

## New Distribution Record of Five Species of *Xylaria* from Tripura, Northeast India

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### Abstract

The present study deals with new distribution record of five *Xylaria* species from Tripura, North-east India. Out of total five species, *X. obovata*, *X. nigripes*, *X. multiplex* and *X. hypoxylon* were not reported from entire Northeast India. Present findings revealed a potential check list of *Xylaria* of the region. It was observed that *Xylaria* species prefer to grow on dead decaying wooden logs situated on the moist deciduous forest floor. In addition, the present study also examines the toxicity test for all *Xylaria* species, and found that all five reported species contain toxic components and recommended as non-edible wild mushroom.

**Keywords:** *Xylaria* species, wild poisonous mushroom, identification key, habitat, toxicity test

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### INTRODUCTION

*Xylariaceae* is generally recognized as one of the most diverse and largest family of Ascomycota and widely distributed throughout the world as evidenced by reports from different regions with broad ecological diversity [1]. Due to the polymorphism, the taxonomy of *Xylaria* species is difficult and names of approximately 550 species available [2]. Index Fungorum has documented 793 epithets of *Xylaria* in the year of 2017 [3]. The Phyla Ascomycotina contains approximately 2000 species in all over the world; among them, 745 species are found in India [4]. The *Xylariaceae* is a large and relatively well-known ascomycete family found in most countries [5]. *Xylaria* grow on a variety of substrates, especially on decaying wood, dead wood and nests of termites or ants, therefore they are mostly saprobic in nature or rarely parasitic in nature [6, 7]. Kshirasagar *et al.* and Karun and Sridhar reported nine and ten species of *Xylaria* from the Western Ghats and Mulashi Forest (Maharashtra), respectively [8, 9]. Koyani *et al.* also reported 19 species of *Xylaria* from all the 33 districts of Gujarat [10]. In this study, a total of six species were found from the small hilly state of Tripura. *Xylariaceae* contains 35 genera and is characterized by perithecial ascocarps bearing

paraphyses and periphyses that are embedded in a stroma [11, 12]. Mushroom poisoning is very common in rural people who consume mushrooms in daily nutrition [13].

The specific goal of this study was to report the occurrence of different species of *Xylaria* from the small hilly state of Tripura, Northeast India, based on morphological as well as anatomical features along with their edibility status.

### MATERIALS AND METHODS

#### Study Area

Tripura is one of the seven states in the north eastern part of India with a geographical area of 10491 km<sup>2</sup>, of which, 6292 km<sup>2</sup> (59.98%) is covered with forest as per legal classification in the state and it geographically lies between 22°57' to 24°33'N and 91°10' to 92°20'E [14]. Samples were collected from forest bed of Jampui Hills, Tripura during 2014 to 2017. Jampui Hills is a hilly area of North District of Tripura and it is covered by Mizoram border in the eastern side.

#### Mushroom Collection and Identification

Different species exhibit different fruiting phenology, which vary at different altitudes and regions. Mushrooms were collected from different habitats with the help of forceps or

trowel or picked by hand along with small part of adhering wood and photographed. Each sample was wrapped in the paper envelop along with field notes, date of collection, habitat, locality and specimen number on tag. The specimens were carefully placed on blotting sheet and brought to the laboratory for further analysis. The measurements of various parts of mushrooms and morphological features were recorded. These samples were dried in hot air oven at 45–55°C for 24 h and after drying, the samples were preserved in polyethylene bag by adding 1,4-dichlorobenzene [14]. These bags were preserved for further analysis. For identifications, macro and micro-morphological features were carefully examined. Studied micro-morphological features included the details of Asci and ascospores. Specimens were identified based on standard literatures [1, 2, 8, 15, 16].

### Toxicity Test

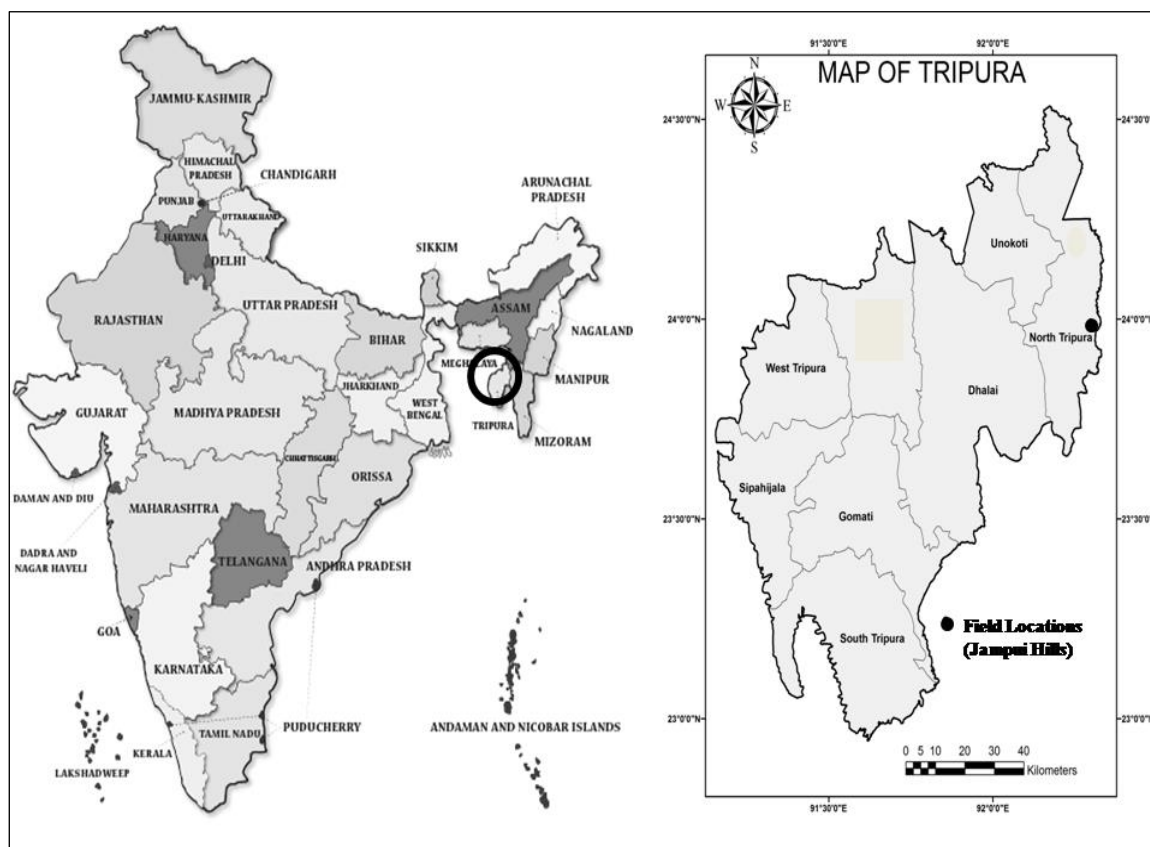
As we know Tripura consist of many tribes (19 tribes) and they use forest plant parts (e.g., leaf, root, rhizomes, bark, fruits, flower, etc.), animals, as well as mushroom as a food and

with their traditional knowledge, they directly consumed the wild mushrooms and also sell to the local market. In Assam, a large number of people died every year due to mushroom poisoning [17]. Therefore it was necessary to confirm the toxicity level of the collected wild mushrooms. Toxicity test was carried out following the standard method [18]. The color change was the primary indicator for presence or absence of toxins. For the conformation of presence or absence of toxins (amatoxins and phallotoxins) in the tested fungi, paper chromatographic method was used [19].

## RESULTS

### Morphological Identification and Processing

A total of five mushroom samples were collected from Jampui Hills of Tripura, North-east India (Figure 1). Identifications of mushroom species were mainly based on details of macro as well as micro-morphological characteristics (Figures 2–6). The present study also documents about the substrates in which they grow and their distribution along with key features.



**Fig. 1:** Field Location of *Xylaria* species, Tripura, Northeast India.

***Xylaria obovata* (Berk.) Berk** (Figure 2, A, B and C)

*Occurrence:* Jampui Hills.

*Habitat:* Saprophytic, solitary, on decaying logs.

*Distinguishing Characters:* Stromata blackish-brown with sub-globose to obovate, round fertile head, narrowing below into a black, short and stout sterile stem firmly attached to the dead wood, flesh whitish, hard, ostiole papillate or semi-papillate and size of stromata was 1.6–2.6×0.7–1.9 cm in diameter. Perithecia black, sub-spherical with fertile head. The size of the head was 1.4–1.9×0.5–1.6 cm and stout sterile stem was 0.3–0.4×0.2 cm. Asci cylindrical, long, stipitate, 8-spored and size measured as 220–237×10–13 µm. Ascospores purple-brown, ellipsoid-inequilateral, aseptate, uniseriate and size measured as 19.15–21.05×5.38–6.12 µm.

*Distribution Range:* It is distributed in tropical and subtropical hilly regions and grows during rainy season. It was reported from Western Ghats, Maharashtra, Kerala states of India.

*Material Examined:* Jampui Hills, North District, Tripura, India, Debnath and Saha, MCCT XY 1 and 13th June, 2015.

*Note:* Hard, odour and taste was absent, and inedible.

***Xylaria nigripes* (Klotzsch) Cooke** (Figure 3, D, E and F)

*Occurrence:* Jampui Hills.

*Habitat:* Saprophytic, solitary, dead wooden log.

*Distinguishing Characters:* Stromata ash to blackish, cylindrical, long, hard, branched or unbranched to gregarious, 4–8×0.2–0.5 cm in diameter, extended fertile apex which is curved and ash in colour. It turns to dark black with maturity and also becomes hard with age. Surface smooth and becomes wrinkled when fully grown. Perithecia black, sub-spherical to spherical, fertile head. The size of the head was 0.009–0.029 cm. Asci cylindrical, long, stipitate, 8-spored and size measured as 60–70×4–5 µm. Ascospores brown, ellipsoid-inequilateral, aseptate, uniseriate and size measured as 5.5–9.0×1.0–2.38 µm.

*Distribution Range:* It is distributed in tropical and subtropical hilly regions and grows during rainy season. It was reported from Western Ghats, Maharashtra, Gujarat states of India.

*Material Examined:* Jampui Hills, North District, Tripura, India, Debnath and Saha, MCCT XY 2 and 13th June, 2015.

*Note:* Hard, odour and taste absent, and inedible.

***Xylaria polymorpha* (Pers.) Grev** (Figure 4, G, H and I)

*Occurrence:* Jampui Hills.

*Habitat:* Saprophytic, grow in unbranched or sparingly branched on dead wooden bark (crack tissue), lignicolous.

*Distinguishing Characters:* Stromata dark brown to black in colour, lower and upper part is sharp, apex round fertile head and narrowing below into a brownish black, short, stout sterile stem firmly attached to the wood. Fruit body 0.8–2.2×0.2–0.62 cm, tough, more or less club shape, stem often proportionally long, but also frequently short or nearly absent. Perithecia black, sub-spherical, fully embedded in fertile head and size measured as 0.052–0.13 cm. Asci cylindrical, long, stipitate, 8-spored and size measured as 155–220×7.5–15 µm. Ascospores purple-brown, aseptate, uniseriate and size measured as 6.15–13.47×5.02–8.33 µm, smooth, fusiform.

*Distribution Range:* It is distributed in tropical and subtropical hilly regions and grows during rainy season. It was reported from Western Ghats, Kerala, Karnataka, Gujarat, Maharashtra, Meghalaya and Tripura states of India.

*Material Examined:* Jampui Hills, North District, Tripura, India, Debnath and Saha, MCCT XY 3 and 20th July 2016.

*Note:* Odour and taste absent and inedible.

***Xylaria multiplex* (Kunze) Fr** (Figure 5, J, K and L)

*Occurrence:* Jampui Hills.

*Habitat:* Saprophytic, grow on dead wooden log, arising in united or solitary, lignicolous.

*Distinguishing Characters:* Stromata blackish to blackish-brown, elongated, cylindrical, undulated, clavate, apex fertile head and narrowing below into a brownish-black, short sterile stem attached to dead wood by long base, the size of stromata measured as 1.76–4.86×0.32–0.64 cm. Perithecia black, sub-spherical, embedded in fertile head, size measured as 0.03–0.04 cm and arranged in a single dense layer. Asci cylindrical, long,

stipitate, 8 spored and size measured as  $90\text{--}120 \times 5.5\text{--}6.5 \mu\text{m}$ . Ascospores black, ellipsoid, aseptate, uniseriate and size measured as  $7.60\text{--}10.42 \times 5.5\text{--}6.01 \mu\text{m}$ .

**Distribution Range:** It is distributed in tropical and subtropical hilly regions and grows during rainy season. It was reported from Western Ghats, Karnataka states of India.

**Material Examined:** Jampui Hills, North District, Tripura, India, Debnath and Saha, MCCT XY 5 and 22nd July 2016.

**Note:** Odour and taste absent and inedible.

***Xylaria hypoxylon* (L.: Fr.) Grev** (Figure 6, M, N and O)

**Occurrence:** Jampui Hills.

**Habitat:** Saprophytic, grow on decaying wooden log single or groups, annual, lignicolous.

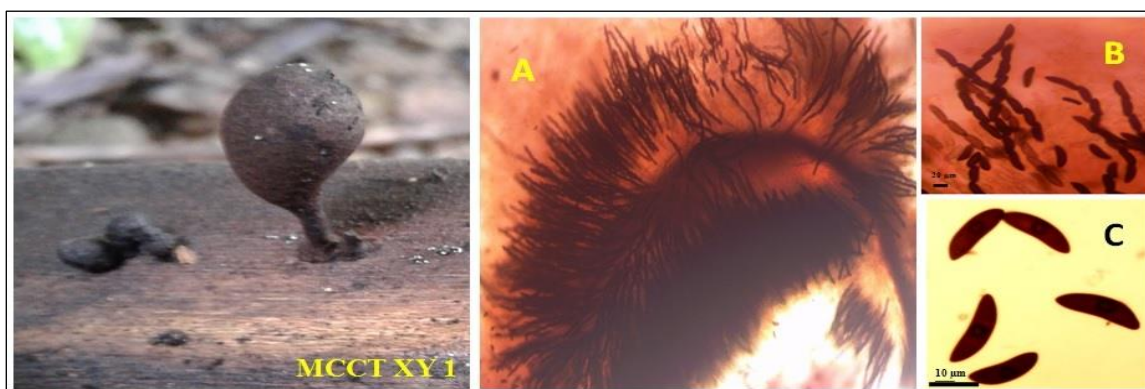
**Distinguishing Characters:** Stromata irregular, single or attached habitually originating from common base, surface roughened, branched

apex, with short or long concolorous horns, stems up to  $4.5\text{--}9.6 \times 0.24\text{--}0.58 \text{ cm}$ , apex first white towards becoming dull black, interior white, woody to carbonaceous. Perithecia black, sub-spherical, embedded, size measured as  $0.02 \times 0.05 \text{ cm}$  and arranged in a single dense layer just below the surface. Asci cylindrical, stipitate, 8-spored and size measured as  $80\text{--}110 \times 4\text{--}6 \mu\text{m}$ . Ascospores blackish to brown, aseptate, ellipsoid, uniseriate and size measured as  $10\text{--}12 \times 4\text{--}6 \mu\text{m}$ .

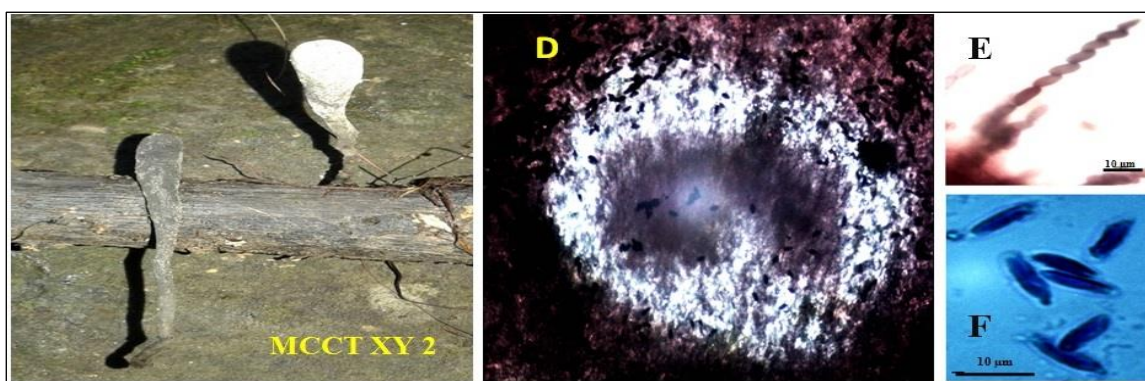
**Distribution Range:** It is distributed in tropical and subtropical hilly regions and grows during rainy season. It was reported from Western Ghats, Kerala, Karnataka, Gujarat states of India.

**Material Examined:** Jampui Hills, North District, Tripura, India, Debnath and Saha, MCCT XY 6 and 5th June 2017.

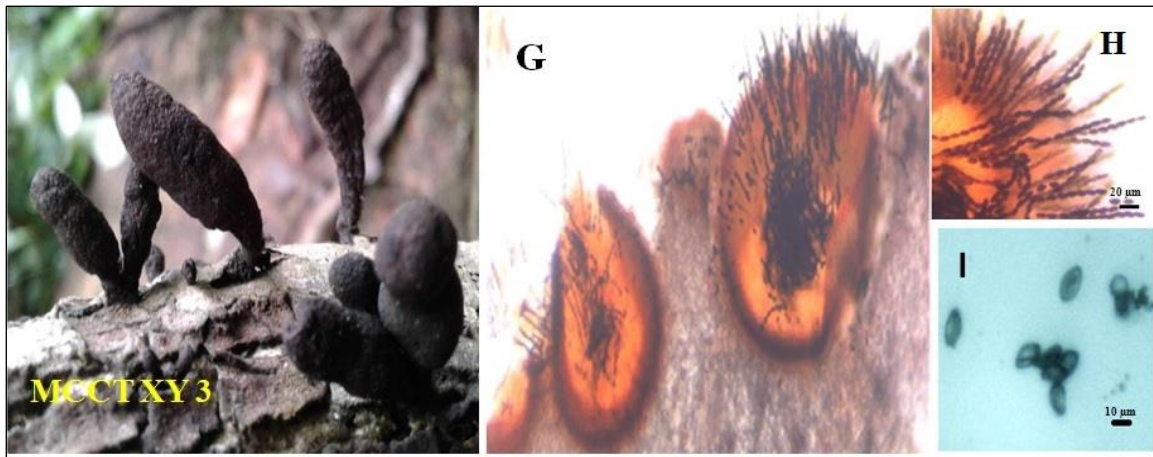
**Note:** Odour and taste not distinctive and inedible.



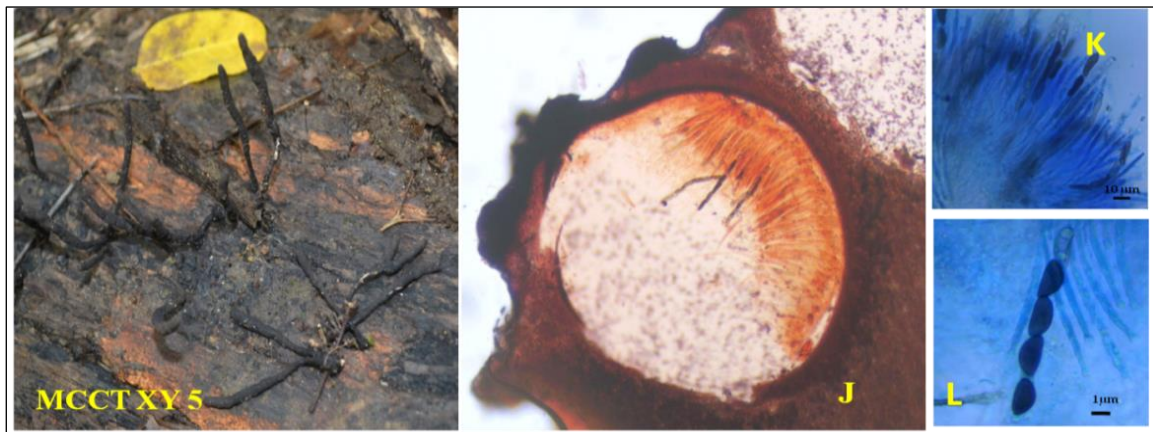
**Fig. 2:** Morphological and Anatomical Features of *Xylaria obovata*. **MCCTXY1:** *X. obovata* Showing Mature Stage of Stromata on Decaying Log, **A:** Cross Section of Stromata Showing Prethecia, Asci and Ascospores, **B:** Immature and Mature Asci ( $\text{--}20 \mu\text{m}$ ), **C:** ascospores ( $\text{--}10 \mu\text{m}$ ).



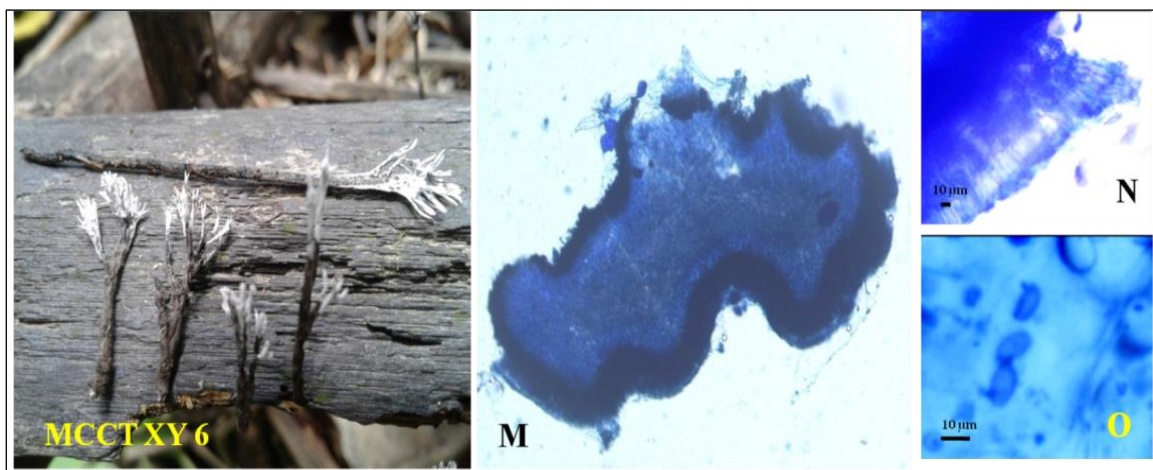
**Fig. 3:** Morphological and Anatomical Features of *Xylaria nigripes*. **MCCTXY 2:** *X. nigripes* Showing Mature Stage of Stromata on Dead Wooden Log, **D:** Cross Section of Stromata Showing Prethecia and Ascospores, **E:** Mature Asci ( $\text{--}10 \mu\text{m}$ ), **F:** Ascospores ( $\text{--}10 \mu\text{m}$ ).



**Fig. 4:** Morphological and Anatomical Features of *Xylaria polymorpha*. **MCCTXY 3:** *X. polymorpha* Showing Mature Stage of Stromata on Dead Wooden Bark (Crack Tissue), **G:** Cross Section of Stromata Showing Prethecia, Asci (—20  $\mu$ m) and Ascospores, **H:** Mature Asci, **I:** Ascospores (—10  $\mu$ m).



**Fig. 5:** Morphological and Anatomical Features of *Xylaria Multiplex*. **MCCTXY 5:** *X. multiplex* Showing Mature Stage of Stromata on Dead Wooden Log, **J:** Cross Section of Stromata Showing Prethecia, Asci and Ascospores, **K:** Mature and Immature Asci (—10  $\mu$ m), **L:** Ascospores (—1  $\mu$ m).



**Fig. 6:** Morphological and Anatomical Features of *Xylaria hypoxylon*. **MCCTXY 6:** *X. hypoxylon* Showing Mature Stage of Stromata on Dead Wooden Log, **M:** Cross Section of Stromata Showing Prethecia, Asci and Ascospores, **N:** Mature and Immature Asci (—10  $\mu$ m), **O:** Ascospores (—10  $\mu$ m).

**Key for Identification of *Xylaria* Species for Present Study**

Based on host, substrate, and teleomorph or anamorph stages (stromatal structure, sporangia and ascospores) of *Xylaria* species were identified with the help of several keys [1, 2, 8, 15, 16]. The following key is the basis for identification of five species of *Xylaria*:

1. Stromata of all known species will be upright, more or less digitate

***Xylaria***

1a. Ascospores exceeding 17  $\mu\text{m}$  in length 2  
 2a. Fertile part usually globose to obovate, up to 1.2 cm in diameter, black, on short stems. Ascospores 19.15–21.05 $\times$ 5.38–6.12  $\mu\text{m}$

***X. obovata***

1b. Ascospores not longer than 17  $\mu\text{m}$  3

3a. Ascospores 5.5–9.0 $\times$ 1.0–2.38  $\mu\text{m}$   
***X. nigripes***

3b. Stromata usually exceeding 1 cm diameter, unbranched or sparingly branched. Ascospores 6.15–13.47 $\times$ 5.02–8.33  $\mu\text{m}$  ***X. polymorpha***

3c. Stromata blackish to blackish-brown, elongated, cylindric, undulated, clavate, apex fertile head and narrowing below into a brownish-black, short sterile stem, ascospores 7.60–10.42 $\times$ 5.5–6.01  $\mu\text{m}$  ***X. multiplex***

3d. Stromata at first white, becoming black, often branched, flattened, irregular, ascospores 10–12 $\times$ 4–6  $\mu\text{m}$ , with straight germ slit  
***X. hypoxylon***

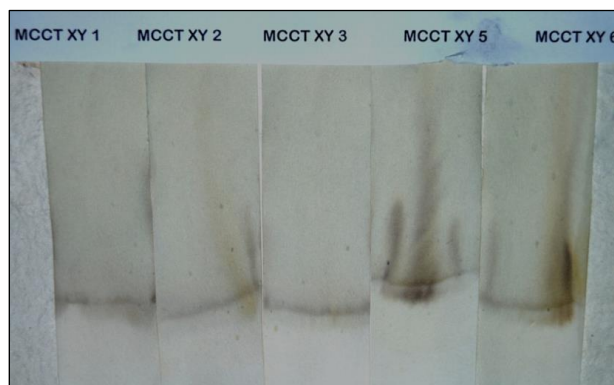
**Toxicity Test**

The primary indicator toxicity test of all the *Xylaria* species showed positive result (Figure 7) which means, it contained toxin

compounds. In confirmatory test, light blue or violet colored spots were found to appear in the chromatographic strips. These observations indicated presence of amanitin and phalloidin toxins in the test fungi.

**DISCUSSION**

Due to various morphological manifestations in the life stages, *Xylaria* species have different environmental distributions. The present study revealed taxonomic investigations at species level. This study also showed that the studied *Xylaria* species were grown on decaying logs, dead wooden log and dead wooden bark (crack tissue). There is necessity to describe the species at different life stages [20]. Different species of *Xylaria* grow on dead logs, stubs, soil, soil embedded with wood, pods, kernels, twigs, humus, wood pieces, leaves and termite mound [21]. We have also observed that dead wooden bark (crack tissue) were suitable substrate for *Xylaria*. Because *Xylaria* can inhabit at low water availability due to controlled growth, moisture content for growth should be initially induced and it followed by a drying period to encourage pigmentation [22]. The stromata of some ascomycetes fungi like *Xylaria* species [23] and basidiomycetes like *Termitomyces* species fungi are grown from dumped termites nest [24]. Actually, termite combs serve as one of the prominent ecological niches of *Xylaria*, mostly owing to microclimatic conditions (temperature, humidity and carbon dioxide) favor growth of *Xylaria* [25]. Bhattacharjee *et al.* [26] and Kumar *et al.* [27] reported *X. polymorpha* from Tripura and Meghalaya of Northeast India, respectively. A record of 28 *Xylaria* species reported from different locations of India (Table 1).



**Fig. 7:** Toxicity Screening Test Showing Positive Results of Five *Xylaria* species.

**Table 1:** Different Species of *Xylaria* Previously Reported from Different Parts of India.

Sl. No.	<i>Xylaria</i> Species	Collected From	References
1.	<i>X. escharoidea</i>	Chandhakkunnu, Nilambur (Kerala)	[28]
		Western Ghats, India	[9]
2.	<i>X. filiformis</i>	Western Ghats, India	[9]
		Gujarat, India	[10]
3.	<i>X. hypoxylon</i>	Western Ghats, India	[9]
		Konaje (Karnataka)	[21]
		Peechi and Vazhani (Kerala)	[29]
		Chandhakkunnu and Iringole Kavu (Kerala)	[28]
		Bhadra Wildlife Sanctuary (Karnataka)	[30]
		Gujarat, India	[10]
4.	<i>X. longipes</i>	Western Ghats, India	[9]
		Gujarat, India	[10]
		Chandhakkunnu, Iringole Kavu and Vadanamkurissi (Kerala)	[28]
		Karnataka, India	[31]
5.	<i>X. multiplex</i>	Western Ghats, India	[9]
		Konaje (Karnataka)	[21]
6.	<i>X. nigripes</i>	Western Ghats, India	[9]
		Gujarat, India	[10]
		Mulashi Forest (Maharashtra)	[8]
7.	<i>X. obovata</i>	Western Ghats, India	[9]
		Mulashi Forest (Maharashtra)	[8]
		Ammayambalam (Kerala)	[28]
8.	<i>X. polymorpha</i>	Western Ghats, India	[9]
		Moist-deciduous forest (Karnataka)	[32]
		Iringole Kavu and Ammayambalam (Kerala)	[28]
		Jampui hills (Tripura)	[26]
		Meghalaya	[27]
		Bhadra Wildlife Sanctuary (Karnataka)	[30]
		Pune (Maharashtra)	[33]
		Mulashi Forest (Maharashtra)	[8]
Gujarat, India	[10]		
9.	<i>X. symploci</i>	Western Ghats, India	[9]
10.	<i>X. acuminatolongissima</i>	Pattambi (Kerala)	[34]
11.	<i>X. anisopleura</i>	Mulashi Forest (Maharashtra)	[8]
12.	<i>X. beccari</i>	Mulashi Forest (Maharashtra)	[8]
13.	<i>X. brevipes</i>	Mulashi Forest (Maharashtra)	[8]
14.	<i>X. carpophila</i>	Semi-evergreen and moist-deciduous forests (Karnataka)	[32]
		Gujarat, India	[10]
15.	<i>X. curta</i>	Courtallum Hills (Tamil Nadu)	[35]
		Western Ghats of Courtallum Hills, Tamil Nadu	[12]
		Gujarat, India	[10]
16.	<i>X. feejeensis</i>	Mulashi Forest (Maharashtra)	[8]
		Gujarat, India	[10]
17.	<i>X. gigantea</i>	Satara and Kas (Maharashtra)	[36]
		Gujarat, India	[10]
18.	<i>X. grammica</i>	Mulashi Forest (Maharashtra)	[8]
19.	<i>X. juruensis</i>	Mulashi Forest (Maharashtra)	[8]
20.	<i>X. oligotoma</i>	Patgaon, Maharashtra	[37]
21.	<i>X. poitei</i>	Vallikkayam and Kuthirn (Kerala)	[29]
22.	<i>X. regalis</i>	Mulashi Forest (Maharashtra)	[8]
		Maharashtra, Western Ghats	[33]
		Gujarat, India	[10]
23.	<i>X. schweinitzii</i>	Anshi National Park (Karnataka)	[38]
24.	<i>X. scruposa</i>	Kaikatty and Thirunelly (Kerala)	[28]
25.	<i>X. apiculata</i>	Gujarat, India	[10]
26.	<i>X. cubensis</i>	Gujarat, India	[10]
27.	<i>X. primorskensis</i>	Gujarat, India	[10]
28.	<i>X. psidii</i>	Gujarat, India	[10]

Nowadays, poisonous mushrooms have attracted much interest of researchers because of the significant physiological properties of their toxic components [39]. In our observation we have found that all the *Xylaria* species contain amatoxins or phallotoxins compounds. But Debnath *et al.* studied the same toxicity test in *Schizophyllum commune* and they found negative result which means *S. commune* is an edible mushroom [40]. Present finding also revealed that the all wild mushrooms are not edible and some of them are toxic for human beings.

### CONCLUSIONS

It is necessary to explore the diversity of edible as well as non-edible mushrooms from different parts of the world. The reports on the occurrence of *Xylaria* species from hilly state of Tripura, Northeast India, would contribute a scientific document to the Indian mycologist. In the present study, *X. obovata*, *X. nigripes*, *X. polymorpha*, *X. multiplex* and *X. hypoxylon* were reported for the first time from hilly state of Tripura, North-east India. Toxicity screening test for the determination of edibility status of five *Xylaria* species is also reported for the first time. The simple and easy toxicity screening test is recommended to determine the edibility status of any macrofungi.

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