



Survey, Morpho-microscopic Characterization of Paddy Straw Mushroom (*Volvariella volvacea*) from Different Locations of Chhattisgarh, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Paddy straw mushroom (*Volvariella volvacea*), commonly known as the straw mushroom, or the Chinese mushroom, belongs to the family Pluteaceae (Kotl. & Pouz) of the Basidiomycetes (Singer, 1961). The present research was undertaken to explore the diversity, distribution, morphological, and microscopic characteristics of *V. volvacea* native isolates from Chhattisgarh. A total of eight isolates were collected in a survey conducted across Chhattisgarh from six districts viz. Rajnandgaon, Raipur, Dhamtari, and Bemetara, Kabirdham, Durg and all the relevant information on habitat, temperature, relative humidity, location (GPS data), habit, and related substrates in native environments; were recorded diligently. *Volvariella* isolates collected were characterized

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morphologically and found possessing pink spores, free lamellae, and a stipe bearing no annulus but was encased at the base by a volva. The gills of all the isolates were freely arranged; consistency was fleshy; colour was moderate brown; and texture was smooth. The stipes of all the isolates were cylindrical, centrally attached to the pileus; the consistency was fleshy; the colour was yellowish white; and the bases were swollen. Pileus characters of all isolates show a light brown outer colour, a moderate brown middle colour, and regular margins. The volva characters of all isolates show a sheathed arrangement and brownish colour. Spore print colours were observed as pink and moderate brown, gill colour varied from light pink to brown for all isolates. Microscopic characterization was done and was found that spore size of all collected isolates ranges between six to nine micrometre and the basidium bears sterigmata.

Keywords: Paddy straw mushroom; survey; morphological characterization; microscopic characterization.

1. INTRODUCTION

Volvariella volvacea, the edible straw mushroom, is a highly nutritious food source that is widely cultivated on a commercial scale in many parts of Asia using agricultural wastes (rice straw, cotton wastes) as growth substrates. However, developments in *V. volvacea* cultivation have been limited due to a low biological efficiency (i.e. conversion of growth substrate to mushroom fruit bodies), sensitivity to low temperatures [1]. *Volvariella volvacea* (Bull. ex Fr.) Sing. is a large pileate edible fungus of the tropics and sub-tropics. It has two kinds of spores, one being the sexual basidiospores in tetrads and the other the asexual chlamydospores [2].

Volvariella Sp. belongs to the family Pluteaceae, order Agaricales, Hymenomycetidae, Eubasidiomycetes, Basidi-omycotina, Eumycota [3]. Most species of *Volvariella* are characterized by 1) the stipe which has a volva at the base and no annulus, 2) lamellae free to the stipe, usually crowded to distance, 3) fruiting bodies rapidly decayed, 4) spore print pink to brownish fresh, 5) spores thin-walled to somewhat thick-walled, and 6) growing on humus soils or on well decayed wood except for *Volvariella surrecta*, which is known to be related with one species of *Clitocybe* belonging to Tricholomataceae. Most species of this genus are worldily distributed and thirty one species have been recorded [4].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Test fungus

The present study was limited to only one species of straw mushroom viz. *V. volvacea*.

The test fungus was procured from the survey done at the different locations and districts of Chhattisgarh. This mushroom may be grown using a variety of cellulosic materials, and it requires a C: N ratio of 40 to 60, which is higher than that of other cultivated mushrooms. On uncomposted substrates like cotton wastes, paddy straw, and other cellulosic organic waste materials, it can grow relatively quickly and easily [5]. It is thought to be one of the easiest mushrooms to grow. In the temperature range of 25–40°C, straw mushrooms (*Volvariella* spp.) can be produced during the summer and rainy season. It needs high temperature (33-35 °C) and 85-90 % relative humidity in the growing environment [6]. The straw mushroom is harvested before the volva breaks or just after reapture. This mushroom usually takes 9-10 days from spawning to first harvest of crop [7].

2.2 Methods

Fruit bodies were gathered from natural habitats (straw heaps) during the rainy season of 2022-23 from various agro-climatic zones in Chhattisgarh in order to investigate the productivity of indigenous isolates of straw mushrooms. The fruiting bodies were collected from their natural habitat and brought to the laboratory to make mother cultures. Most of the fruiting bodies were collected in the morning and brought to the laboratory by afternoon or evening for culturing the fungus. A juvenile fruit body at the button stage was newly plucked and delivered to the lab for tissue culture [8]. Cotton swabs were used to help remove the dust that had become attached. To get rid of the microorganisms on the fruit's surface, it was submerged in 70% ethanol for 30 seconds while being grasped with sterilised forceps. The pileus-stipe junction (collar region) was then cut longitudinally through the fruit body using a sterile knife to

separate tissue fragments of about 3-5 mm. With the use of sterilised forceps, the parts were aseptically deposited onto presterilized petriplates containing potato dextrose agar growth medium. Parafilm- wrapped petriplates were incubated for a week at 30-32°C in a BOD incubator [9]. A number of test tubes were filled with actively growing mycelium from the edges and culture medium that was approximately 5 mm in size. These test tubes were then infected at 30-32°C for a week [10]. This pure culture served as the spawn preparation's inoculum. The culture was maintained by sub-culturing or re- culturing every 15–20 days. The photograph with a geo-location tag has been taken; date, time, and habitat were noted. Morphological characters (Plate 1 a), viz., Gill character (Table 1 b), Stipe character (Table 1 c), Pileus character (Table 1 d), and volva character (Table 1 e) were recorded, and morphometry was done. The spore print (Plate 1 b) of all the isolates were taken. The isolates were given numbering for future references, which is given in the survey data mentioned below in the Table 1(a). The spore size was

measured by average of divisions covered by ten spores multiplied by correction factor (Table 2).

3. RESULTS

3.1 Survey, Morpho-microscopic Characterization of Paddy Straw Mushroom (*Volvariella volvacea*) from Different Locations of Chhattisgarh.

3.1.1 Survey and morphological characterization

A systematic survey was conducted in 2022-23 in the different districts of Chhattisgarh, viz., Raipur, Rajnandgaon, Durg, Dhamtari, Kabirdham, and Bemetara. The survey was done during the rainy season, and the fruiting bodies were collected from their natural habitats, each of which has a different habitat. Three strains were collected from Raipur district, and one strain was collected from each of Bemetara, Dhamtari, Durg, Kabirdham, and Rajnandgaon districts. The details of the survey is given below in the Table 1(a).



Plate 1 (a). Morphology of paddy straw mushrooms collected from survey

Table 1(a). Details of native isolates of paddy straw mushroom under natural conditions

S.N	Isolates (Acce. No. DMR)	Collection date	Location	Habitat	Morphologica l character
1.	VV-22-1003 (DMRX-2212)	31/08/2022	21.0105556,81.052615 Rajnandgaon	Paddy straw(wet)+cow dung	Stipe-5cm Pileus -3x4cm Volva-1.2cm The colour of the fully grown pileus is greyish whitewith a reddish tinge.
2.	VV-22-1004 (DMRX-2213)	01/10/2022	21.211028,81.715327 Dharampura, Raipur	Paddy straw(Dry)+cow dung	Stipe-8cm Pileus -1x1cm Volva-1.8cm Silvery white at the top and slightly white at the stipe.
3.	VV-22-1006 (DMRX-2215)	13/09/2022	20.942728,81.709586 Gatapar, Dhamtari	Paddy straw(wet)	Stipe-6cm Pileus 3x2.5cm Volva-1cm Dark grey in the centre, whitish towards the margins.
4.	VV-22-1008 (DMRX-2216)	01/10/2022	21.141054,81734735 Nimora, Raipur	Paddy straw(Dry)	Stipe-6cm Pileus -3x2cm Volva-1cm Fully grown pileus reddish brown at centre.
5.	VV-22-1009 (DMRX-2217)	04/10/2022	21.31775,81.790965 Pacheda, Raipur	Paddy straw(wet)	Stipe-10cm Pileus -5x5cm Volva-2.5cm Slightly brown at the centre and pure white at the margin.
6.	VV-22-1010 (DMRX-2218)	25/09/2022	21.782454,81.488256 Garra, Bemetara	Paddy straw(De composed)	Stipe-6.2cm Pileus - 3.5x3cm Volva-2cm dark grey in the centre, becoming silvery-white or brownish- grey towards the margins.

S.N	Isolates (Acce. No. DMR)	Collection date	Location	Habitat	Morphologica l character
7.	VV-22-1011 (DMRX-2219)	25/09/2022	21.947061,81.183338 Maharajpurdih, Kabirdham	Paddy straw(wet)	Stipe-4cm Cap-3x2.5cm Volva-2cm Reddish brown at centre, white at the margins.
8.	VV-22-1012 (DMRX-2220)	25/09/2022	21.63281,81.136976 Jagmadwa, Durg	Paddy straw(De composed)	Stipe-2cm Cap-1x1cm Volva-2cm Brownish cap with slight reddish margins

Table 1 (b). Gill characters of native isolates

S.N. Isolates	Arrangement	Consistency	Colour	Texture
1. VV-22-1003 (DMRX-2212)	Free	Fleshy	Moderate brown	Smooth
2. VV-22-1004 (DMRX-2213)	Free	Fleshy	Moderate brown	Smooth
3. VV-22-1006 (DMRX-2215)	Free	Fleshy	Moderate brown	Smooth
4. VV-22-1008 (DMRX-2216)	Free	Fleshy	Moderate brown	Smooth
5. VV-22-1009 (DMRX-2217)	Free	Fleshy	Moderate brown	Smooth
6. VV-22-1010 (DMRX-2218)	Free	Fleshy	Moderate brown	Smooth
7. VV-22-1011 (DMRX-2219)	Free	Fleshy	Moderate brown	Smooth
8. VV-22-1012 (DMRX-2220)	Free	Fleshy	Moderate brown	Smooth

Table 1 (c). Stipe characters of native isolates

S.N.	Isolates	Shape	Attachment to pileus	Consistency	Colour	Base
1.	VV-22-1003 (DMRX-2212)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
2.	VV-22-1004 (DMRX-2213)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
3.	VV-22-1006 (DMRX-2215)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
4.	VV-22-1008 (DMRX-2216)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
5.	VV-22-1009 (DMRX-2217)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
6.	VV-22-1010 (DMRX-2218)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
7.	VV-22-1011 (DMRX-2219)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen
8.	VV-22-1012 (DMRX-2220)	Cylindrica	I Central	Fleshy	Yellowish white	Swollen

Table 1 (d). Pileus characters of native isolates

S.N.	Isolates	Outer colour	Middle colour	Margin
1.	VV-22-1003 (DMRX-2212)	Light yellow	Moderate brown	Regular
2.	VV-22-1004 (DMRX-2213)	Light yellow	Moderate brown	Regular
3.	VV-22-1006 (DMRX-2215)	Light yellow	Moderate brown	Regular
4.	VV-22-1008 (DMRX-2216)	Light yellow	Moderate brown	Regular
5.	VV-22-1009 (DMRX-2217)	Light yellow	Moderate brown	Regular
6.	VV-22-1010 (DMRX-2218)	Light yellow	Moderate brown	Regular

S.N.	Isolates	Outer colour	Middle colour	Margin
7.	VV-22-1011 (DMRX-2219)	Light yellow	Moderate brown	Regular
8.	VV-22-1012 (DMRX-2220)	Light yellow	Moderate brown	Regular

Table 1 (e). Volva characters of native isolates

S.N.	Isolates	Volva arrangement	Colour
1.	VV-22-1003 (DMRX-2212)	Sheathed	Brownish
2.	VV-22-1004 (DMRX-2213)	Sheathed	Brownish
3.	VV-22-1006 (DMRX-2215)	Sheathed	Brownish
4.	VV-22-1008 (DMRX-2216)	Sheathed	Brownish
5.	VV-22-1009 (DMRX-2217)	Sheathed	Brownish
6.	VV-22-1010 (DMRX-2218)	Sheathed	Brownish
7.	VV-22-1011 (DMRX-2219)	Sheathed	Brownish
8.	VV-22-1012 (DMRX-2220)	Sheathed	Brownish

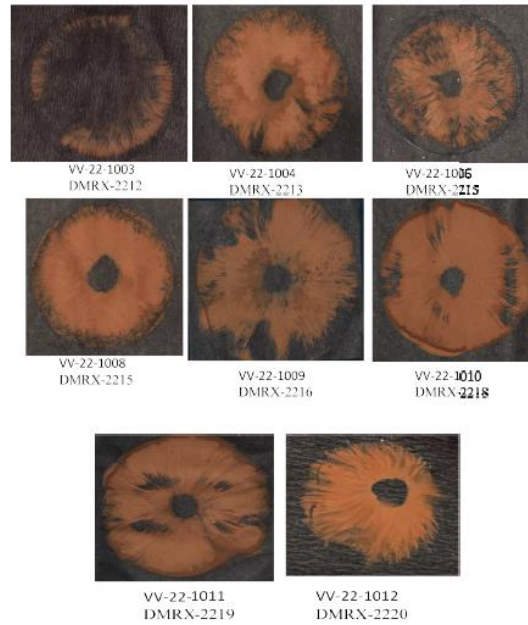


Plate 1 (b). Spore Print of *V. volucae* collected from survey

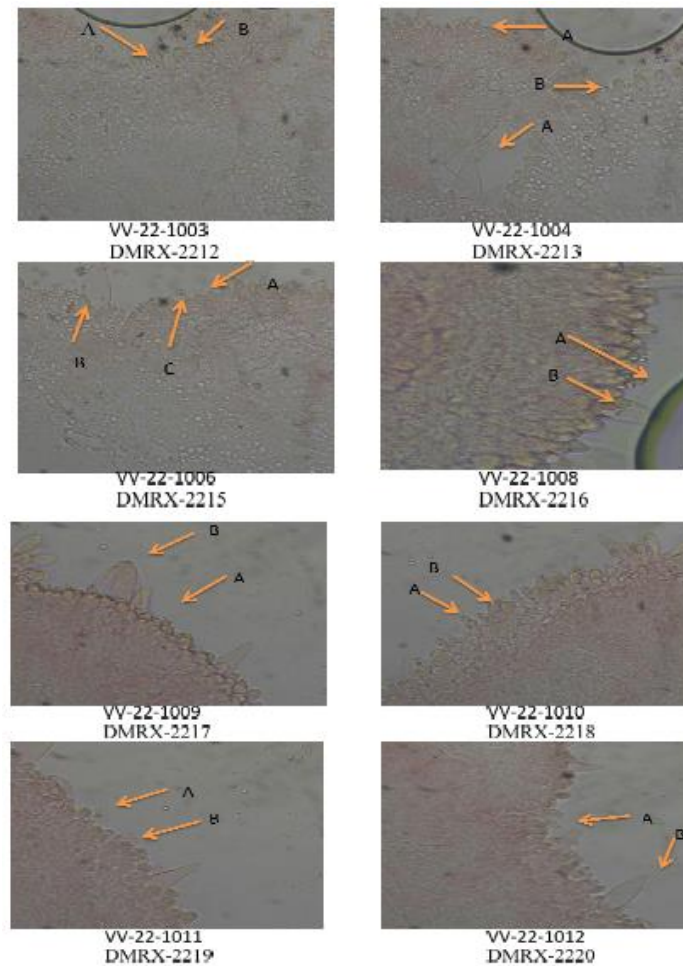
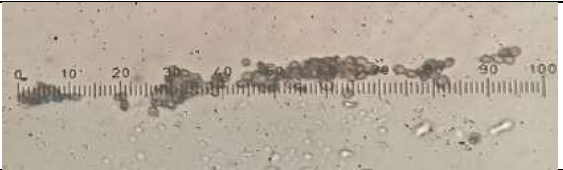

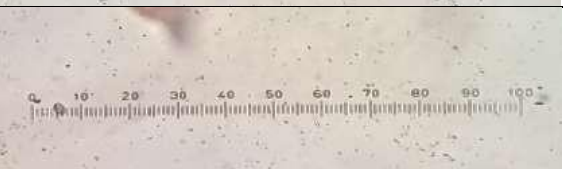
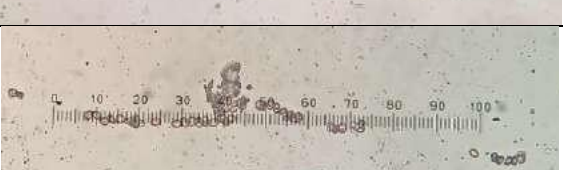






Plate 2. Microscopic images of collected isolates
A= Sterigmata, B= Basidium, C= Spore

Table 2. Microscopic characterizations of collected isolates

S.N.	Isolates	Microscopic character	Image
1.	VV-22-1003 (DMRX-2212)	Spore size - 6.76 μm Spore Shape - Oval	
2.	VV-22-1004 (DMRX-2213)	Spore size - 7.54 μm Spore Shape - Oval	
3.	VV-22-1006 (DMRX-2215)	Spore size - 7.02 μm Spore Shape - Oval	
4.	VV-22-1008 (DMRX-2216)	Spore size - 7.54 μm Spore Shape - Oval	
5.	VV-22-1009 (DMRX-2217)	Spore size - 8.06 μm Spore Shape - Oval	
6.	VV-22-1010 (DMRX-2218)	Spore size - 8.06 μm Spore Shape - Oval	
7.	VV-22-1011 (DMRX-2219)	Spore size - 7.02 μm Spore Shape - Oval	
8.	VV-22-1012 (DMRX-2220)	Spore size - 6.5 μm Spore Shape - Oval	

3.2 Microscopic Characterization

The microscopy of each strain has been done to observe its microscopic characterization. Hence, it is a basidiomycetes fungus; Basidium, basidiospores were seen on the microscope, and the size of basidiospores was also observed. The Isolate-wise microscopic characterizations are mentioned below (Table 2, Plate 2).

4. CONCLUSIONS

In 2022 monsoons a total of eight isolates were collected in a systematic survey conducted across Chhattisgarh from six districts viz. Raipur, Rajnandgaon, Durg, Dhamtari, Kabirdham, and Bemetara. The survey shows the diversification and distribution of *Volvariella* spp. in various agroclimatic regions of

Chhattisgarh. It shows the habit and habitat behaviour of the mushroom. Morphological analysis defines the major characteristics such as shape, size, and other taxonomic characters such as gills, stipe, pileus, and volva. *Volvariella* isolates collected were characterized macroscopically and found possessing pink spores, free lamellae and a stipe bearing no annulus but was encased at the base by a volva. Spore print colours were observed as pink and moderate brown, gill colour varied from light pink to brown for all isolates. The spore print not only shows the colour of the spore, but it also shows the arrangement of gills and the attachment of pileus to the stipe.

All the collected strains were characterized microscopically. The spore size was measured shape was observed. The spore size of all *V. volvaceae* isolates ranges between 6-9 µm and the shape of the spores were oval. A small horn like structure present over the basidium which is called sterigmata.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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