## Inocybe poisoning from Kerala-a case study

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Cases of poisoning by *Inocybe carnosibulbosa* is reported from the State of Kerala. A morphological description of the material causing the poisoning is provided and the associated case history is detailed.

Key words: Agaricales, mycetismus, systematics

#### INTRODUCTION

The incidence of mushroom poisoning varies a lot over the world depending on local traditions, lifestyles, nutritional factors, climatic conditions and the occurrence of wild mushrooms. Disregarding mushrooms containing psychoactive toxins, ingestion of toxic fungi is almost invariably accidental. At present mushroom poisoning is quite common in Kerala and Chlorophyllum molybdites (G. Mey.) Massee, is reported to be the frequent cause of mycetism in Kerala (Bijeesh et al. 2017). Almost all species of the genus Inocybe are poisonous and contain 'muscarine' which is one of those toxins that is not usually regarded as 'deadly'. Muscarine was first discovered in Amanita muscaria but the toxin is present at very low levels in Amanita muscaria, however, it is known to occur at much higher levels in other mushrooms and is particularly high in a number of species of the genus Inocybe (even up to 100 times). The symptoms of muscarine poisoning are: a dramatic increase in salivation, lacrimation and perspiration accompanied by vomiting, diarrhea and stomach pain. Death rarely occurs with this type of poisoning but on rare occasions, muscarine poisoning can be fatal and this is often explained on the basis that the person had some other debilitating disease. Moreover if taken along with alcohol or other drugs it may be fatal. After treatment, the patient will make a 100% recovery with no lasting ill effects or damage to the vital organs like liver, kidney etc. (Lurie *et al.* 2009).As with any kind of poisoning, the two most important things to do are to seek immediate medical attention and identify the agent responsible.

#### MATERIALS AND METHODS

Based on reports on wild mushroom poisoning in all the leading dailies of Kerala, the mushroom research team of JNTBGRI visited the Medical College Hospital at Thiruvananthapuram and collected as many details as possible from the hospital by examining the files of wild mushroom poisoning patients who were admitted to the Medical College Hospital, Thiruvananthapuram, and interviewing the victims of poisoning and the wayside seller. Mushroom specimens responsible for the poisoning were immediately collected from the same localities from where the victims had collected them. Collected specimens were systematically analyzed and identified following standard taxonomic methodology (Singer, 1986). Microscopic examination of tissues was made from thin free-hand sections revived in 3% aqueous KOH and stained in 1% Congo red. Examinations were made directly using a Leica DME 1000 research microscope. Basidiospores were examined in Melzer's reagent or 3% aqueous KOH. Spore measurements were taken from hymenial tissues and 30 basidiospores from

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sections were measured. Colour codes and notations are from Kornerup and Wanscher (1978). All the collections examined are deposited at the Mycological Herbarium of TBGRI [TBGT (M)].

## **RESULTS AND DISCUSSION**

*Inocybe carnosibulbosa* C.K. Pradeep and Matheny, *Mycol. Progr.* 15 (24): 16 (2016).

*Pileus* 4.5–10.5 cm wide, convex when young, becoming plano-convex to applanate with a broad obtuse umbo or with a slightly depressed centre, becoming a little uplifted with age; uniformly yellowish white to cream (3A2/4A3) when young, becoming more brownish at the disc (6C3/6C4/6D4/6E5/6E6) and cream to yellowish white (4A2/4A3) elsewhere, smooth at the disc, appressed fibrillose-striate elsewhere, appearing squamulose when dry, rimose, often splitting radially exposing the underlying white context below, dry; margin



Fig. 1. Inocybe carnosibulbosa. A. Habit in situ; B. Basidia; C. Basidiospores; D. Cheilocystidia; E. Pileipellis. Scale bar: A = 20mm, B-E = 10 μm.

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incurved when young becoming straight, entire to incised. Lamellae adnexed, white to yellowish white when young, becoming brownish orange (5C3/ 5C4) with age, up to 1.4 cm wide, crowded, with lamellulae of different lengths; edge paler, entire. Stipe 5–12  $\times$  0.7–1.7 cm, central, cylindric, with or without a marginate basal bulb, tapering abruptly below, white to yellowish white (5A2/5A3), becoming brownish (6C4/6D4/6F4) on handling, fibrillose striate. Context solid, white, soft, up to 0.8 cm wide. Odour mild. *Spore print* snuff brown. Basidiospores (5.5-) 6-8 × 5-6.5 [7.2 ± 0.66 × 5.4  $\pm$  0.30]  $\mu$ m, Q= 1.07–1.42, Q'= 1.26, smooth, subglobose to broadly ellipsoid, rarely phaseoliform, yellowish brown, thick-walled. Basidia 27-31× 7-8 µm, clavate, 4-spored, thinwalled, hyaline. Lamella-edge sterile with tufts of cheilocystidia. Cheilocystidia 29–140 × 6–13  $\mu$ m, clavate, vesiculose, lageniform with a long narrow neck, multi-septate, thin-walled, hyaline. Pleurocystidia absent. Hymenophoral trama regular, composed of thin-walled, hyaline hyphae, 5.5–14 µm wide. Subhymenium pseudoparenchymatous, 2-3 cells thick. Pileal trama composed of loosely arranged hyphae, 2.5-12.5  $\mu$ m wide, inflated up to 22  $\mu$ m wide, thin-walled, hyaline. *Pileipellis* an interrupted epicutis, consisting of parallel 3–5  $\mu$ m wide, incrusted hyphae, with yellowish brown contents. Stipitipellis a cutis of parallel, thin-walled, hyaline hyphae, 2.5-5  $\mu$ m wide, incrusted at places. Stipe trama with parallely arranged, thin-walled, hyaline, 7.5–22  $\mu$ m wide hyphae. Caulocystidia absent. Clamp connections present in all tissues.

## Habitat and phenology

Scattered, in groups or in pairs, on forest floor, under *Hopea parviflora* Bedd. (Dipterocarpaceae) and *Xanthophyllum* sp. (Polygalaceae). July-November.

#### Specimens examined

India, Kerala State, Thiruvananthapuram district: Palode, 20 July 2015, TBGT (M) 15769.

## Additional specimens examined

India, Kerala State, Thiruvananthapuram district: JNTBGRI campus, 25 September 2008, TBGT(M) 12047; 20 November 2008, TBGT(M) 12276; 14 October 2009, TBGT(M) 12976; 23 October 2009, TBGT(M) 13011; 16 November 2011, TBGT(M) 13898; 17 November 2011, TBGT(M) 13906; 18 November 2011, TBGT(M) 13909; 30 September 2015, TBGT(M) 15878; 07 November 2016, TBGT(M) 16681; 01 August 2018, TBGT(M) 17616.

The species is so far known only from the type locality.

The genus *Inocybe* (Fr.) Fr., was originally placed family Cortinariaceae Heim the in ex Pouzar. Later, phylogenetic analyses, suggested that the genus was better placed as the type genus of the family Inocybaceae Jülich, as Cortinariaceae was not a monophyletic group. Most Inocybaceae members can be recognized in the field by a combination of features of their fruit bodies, which are typically brown in colour, small to medium sized with a stipe, a fibrillose to scaly pileus, brownish attached lamellae with whitish fimbriate edges and dull brown spore deposit (Matheny and Bougher, 2017).

Inocybe carnosibulbosa C.K. Pradeep & Matheny, was originally described from Kerala (Pradeep et al. 2016). The species is characterized by its large, fleshy tricholomatoid basidiomes, cream to yellowish white pileus with a brownish disc, small subglobose, smooth basidiospores, conspicuous cheilocystidia with a long neck and association with indigenous trees like Xanthophyllum and Hopea. Inocybe virosa C.K. Pradeep, K.B. Vrinda & Matheny, another species described from Kerala is a poisonous look-alike (Pradeep et al. 2016) of I. carnosibulbosa. Though both the species cannot be readily distinguished based on morphological characters, microscopically they are distinct by virtue of the size and shape of their cheilocystidia. *Inocybe virosa* has comparatively smaller cystidia which are clavate to inflated clavate, where as in I. carnosibulbosa they are large and conspicuous, often with a long narrow neck.

*Inocybe cutifracta* Petch, described from Sri Lanka, is another closely related species, with a similar morphology, differing in the smaller size of the basidiomata, larger basidiospores [7–11 × 4.5-6 (8 ± 0.5 × 4.8 ± 0.3)  $\mu$ m, Q= 1.6], and cheilocystidia with a globose capitellum.

*Inocybe gregaria* K.P.D. Latha & Manim., yet another species described from Kerala (Latha and Manimohan 2016) also shows some affinity to *I*.

*carnosibulbosa* in its general morphology but the former can be separated macroscopically by the presence of occasionally bifurcated lamellae and pruinose stipe apex and microscopically by the presence of caulocystidia.

#### Reports of mushroom poisoning cases

On July 19, 2015, two poisoning cases occurred in Thiruvananthapuram district due to the consumption of mushrooms collected from a rural property at Palode by a tribal wayside seller. Eleven people belonging to two different families were the victims, including a lactating mother (who had consumed the mushrooms) and her six month old baby, feeding her mother's breast milk. All of them were admitted to the Medical College Hospital, Thiruvananthapuram, due to heavy perspiration, salivation, vomiting, giddiness, diarrhea, headache, anxiety and disturbances of vision. In both the cases, the same mushroom species was involved as both the families had purchased the mushrooms from the same tribal wayside seller. After supportive treatment involving intravenous normal saline, antiemetic and intravenous atropine, all the patients made a full clinical and laboratory recovery within 12 hours. They were kept in observation for another 12 hours and were discharged from the hospital afterwards. The severity of the toxic effects seemed to be related to the amount of mushroom consumed; however, this could not be accurately determined. All patients recovered with supportive treatment, and low-dose atropine was required in five patients. A cat and a chicken also died after eating the mushrooms raw. These poisoning cases were reported by all the newspapers of Kerala with great significance. Based on the reports, the Mushroom Research team of JNTBGRI made a thorough investigation about these cases. The site was visited, met the victims and collected the leftover mushroom samples they consumed. A critical study of the specimens in the laboratory revealed that the mushroom was a hitherto undescribed species, belonging to the genus Inocybe, a member of the family Inocybaceae. The species was described and later published as Inocybe carnosibulbosa, in reference to the large fleshy basidiomes with a pronounced bulbous stipe base (Pradeep et al. 2016).

The symptoms mentioned in the above case history, particularly the intensive vomiting, nausea, diarrhea, abdominal pain, hyper salivation, blurred vision, chills, headache, and anxiety and the moment of their appearance, rather soon after the ingestion, are typical muscarine poisoning syndromes. Many species of the genus *Inocybe* are muscarine-containing mycorrhizal mushrooms, ubiquitous around the world. Presence of muscarine in relatively high concentrations in many species of *Inocybe* were also recorded.

Species of *Inocybe* are quite common in the forests of Kerala and the scientists of JNTBGRI have already documented 17 species from the State, none being edible. All the species appear in large numbers, forming ectomycorrhizal association with indigenous tree species like *Hopea parviflora, Vateria indica* (Dipterocarpaceae), *Aporosa acuminata* (Phyllanthaceae), *Knemaattenuata* (Myristi-caceae), *Calophyllum* (Calophyllaceae) and *Xanthophyllum* (Polygalaceae). Members of the genus *Inocybe* are quite distinct in appearance and do not resemble any of the common edible species in Kerala.

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Mega (10 <sup>6</sup> )	М	day	day
Giga (10 <sup>9</sup> )	G	month	month
Tera (1012)	Т	year	yr
deci (10 <sup>-1</sup> )	d		
centi (10 <sup>-2</sup> )	с	Units of concentration	
mili (10⁻³) m	molar	М	
micro (10 <sup>-6</sup> )	μ	millimolar mM	
nano (10 <sup>-9</sup> )	n	micromolar	μM
pico (10 <sup>-12</sup> )	р		
femto (10 <sup>-15</sup> )	f	Units of length	
atto (10 <sup>-18</sup> )	а	meter	m
liter	I	centimeter	cm
milliliter	ml	millimeter mm	
microlitre	μΙ	micrometer	μm
Units of mass		nanometer	nm
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