cell-degrading enzymes, exopolysaccharides, xylaramide, xylarin xyloketal, xylaric acids and helvonic acid which exhibited antifungal and antibacterial activities. So, exploitation of *Xylaria* fungi creates new opportunities to develop bio-based commercial products to combat global crop and human pathogens.

Key words: *Xylaria*, stromata, morphology, pileus, ascospore, stipe

Introduction

Xylaria Hill ex Schrank (1789) is the largest and first described genus of *Xylariaceae* which is generally identified as one of the most diverse and comparatively well known largest family of ascomycota and widely distributed throughout the globe evidenced by reports from different regions with broad ecological diversity (Lee *et al.* 2002). *Xylaria* differs from other genera in *Xylariaceae* by producing upright, barrel shaped to clavate, multi-peritheciate stromata (Maharachchikumbura *et al.* 2016). At present, about 300 estimated species are thought to have a place in *Xylaria* (Kirk *et al.* 2008). They are mostly saprophytic, may be pathogenic (Proffer 1988; Hartman *et al.* 2008) and endophytic in nature and appeared to exist on decayed wood, dung, litter and soil (Davis *et al.* 2003; Okane *et al.* 2008). Kshirsagar *et al.* (2009); Ramesh *et al.* (2012) and Hande & Hiwarale (2013) reported more than 10 *Xylaria* species in western and northern states of India. However, existing awareness and research on these fungi in Gujarat state (India) is at their primary stage with only few reports (Korat *et al.* 2013; Nagadesi and Arya 2014; Rajput *et al.* 2015).

Gujarat shares unique diversity of various flora and fauna because Gujarat is known to have varying climatic conditions like moist deciduous (ending part of Western Ghat) to extreme desert conditions (Rann of Kutchh), Savanna type grassland, arid to semiarid conditions in northern part of the state and also has the longest seacoast in country. Angiosperm flora of Gujarat documented and studied thoroughly but mycoflora has always been neglected by the botanists (Rajput *et al.* 2015). The reports on fungal diversity of Gujarat could be found in the literature but they are only restricted to pathogenic fungi associated to agricultural crops or human beings (Bhavsar *et al.* 2012; Assudani 2013; Dhingani *et al.* 2013; Nagadesi and Arya

2014). In the present study we attempted to document the diversity of *Xylaria* which is a rich source of various secondary metabolites due to endophytic nature of many species (Hacioglu *et al.* 2011; Nilza 2013; Fan *et al.* 2014). The diversity of *Xylaria* species in this region invokes and unveils the opportunities for exploring rich source of bioactive compounds such as alkaloids, terpenoids, cytochalasins, exopolysaccharides, cell-degrading enzymes, xylaramide, xylarin, xyloketals, xylaranic acids and helvonic acid which exhibited antifungal, antibacterial and also antiviral activities. Thus, it will be a new opportunity to develop bio-based commercial products to combat global crop and human pathogens. It is also valued for various lignocellulosic enzymes that have a wide application in pharmaceutical, in textile industry, paper and pulp industry etc. (Soto *et al.* 2015).

Materials and Methods

Survey was conducted at the different locations of Navsari, Gujarat for the collection of naturally growing *Xylaria* during the rainy seasons of 2017 and 2018 when the temperature was around 28- 30°C and relative humidity was more than 85%. The sites of collection were soil, open lands, farm lands, roadside, research field etc. The fruiting bodies were kept in the sterile polyethylene bags labeled with the location, name of the host substrate and date of collection. These were carried to the laboratory. All the fruiting bodies were micro-photographed. All the specimens were examined for their morphological characters based on shape, size and colours of pileus and length and diameter of stipe followed by detailed microscopic examination of perithecia and ascus with light microscope and were identified.

Results

From the total collection seven species were identified. Among which five were identified upto species level and two upto genus level. These were *X. hypoxylon*, *X. longipes*, *X. polymorpha*, *X. filiformis* and *X. carpophila*.

***Xylaria carpophila*:** The pileus was long, slender, rough texture, black, 1.6-3.8mm width, unbranched and the tip was pointed. Stipe was black in colour with 15.67cm long and diameter was 0.5cm. Perithecia were barrel shaped with papillate ostioles. Asci 8 spored. Ascospores pale yellow to dull white and 8-10 × 4-5 µm in size.

Host: on humus rich soil and leaf litter.

***Xylaria filiformis*:** Pileus was simple, long, slender, 2 mm width, filiform and white smooth surfaced, which become wrinkled at maturity with very thin apical tip and distinct. The stipe was 38cm long, smooth, black in colour and diameter was 1.9cm. Perithecia were solitary with papillate ostioles. Asci are cylindrical. Ascospores are black and 9-14 × 4-6 µm in size,

Host: seeds of royal palm tree.

***Xylaria hypoxylon*:** Pileus was slender, finger like (2-5 pointed) shaped, white or ashy white in colour with smooth surface, 1-1.9mm in diameter. Stipe was 19.67-53cm length × 0.8-3.82cm width, smooth and black in colour. . Perithecia were barrel shaped with papillate ostioles. Asci cylindrical and 8 spored. Ascospores black and 3.5-7.5 × 2.5-5.5µm in size.

Host: decaying wood log/sticks/chips, soil, dry fruits shells of *Cassia fistula*

Xylaria longipes: Fruiting bodies were found in single or in-group. Pileus was club shaped, tough, 7-19mm in diameter and lengthened to round apex. Surface was smoky white to greyish white and turns black, cracky and scaly with age. Stipe was 22-62cm length \times 1.9-2.87cm width and black in colour. Perithecia with papillate ostiole. Asci 8 spored. Ascospores brown to black and $8-11 \times 3-5 \mu\text{m}$ in size.

Host: soil, decaying woodlog/sticks.

Xylaria polymorpha: Fruiting body commonly grows on the decaying wood. Pileus was finger shaped. The tip of the fruiting body is rounded and white in colour when young and turns to black eventually with the age. Stipe was short (12.67-13cm), cylindrical and black in colour. Surface was rough and wrinkled at maturity. Asci cylindrical with 8 spores. Ascospores are $8-11 \times 4-6 \mu\text{m}$ in size.

Host: on unknown wood logs, chips

Table 1: Characteristics of *Xylaria* species

Sr. No.	<i>Xylaria sp.</i>	Pileus colour	Pileus diameter (mm)	Pileus shape	Stipe length (mm)	Stipe diameter (mm)	Stipe colour	Size of spore (μm)	Host
1	<i>X. carpophila</i>	Black	1.6 -3.8	pointed	15.67	0.5	black	8-10 X 4-5	Soil
2	<i>X. filiformis</i>	White	1-1.2	Hairy to slender	38	1.9	black	9.14 X 4-6	Royal palm seed
3	<i>X. hypoxylon</i>	White or ash white	1-1.9	Finger like(2-5 pointed)	19.67 - 53	0.8- 3.82	black	3.5- 7.5 X 2.5 – 5.5	Wood chips, soil
4	<i>X. longipes</i>	Smoky white to greyish white	7-11	Culb shaped	22-62	1.9 – 2.87	black	8-11 X 3-5	Soil
5	<i>X. polymorpha</i>	Black	1.8–2.9	Finger like (single)	12.67 - 13	1 – 1.3	black	8-11 X 4-6	Wood chips



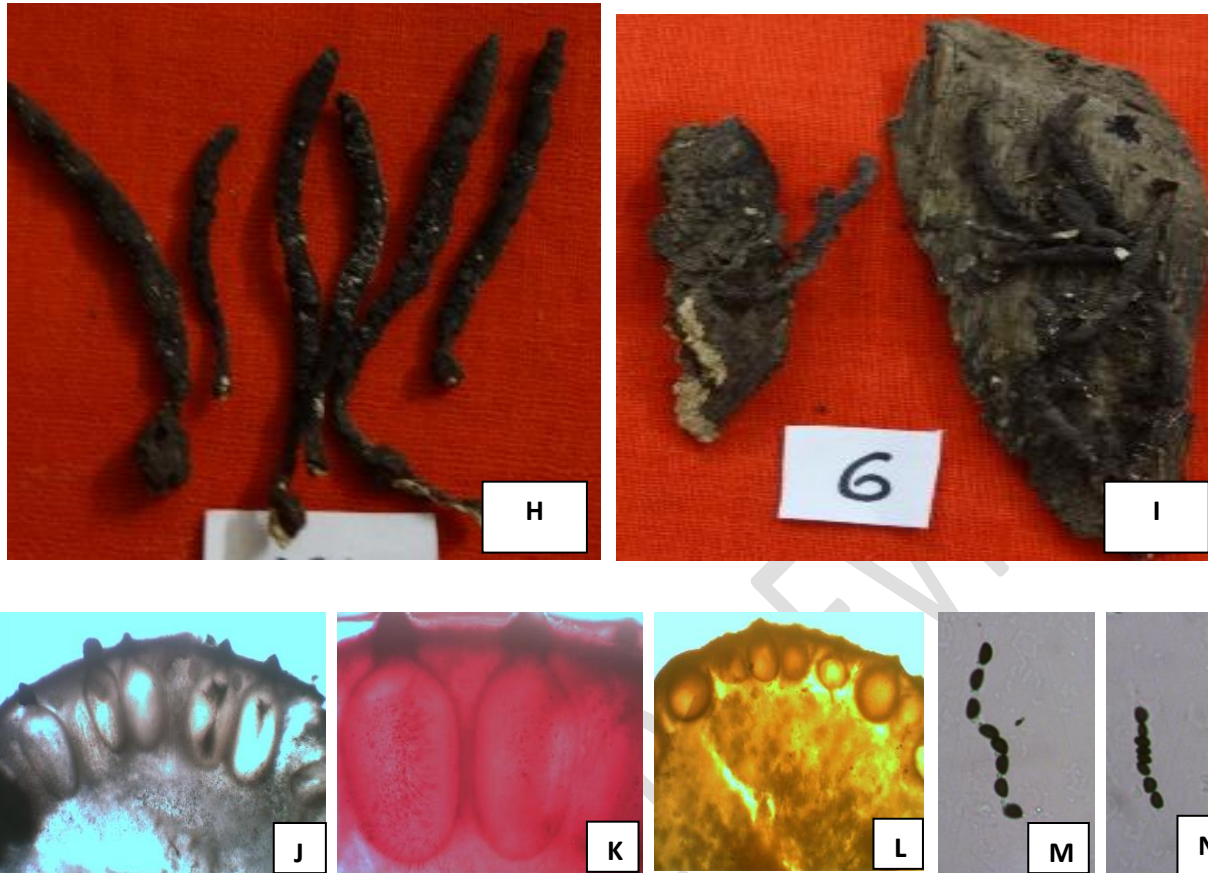


Figure I: A-*Xylaria carpophila*, B-*Xylaria filiformis*, C to E-*Xylaria hypoxylon*, F&G-*Xylaria longipes*, H&I - *Xylaria polymorpha*, J to L-*Perithecia*, M&N-*Ascus* with ascospores

Discussion

Few studies on fungal diversity of Gujarat have been carried out earlier (Nagadesi & Arya 2014; Koyani *et al.* 2016). Koyani *et al.* (2016) studied 14 species of genus *Xylaria* which were collected from different districts of Gujarat. Species mentioned in this paper have already been documented in the previous studies while existence of these five species of genera *Xylaria* in Navsari is being reported for the first time. Current study is preliminary but very essential contribution to fungal diversity of Gujarat state and further studies are needed. More species are expected from this genus and therefore; further studies are warranted to investigate the diversity of macro-fungi occurring within the state.

References

Assudani, H.J., Pandya, J.M., Sarvan, R.R., Sapre, A.M., Gupta, A.R. and Mehta, S.J. 2013. Etiological diagnosis of microbial keratitis in a tertiary care hospital in Gujarat. *N J Med Res* 3: 60-62.

- Bhavsar, H.K., Modi, D.J., Sood, N.K. and Shah, H. 2012. A study of superficial mycoses with clinical mycological profile in tertiary care hospital in Ahmedabad, Gujarat. *N J Med Res* 2(2): 160-164.
- Davis, E.C., Franklin, J.B., Shaw, A.J. and Vilgalys, R. 2003. Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: Phylogenetics, evolution and symbiosis. *American J Bot* 90(11): 1661-1667.
- Dhingani, J.C., Solanki, K.U. and Kansara, S.S. 2013. Management of root rot disease [*Macrophomina phaseolina* (Tassi.) Goid] of chickpea through botanicals and oil cakes. *The Bioscan* 8(3): 739-742.
- Fan, N.W., Chang, H.S., Cheng, M.J., Hsieh, S.Y., Liu, T.W. and Chen, I.S. 2014. Secondary metabolites from the endophytic fungus *Xylaria cubensis*. *Helvetica Chimica Acta* 97(12): 1689-1699.
- Hande, D.V. and Hiwarale, S.V. 2013. Diversity of *Xylaria* species from Amravati region, Amravati, MS, India. *Inter Res J Biol Sci* 2(1): 67-69.
- Hartman, J., Beale, J. and Bachi, P. 2008. Root and Collar Rots of Tree Fruits. UK Cooperative Extension Service, Plant Pathology Fact sheet. Agriculture College, University Kentucky (PPFS-FR-T-10).
- Hacioglu, N., Akata, I. and Dulger, B. 2011. Antimicrobial potential of *Xylaria polymorpha* (Pers.) Grev. *African J Microbiol Res* 5(6): 728-730.
- Kirk, P.F., Cannon, P.F., Minter, D.W. and Stalpers, J.A. 2008. Dictionary of the fungi, 10th edn. CABI, Egham CABI Bioscience, CBS, Landcare Research.
- Korat, C., Chopada, G. and John, P. 2013. Studies on biodiversity of fleshy fungi in Navsari (South Gujarat), India. *Inter J Biodiversity Conservation* 5(8): 508-514.
- Koyani, R.D., Patel, H.R., Vasava, A.M. and Rajput, K.S. 2016. Xylariaceae: Overview and addition to fungal diversity of Gujarat state. *Studies Fungi* 1(1): 69-79.
- Kshirsagar, A.S., Rhatwal, S.M. and Gandhe, R.V. 2009. The genus *Xylaria* from Maharashtra, India. *Indian Phytopath* 62(1): 54-63.
- Lee, Y., Han, S.S. and Jeong, I.S. (2002) Taxonomical characteristics of *Xylaria* spp. collected from Malaysia. *Mycobiol* 30(4):193-196.
- Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.B.G., McKenzie, E.H.C. *et al.* 2016. Families of Sordariomycetes. *Fungal Diver* 79: 1-317.
- Nagadesi, P.K. and Arya, A. 2014. New records of lignicolous fungi from Ratanmahal Wildlife Sanctuary, Gujarat, India. *Inter Letters Natural Sci* 8: 23-29.
- Nilza 2013. Screening of endophytic fungi *Xylaria* for antibacterial and antifungal activity. PhD Thesis, Patiala, Punjab, India: Thapar University.

- Okane, I., Srikitikulchai, P., Toyama, K., Laessoe, T., Sivichai, S., Nigel, H., Nakagiri, A., Potacharoen, W. and Suzuki, K. 2008. Study of endophytic Xylariaceae in Thailand: diversity and taxonomy inferred from rDNA sequence analyses with saprobes forming fruit bodies in the field. *Mycosci* 49: 359-372.
- Proffer, T.J. 1988. *Xylaria* root rot of urban trees caused by *Xylaria polymorpha*, *Pl Dis* 72: 79.
- Rajput, K.S., Koyani, R.D., Patel, H.R., Vasava, A.M., Patel, R.S., Patel, A.D. and Singh, A.P. 2015. A preliminary checklist of fungi of Gujarat State, India. *Cur Res Environ Appl Mycol* 5 (4): 285-306.
- Ramesh, V., Thalavaipandian, A., Karunakaran, C. and Rajendran, A. 2012. Identification and comparison of *Xylaria curta* and *Xylaria* sp. from Western Ghats-Courtallum Hills, India. *Mycosphere* 3: 607–615.
- Soto, G.G., Medina-Gonzalez, G.E., Trevino-Ramirez, J.E. and Hernandez-Luna, C.E. 2015. Native macrofungi that produce lignin-modifying enzymes, cellulases, and xylanases with potential biotechnological applications. *Bioresources* 10(4): 6676-6689.