Faculty of Agriculture, University of Poonch Rawalakot

Jammu Kashmir Journal of Agriculture

ISSN: 2312-9344 (Online), 2313-1241 (Print)

https://jkjagri.com/index.php/journal

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGENS ASSOCIATED WITH CULTIVATED MUSHROOMS

[a]Abdul Nasir, [b]Farhan Fareed Qureshi, [c]Hafiz Muhammad Umar Qasmi

[a] *Botanical Science Division, Pakistan Museum of Natural History, Islamabad, Pakistan.*
[b] *Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi, Pakistan.*
[c] *Al-Beruni Education System, Pakistan.*

A B S T R A C T

Mushrooms, classified as fungi, boast a distinctive fruiting body and have been cultivated since ancient times due to their nutritional richness and unique texture. Serving as a unique source of proteins, vitamins, minerals, crude fibers, carbohydrates, and more, mushrooms offer a novel and valuable addition to the dietary spectrum. Boasting all essential amino acids and encompassing nearly all vital minerals, mushrooms exhibit diverse therapeutic properties. Samples afflicted with mushroom diseases and contaminated compost were gathered from the Pakistan Museum of Natural History (PMNH) mushroom garden, where the infected specimens exhibited various symptomatic features through visual observation. The virulence of the infected samples varied, indicating diverse degrees of fungal diseases. Fungal pathogens were isolated, identified, and purified on PDA medium. Post-isolation, both compost and mushroom (button) samples were prepared for visual confirmation of the fungal pathogens. It was observed that the aerial mycelium exhibited variations from dense to sparse, while the colony colors transitioned from white to pale brown and eventually dark brown after an incubation period of 10-12 days at 24±1 °C. The hyphae displayed branching and interweaving, with a size of 3-5 µm. Conidiophores were characterized as short, thin, branched, hyaline, sub-verticillate to verticillate, featuring an apical conidium measuring 150 to 180 x 3 to 5 µm. Conidia were oblong, singular, 2-celled, possessing thin walls, and were relatively short-lived, measuring 12-14 x 4-5 µm. By enhancing our understanding of the diversity and behavior of these pathogens, this study contributes to the advancement of sustainable and resilient practices in mushroom farming, ultimately promoting the stability and productivity of this economically significant agricultural sector.

Keywords: Button mushroom; Compost; Fungal diseases; Mushroom

Corresponding Author: Farhan Fareed Qureshi
Email: farhan.fareed10@gmail.com

Article history
Received: June 09th, 2023
Revised: July 17th, 2023
Accepted: July 24th, 2023

INTRODUCTION

Various types of mushrooms are known for their ease of cultivation, delightful taste, and nutritional benefits, making them a popular choice for consumption. Mushrooms, classified as fungi, feature a distinctive fruiting body and have been cultivated since ancient times for their unique texture, nutritional value, and flavorful qualities. This historical significance is evident in ancient Roman history (Smith et al., 2002). The cultivation of the Button mushroom (*Agaricus bisporus*) is widespread globally; nevertheless, the success of cultivation is influenced by the proliferation of various bacterial and fungal pathogens. These pathogens pose challenges in both the cultivation processes and storage phases. Largeteau and Savoie (2010) stated that button mushroom; *Agaricus bisporus* is very common mushroom which is susceptible for many different types of virus, fungal and bacterial diseases. Mills et al. (2008) reported the interaction between *Agaricus bisporus* and *Verticillium fungicola* may be the most economically significant interaction between two fungi. Pathogen variability has been assessed, and specific genotypes important to major mushroom-producing countries have been characterized. The living body of the fungus is mycelium made out of a tiny web of threads called hyphae.

Under specific conditions, sexually compatible hyphae will fuse and start to form spores. The larger spore producing structures are called mushrooms (Alexopoulos et al., 1996; Oei and Nieuwenhuijzen, 2005). Under favorable conditions, this mushroom exhibits rapid growth, completing its entire crop cycle within 4-5 weeks. It can be easily and quickly cultivated on non-composted materials like cotton waste, paddy straw, or other cellulose-rich natural waste materials (Ahlawat and Kumar, 2005).

A vast range of cell-wall-degrading nutrients from *Verticillium fungicola* have been discovered and main component analysis showed a complex correlation between chemical production and symptom phrase. It is likely that some or indeed many of these enzymes play a critical role in the pathogencity of Verticillium. Komon-Zelazowska et al. (2007) reported that production of the button mushroom *Agaricus bisporus* currently threatened by massive attacks of renewable mold disease. They determined the causal agents of this disease were two genetically closely related, but phenotypically strongly different, types of *Trichoderma*, which have recently been recently identified as *Trichoderma pleurotum* and *Trichoderma pleurotico* (Kou, 2007). The primary objective of the study was to systematically identify and characterize fungal pathogens prevalent in mushroom cultivation environments, employing morphological techniques for precise taxonomic classification.

MATERIALS AND METHOD

Cultivated mushroom samples

On the basis of symptomatology infected button mushrooms and infected substrates were collected (Figure 1). Samples were placed in polythene bags, labeled and were brought in Laboratory of Botanical science division, PMNH Islamabad, Pakistan for the isolation and characterization of fungal pathogen.

Figure 1. Infected compost samples for isolation of fungal pathogens.

Incidence of disease

Percentage disease incidence was calculated with the help of the following formula;

$$\text{Incidence } \% = \frac{\text{No of infected mushroom composts}}{Total \text{ no of mushroom composts samples}} \times 100$$

Isolation of fungal pathogens and Preservation

The fungal pathogens were isolated from the mushroom samples and from the substrate using direct plating method. Samples were surface sterilized using 2% clorox solution followed by two washings with sterilized distilled water. After complete drying, samples were placed on artificially nutritional media plate. Plates were then incubated for 7-10 days and the fungal growth was observed visually. Samples were desiccated using silica gel. A seven-day-old fungal culture was chosen from the incubator for preservation within a laminar flow cabinet, with a spirit lamp present. Plugs were extracted from Petri plates harboring the fungal pathogen and subsequently transferred to plastic vials containing silica gel. These vials were appropriately labeled and stored for future use.

Cultural and Morphological characterization

Cultural and morphological characterization was conducted, encompassing the observation of pathogenicity features. For the examination of morphological traits, the pathogen's growth from the mushroom sample was carefully positioned on a slide using an inoculating needle. Subsequently, the slide was scrutinized under a microscope to assess

morphological characteristics such as colony color, mycelial texture, sporulation, and conidial shape and size (length and width). Slides were meticulously prepared, incorporating lacto phenol cotton blue stain for enhanced clarity.

RESULTS AND DISCUSSION

Multiple visits were undertaken to gather infected samples, aiming to document the prevalence of fungal diseases in mushrooms at the Mushroom Garden, PMNH, based on observed symptoms. The cultivation of button mushrooms was carried out, and the incidence of fungal infection was documented, ranging from 30-40% (Table 1). Notably, the overall incidence of fungus varied, with the highest recorded incidence found in compost derived from wheat straws originating from Punjab, reaching 40% (Table 1).

Table 1. Percent disease incidence of fungal infection in compost samples.

Mushroom Type	Composts	
	Compost (Punjab)	Compost (Sindh)
Button Mushroom	40%	30%

Morphological characterization of *Trichoderma spp.*

The characteristics of *Trichoderma* isolates were examined by cultivating 7-day-old Potato Dextrose Agar (PDA) cultures at temperatures of 28°C and 35°C. It was noted that all Trichoderma isolates exhibited robust growth at 28°C, forming conidia within an 8-day period. Morphological observations were conducted on a 5-day-old culture of *Trichoderma* spp. cultivated on PDA, revealing the following traits. The conidia of *Trichoderma* spp., measuring 3.0x2.8 µm, exhibited a globose shape, and their coloration was noted as light green (Table 2). The mold disease manifests as a dense, cushioned white patch with greenish fungal growth on spawned and cased bags, eventually transforming into a bluish-green hue. In its initial stage, Trichoderma spp. exhibits a dense, purely white mycelium that closely resembles the mycelium of the mushroom, posing a challenge for growers in distinguishing between the two (Shah et al., 2013). The green mold fungus demonstrates robust colonization capabilities of organic material and deceased mushroom tissue. The proliferation of this disease is attributed to inadequate Phase II composting and elevated humidity levels. The dispersal of the fungus's spores occurs through air, water, and careless handling practices (Munshi et al., 2010).

Table 2. Morphological characterization of *Trichoderma viride.*

Isolates	Conidial Length (µm)	Spore Colour	Mycelium
1	3.0x2.8 µm	Light Green	Globose
3	3.0x2.7 µm	Light Green	Globose
4	3.0x2.8 µm	Light Green	Globose

Morphological characterization of *Sepedonium* spp.

Initially, the mycelium presents as white but undergoes a color transformation to yellow-tan as it matures. The hyphae exhibit characteristics of being septate, branched, hyaline, moderately thick, and measuring 3-5 mm in width. Erect conidiophores are observed, featuring lateral simple or botryose clusters of branches measuring 4-4.5 mm in width. These conidiophores are typically septate and bear spores singly and terminally on the branches. There are two prominent types of spores produced abundantly: hyaline, thin-walled conidia with an ellipsoid or pyriform shape, originating singly from the tips of the phialids, and chlamydospore-like structures characterized by being globose, warted, dark yellow, thick-walled, and measuring 13-17 mm in diameter (Table 3). The results of current study was parallel with the previous findings that the conidia are produced individually at the tip of the phialids on these branches. Chlamydospores, measuring 13 to 21 µm in diameter, are spherical, with a textured surface, exhibiting a dark yellow color and possessing thick walls (Rajan and Sivakumar, 2020). Sepanodium yellow mold disease occurs in spent compost when the moisture content exceeds 70%, and the temperature ranges between 19-20 ℃ (Sharma and Kumar, 2008).

Morphological characterization of *Cladobotrym* spp.

The conidia from the isolates were categorized into two groups based on size: one group ranged from 11~26 × 7~12 µm, and the other ranged from 8~14 × 6~11 µm. Identification of the two isolates as *Cladobotrym* spp. was established through the examination of observed

morphological characteristics of the conidia. The color of the mycelia displayed variations over time, with the isolates initially exhibiting white or grayish hues and later transitioning to light yellow (Table 4). *Cladobotryum dendroides*, also known as *Dactylium dendroides*, is responsible for causing cobweb disease. This ailment is characterized by the distinct coarse growth of mycelium covering the affected mushrooms, giving it the name "cobweb disease." Mushrooms can be susceptible to this disease at any stage of development. The pathogen rapidly takes over the mushroom, leading to a transformation in color to brown, accompanied by decay (Munshi et al., 2010). The initial infection occurs through casing soil contaminated with spores carried by air. Subsequent spread of the disease is facilitated by factors such as air movement, the activities of pickers, and splashes of water (Gupta et al., 2018).

Table 3. Morphological Characterization of *Sepedonium* spp.

Isolates	Colony Size (mm)	Spore Colour	Mycelium
5	12-15	Dark Yellow	Pyriform
6	13-17	Dark Yellow	Pyriform

Table 4. Morphological characterization of *Cladobotrym* spp.

Isolates	Conidial Length (µm)	Spore Colour	Mycelium
7	11~26 × 7~12	Whitish	Obovoid
8	8~14 × 6~11	Light yellow	Obovoid

Morphological characterization of *Alternaria* spp.

Morphological observations of the fungus were recorded by adopting slide culture technique. The measurements of different morphological structures of *Alternaria* spp. were observed. The fungus produced profuse mycelial growth on PDA. Initially, the mycelium was turned to grey brownish, multicelled and irregularly branched. Early growing stage, spores were thin (2.84 µm), narrow but became slightly thick (4.42 µm) as grew old. Conidiophores arised singly or in clusters, usually 2-6 and were long or short. The conidiophores measured 42.26 µm (27.30-112 µm) in length and 4.29 µm (3.12-8.43 µm) in width. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa (Table 5).

Table 5. Morphological characterization of *Alternaria* spp.

`	Conidial Length (µm)	Spore Colour	Mycelium
9	42.26 µm	Brown	Ellipsoidal
10	41.20 µm	Brown	Obclavate
11	42.20 µm	Brown	Ellipsoidal
12	42.26 µm	Brown	Obclavate

The results were similar with the findings of previous studies that competing molds such as *Aspergillus* spp., *Coprinus* spp., *Alternaria alternate*, *Fusarium* spp., *Mucor* sp., *Penicillium* sp., *Rhizopus* spp., *Drechslera bicolor*, *Trichoderma viride*, *Sclerotium rolfsii*, *Oedocephalum* spp. absorb essential nutrients required for the growth of Oyster mushrooms, resulting in a significant loss of approximately 70% in Oyster mushroom yields (Seth and Bhardwaj, 1989; Seth and Dar, 1989; Sharma and Vijay, 1993).

CONCLUSION

The knowledge of fungal pathogens associated with cultivated mushrooms is essential for promoting sustainable and resilient mushroom farming practices, ensuring the continued success of this important agricultural sector. Further research and ongoing monitoring are imperative to stay ahead of emerging threats and enhance the overall health and productivity of cultivated mushroom crops.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed and supported in this manuscript.

REFRENCES

Ahlawat, O., Kumar, S., 2005. Traditional and modern cultivation technologies for the paddy straw mushroom (*Volvariella* spp.), in: Rai, R., Upadhyay, R., Sharma, S. (Eds.), Frontiers in Mushroom Biotechnology, pp. 157-164.

Alexopoulos, C.J., Mims, C., Blackwell, M., 1996. Introductory Mycology. John Wiley and Sons, Inc., USA.

Gupta, S., Summuna, B., Singh, R., Gupta, M., Gupta, A., 2018. Mushroom Diseases: A potential threat to mushroom cultivation, Transformation of Indian agriculture through Innovative technologies, pp. 57-70.

Komon-Zelazowska, M., Bissett, J., Zafari, D., Hatvani, L., Manczinger, L., Woo, S., Lorito, M., Kredics, L., Kubicek, C.P., Druzhinina, I.S., 2007. Genetically closely related but phenotypically divergent *Trichoderma* species cause green mold disease in oyster mushroom farms worldwide. Applied and Environmental Microbiology 73, 7415-7426.

Largeteau, M.L., Savoie, J.-M., 2010. Microbially induced diseases of *Agaricus bisporus*: Biochemical mechanisms and impact on commercial mushroom production. Applied Microbiology and Biotechnology 86, 63-73.

Mills, P., Thomas, J., Sergeant, M., Costa, A., Collopy, P., Bailey, A., Foster, G., Challen, M., 2008. Interactions between *Agaricus bisporus* and the pathogen *Verticillium fungicola*, British Mycological Society Symposia Series. Elsevier, pp. 1-17.

Munshi, N.A., Dar, G.H., Ghani, M., Kauser, S., Mughal, N., 2010. Button mushroom cultivation. Communication and Publication Centre: SKUAST 1, 1-28.

Oei, P., Nieuwenhuijzen, B.v., 2005. Small-scale mushroom cultivation, Oyster, Hiitake and Wood ear Mushrooms of Digigrafi, Wageningen. Agromisa, Wageningen, The Netherlands.

Rajan, S., Sivakumar, N., 2020. Mushroom Technology. CBS Publishers & Distributors.

Seth, P., Bhardwaj, S., 1989. Studies on vert-de gris caused byb Myceliophthora lutea Coast on *A. bisporus* and its control. Mushroom Science 12, 725-733.

Seth, P., Dar, G., 1989. Studies on *Cladobotryum dendroides* (Bull: Merat) W. Gams et Hoozem, causing cobweb disease of *Agaricus bisporus* and its control. Mushroom Science 12, 711-723.

Shah, S., Nasreen, S., Kousar, S., 2013. Efficacy of fungicides against *Trichoderma* spp. causing green mold disease of oyster mushroom (*Pleurotus sajor*-caju). Research Journal of Microbiology 8, 13-19.

Sharma, J., Kumar, S., 2008. Evaluation of strains of milky mushroom (*Calocybe indica*) for cultivation in Jharkhand. Mushroom Research 17, 24-37.

Sharma, S., Vijay, B., 1993. Competitor moulds: A serious threat to *A. bisporus* cultivation in India, Proceedings of Golden jubilee Symposium of Horticultural Society of India, Bangalore, India, pp. 312-313.

Smith, J.E., Rowan, N.J., Sullivan, R., 2002. Medicinal mushrooms: A rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnology Letters 24, 1839-1845.