One of the main culprits of Morchella (Morohellaceae: Morchella) pileus rot disease: Pestalotiopsis trachicarpicola

zhen liang (lz2497818016@163.com)  
China West Normal University  https://orcid.org/0000-0001-9938-7003

linhui li  
https://orcid.org/0000-0002-0915-9793

Research Article

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Abstract

We found some diseased fruiting bodies when cultivating Morchella. The infected ascomata were covered by white mycelia mainly on the pileus, and the infection cause the fruiting body to rot and die. A total of 5 fungi were separated from symptomatic tissue. One of them was proved to be pathogenic by Koch’s postulates. The pathogen was identified as *Pestalotiopsis trachicarpicola* based on its morphology and Multi-gene (ITS, β-tubulin and *tef1*) analysis. This is the first report of pileus rot disease on cultivated Morchella caused by *P. trachicarpicola* in China, which is of positive significance for the quarantine of cultivated soil of Morchella.

Introduction

Morchella is a kind of edible and medicinal mushrooms with high economic and scientific research value (Badalyan SM and Borhani A, 2019, Tietel Z and Masaphy S, 2018). With the increasing scarcity of wild resources and the expansion of artificial cultivation area by years, there are more and more reports on the cultivation, ecology, genetics, physiology about Morchella (Liu Q *et al.*, 2018, Du XH and Yang ZL, 2021, Du XH *et al.*, 2015). However, so far, Morchella has not yet achieved controllable indoor cultivation, and the open cultivation environment made Morchella vulnerable to pathogenic microorganisms (Liu W *et al.*, 2019). And there are few studies about Morchella diseases. To our knowledge, Morchella pathogens reported at present include *Fusarium incarnatum* (Guo MP *et al.*, 2016), *Fusarium proliferatum* (Lu QY *et al.*, 2019) *Diploëspora longispora* (Peixin *et al.*, 2018), *Cladobotryum protrusum* (Lan YF *et al.*, 2019), *Rhizopus stolonifer* (Zhang YC *et al.*, 2020), these might be only a part of Morel pathogenic fungi, and more remain to be discovered.

During the cultivation of *M. sextelata*, we found a very harmful fungal disease, which caused huge economic losses to the farmers. We speculate that the pathogen may come from the soil, in the early development of *M. sextelata*, it attached to the fruiting body cap, as *M. sextelata* grows, its mycelia gradually invade the interior of the ascomata pileus, eventually make whole fruit body rot and die, result in a serious decline in the quality and yield of *M. sextelata*. We isolated a pathogenic fungi from this nidus, through morphological identification and Multi-gene (ITS, β-tubulin and *tef1*) analysis, it was identified as *Pestalotiopsis trachicarpicola*.

Materials And Methods

Pathogen isolation

The infected *M. sextelata* was placed in Clean Bench and the surface of fruiting body was washed with sterile water, then cut the diseased plaque and inoculated on potato-dextrose agar (PDA) medium, cultured at 25 °C under dark conditions. The marginal mycelia were picked for isolation of pure culture, when the mycelia began to germinate. The purified pathogen strain is stored at 4 °C.
**Pathogenicity tests**

Koch's postulates was used to verify the pathogenicity of pathogen. Firstly, healthy ascomata were selected in the cultivation field of *M. sextelata* and a 5 mm hole was inserted on the cap with sterile toothpick. Meanwhile, conidia were cultured on PDA plate and eluted to prepare suspension. Then, 20µL suspension was inoculated into the hole and sterile water was used as negative control.

After the fruiting body of *M. sextelata* showed symptoms, isolation of pure culture was carried out again from the diseased spot, and if the pure culture was completely consistent with the previous culture, the fungus was identified to be pathogen.

**Morphological observation**

The pathogen was inoculated on PDA plate and cultured at 25°C in Darkness for 3–15 days to visually observe the color, diameter and characteristics of hyphae. Approximately 25 random conidia were examined for morphological characters (the size, the shape, color type, the number and length of apical and basal appendages) using Optical Microscope. Identification was based on the keys and descriptions provided by *General Mycology* (Xing LJ et al., 2021) and *FLORA FUNGORUM SINICORUM VOL.38* (Ge QX et al., 2009).

**DNA extraction, PCR amplification and nucleotide sequencing**

The pathogenic fungi were grown on PDA plate and cultured at 25°C in Darkness for 9 days. The fresh mycelium was scraped from the surface of the culture medium, and the whole genomic DNA of the pathogen was extracted with a fungal DNA extraction Kit(sangon Biotech, shanghai). The internal transcribed spacer regions, interval 5.8 S nrRNA gene (ITS) was amplified using primer pairs ITS1(5′-TCCGTAGGTAACTGCGG-3′) and ITS4(5′-TCCTCGCTATTGATATGC-3′)(White TJ et al., 1990), partial β-tubulin(*tub2*) gene region was amplified with primer pairs BT2A (5′-GGTAACCAATCGTGCTGCTTTCC-3′) and BT2B (5′ACCCTCAGTGTAGTGACCCTTGC-3′)(Maharachchikumbura SSN et al., 2012), and part of translational elongation factors(*tef1*) gene was amplified using the primer pairs EF1-526F (5′-GTGTYGTYATYGHCAYGT-3′) and EF1-1567R(5′-ACHGTRCCRTACACCRATCTT-3′) (Maharachchikumbura SSN et al., 2012).

PCR was performed in a 25µL reaction system containing 10.3µL of double distilled water, 12.5µL 2×Taq MasterMix(sangon Biotech,shanghai),0.6µl of each primer (10µM) and 1µL DNA template. The thermal cycling program was as follows: For ITS an initial denaturing step of 95°C for 3 min, followed by 30 amplification cycles of 95°C for 30 s, 52°C for 45 s and 72°C for 90 s, and a final extension step of 72°C for 10 min. For β-tubulin PCR conditions were an initial step of 3 min at 95°C, 30 cycles of 30 s at 95°C,
50 s at 55°C, and 1 min at 72°C, followed by 10 min at 72°C. For *tef1*, an initial step of 3 min at 95°C, 30 cycles of 30 s at 95°C, 55 s at 58°C, 90 s at 72°C (Maharachchikumbura SSN et al., 2012). The PCR products were detected by 1% agarose gel electrophoresis and sent to Sangon Biotech for sequencing.

**Phylogenetic analysis**

The sequencing results were compared and analyzed in NCBI database by Blast. Similar strains were selected for multi gene joint tree construction. Combination of multi-locus dataset of three gene regions was phylogenetic analyzed by using MEGA7 software (Kumar S *et al.*, 2016). The sequence alignments algorithm adopts ClustalW to delete the unaligned sequence manually after the alignment, and then uses the method of Maximum Likelihood (ML) to build the phylogenetic trees. The branch support of ML analysis was evaluated by 1,000 bootstrap replications.

**Results**

**Pathogen isolation and Pathogenicity tests**

In this study, five strains of fungi were isolated from the fruiting body of diseased *M. sextelata* and numbered M-1, M-2, M-3, M-4 and M-5 respectively. It was found that the fruiting body of *M. sextelata* inoculated with M-5 appear obvious lesions by Koch's postulates verification (Fig. 1b), and the symptom of the lesions was consistent with that of the naturally diseased ascomata (Fig. 1a), however, there was no symptom in the control group (Fig. 1c), this showed the fungus M-5 really was a Morchella pathogen.

**Morphological characteristics of pathogen**

The colony on the PDA reached 90 mm in diameter after 9 days at 25°C, aerial mycelium on surface, edge fimbriate, white and dense, the back was yellow white, and the conidioma was black, round and mucinous (Fig. 2a, b and c). Conidia fusiform, straight to slightly curved, consists of 5 cells, 4-septate, 21.0-28.6×6.0-7.6µm (x = 24.8×7.0µm); apical cell hyaline, conic to subcylindrical 2.7–4.5µm long (x = 3.7µm); with 2–4 tubular apical appendages, arising from the apex of the apical cell, 6.0-19.3µm long (x = 10.2µm); the three intermediate cells of conidia, concolorous to versicolorous, brown to olivaceous, melanin deposition, doliiform to cylindrical, constricted at septa, peripheral and diaphragm walls darker than the rest of the cell, together 13.9–17.6µm long (x = 15.9µm); second cell 4.6–5.9µm long (x = 5.4µm); third cell 4.4-6.0µm long (x = 5.1µm); fourth cell 4.1–6.5µm long (x = 5.39µm); basal cell conic to acute, 3.3–7.2µm long (x = 5.2µm), with a basal appendage, 2.4–6.9µm long (x = 4.5µm) (Fig. 2d-g). After consulting (Maharachchikumbura SSN et al., 2012, Ge QX et al., 2009) the morphology of the pathogen M-5 was consistent with that of *Pestalotiopsis spp.*

**Phylogenetic study**
The PCR amplified fragments were sent to the company for sequencing to obtain the partial sequence of ITS of 586bp, the partial sequence of \(\beta\)-tubulin gene of 458bp and the partial sequence of \(tef1\) gene of 965bp. The three sequences were uploaded to GenBank and the accession numbers were OL362082, OL828342 and OL905009, respectively. The sequencing results were compared on the NCBI website, and the results showed that the three sequences of M-5 strain were identical with \textit{Pestalotiopsis trachicarpicola}, homology is as high as 99% – 100%. The phylogenetic tree was constructed by using MEGA7 software, and the information of the tree was shown in Table 1. The pathogen M-5 strain and \textit{Pestalotiopsis trachicarpicola} strain YB1 were clustered in the same branch, and the test value was as high as 100% (Fig. 3).

The pathogen M-5 was identified as \textit{Pestalotiopsis trachicarpicola} by morphological identification and multi-gene analysis.

**Discussion**

The pathogen of \textit{M. sextelata} is classified in \textit{Pestalotiopsis spp.}. This genus, is characterized by five cells and the middle three by dark color, which is a fungi imperfecti and was established and named by Steyaert in 1949 (Steyaert RL, 1949). It is very difficult to classify and identify (Maharachchikumbura SSN et al., 2011). At present, the morphology of conidia is the most widely used taxonomic feature of \textit{Pestalotiopsis spp.}, which is classified by the length, width, cell color, number and length of attached filaments of conidia (Jeewon R et al., 2004). However, because of the morphological similarity between species, only observing the morphology of conidia is not enough to accurately identify the species. Therefore, the molecular data are also gradually used in the classification of this genus, and the classification and identification system of this genus is gradually improved from single analysis of conserved ITS sequence to joint analysis of multiple genes (Liu AR et al., 2010). Now, the most commonly used multi-gene joint analysis is through the combination of three sequences: ITS sequence, \(tub2\) and \(tef1\), which shows better differences among different species (Maharachchikumbura SSN et al., 2012). In this study, the pathogen of \textit{M. sextelata} was identified as \textit{Pestalotiopsis trachicarpicola} by the method described above.

\textit{Pestalotiopsis spp.} is an important plant pathogen, which causes diseases all over the world. It can not only cause diseases of seeds and seedlings, but also cause leaf spot, branch and trunk blight and fruit rot. Among them, leaf spot is the most common, and the main host includes \textit{Camellia sinensis} (Maharachchikumbura SSN et al., 2016, Wang YC et al., 2019, Chen YJ et al., 2017), \textit{Photinia × fraseri} (Zhu YH et al., 2021, Xu XL et al., 2021), \textit{Paris polyphylla} (He X et al., 2020), \textit{Vitis davidii} (Tang XB et al., 2020), \textit{Banana} (Bhuiyan MAB et al., 2021), \textit{Strawberry} (Morales-Mora LA et al., 2019), \textit{Eucommia ulmoides} (Li SJ et al., 2018), \textit{Mango} (Shu J et al., 2019) and so on. Its typical disease symptoms on the leaf consisted of circular, semicircular or irregular shape of the disease spot, brown at early stage and grayish brown to grayish white at middle stage and eventually lead to leaf abscission. Branch and trunk blight often occurs on \textit{Blueberry Bushes} (Borrero C et al., 2017), \textit{Chinese Hickory} (Liu YJ et al., 2014), \textit{Loquat} (Fang L et al., 2013), \textit{Pinus bungeana} (Qi M et al., 2021) and grapevine (Jayawardena RS et al., 2014).
2015). Initially, the necrotic lesions appeared on the branch and trunk, which led to the discoloration and cracking of the bark with the development of the disease, branch and trunk with the lesion were wilt at the end. Fruit rot mainly occurs in the harvest and storage period of *Grape* (Ghuffar S et al., 2018, Jayawardena RS et al., 2015), *kiwifruit* (Li L et al., 2016), *rambutan* (Keith LM, 2008), *Guava* (Keith LM et al., 2006), *Persimmon* (Palou L et al., 2015) etc. Dark brown to black spots appear in the disease spot at early stage and white hyphae grow in the later stage, whole fruit rots at the end. If the fruit is damaged, it is more likely to be infected. The infection phenomenon of each species will be different. In addition, it also causes the problems of *Cunninghamia lanceolata* (Zeng SH, 1998, Huang TZ, 1983), *strawberry* (Hua XF et al., 2021) rotten seed, rotten root and seedling growth decline. However, so far, the pathogenic effect of this genus on macrofungi has not been found.

It has been reported that the *Pestalotiopsis spp.* mainly take the soil, diseased seedlings and organization as the transmission carriers, which prefer to grow in the high humidity environment. The younger the infected tissue is more likely to get infected and tissue damage is also conducive to pathogen infection (Ge QX et al., 2009). Morchella is suited to grow in high humidity environment, and its tissue is very fragile and vulnerable to mechanical and insect damage, which increase the possibility of Morchella infection (Ruiz-Herrera J and Osorio E, 1974, Sambyal K and Singh RV, 2021). Most importantly, as an edible commercial mushroom, Morchella grows and is harvested in a short time (Zhi CY et al., 2021). Once infected, it loses all commercial value, therefore, we should pay more attention to the soil quarantine work in the cultivation process of Morchella.

**Declarations**

**Informed consent statement**

All authors have seen a copy of the manuscript and have approved its submission.

**Author contribution**

LHL conceived the idea and designed the experiments. ZL carried out the research experiments and analyzed the results. ZL drafted the manuscript. LHL revised the manuscript.

**Data Availability Statement**

Some or all data, models, or code generated or used during the study are available from the corresponding author by request. (List items).

**Compliance with ethical standards**
Conflict of interest

The authors declare that they have no conflict of interest.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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References

1. Badalyan SM & Borhani A (2019), "Medicinal, Nutritional, and Cosmetic Values of Macrofungi Distributed in Mazandaran Province of Northern Iran (Review)", *International Journal of Medicinal Mushrooms*, Vol. 21 No. 11, pp. 1099-1106.
2. Bhuiyan MAB, Islam SMN, Bukhari MAI, Kader MA, Chowdhury MZH, Alam MZ, Abdullah HM & Jenny F (2021), "First Report of Pestalotiopsis microspora Causing Leaf Blight of Banana in Bangladesh", *Plant Disease*.
3. Borrero C, Casta OR & Aviles M (2017), "First Report of Pestalotiopsis clavispora (Neopestalotiopsis clavispora) Causing Canker and Twig Dieback on Blueberry Bushes in Spain", *Plant Disease*, Vol. 102 No. 6, pp. 1178-1178.
4. Chen YJ, Zeng L, Shu N, Jiang MY, Huang YJ, Wang H & Tong HR (2017), "Pestalotiopsis-Like Species Causing Gray Blight Disease on Camellia sinensis in China", *Plant Disease*, Vol. 102 No. 1, pp. 98-106.
5. Du XH & Yang ZL (2021), "Mating Systems in True Morels (Morchella)", *Microbiology And Molecular Biology Reviews*, Vol. 85 No. 3, pp. e0022020.
6. Du XH, Zhao Q & Yang ZL (2015), "A review on research advances, issues, and perspectives of morels", *Mycology*, Vol. 6 No. 2, pp. 78-85.
7. Fang L, Wang HR & Feng JJ (2013), "Branch Blight of Loquat Caused by Pestalotiopsis sydowiana in China", *Plant Disease*, Vol. 97 No. 7, pp. 990.
8. Ge QX, Chen YX & Xu T (2009), FLORA FUNGORUM SINICORUM VOL.38 PESTALOTIOPSIS REDACTORES PRINCIPALES, Science Press(in Chinese).
9. Ghuffar S, Irshad G, Naz F, Zhang XY, Bashir A, Yang HL, Zhai FY & Gleason ML (2018), "First Report of Postharvest Rot Caused by Pestalotiopsis sp. on Grapes in Punjab, Pakistan", *Plant Disease*, Vol. 102 No. 6, pp. 1175-1175.
10. Guo MP, Chen K, Wang GZ & Bian YB (2016), "First Report of Stipe Rot Disease on Morchella importuna Caused by Fusarium incarnatum – F. equiseti Species Complex in China", *Plant Disease*, Vol. 100 No. 12, pp. 2530-2530.

11. He X, Li A, Li C, Zhang Q, Yang QH, Zhu HY, Chen QB & Yang PW (2020), "Pathogen identification and biological characteristics of Paris polyphylla var.yunnanensis leaf spot disease caused by Pestalotiopsis oryzae", *Plant Protection*(in Chinese), Vol. 46 No. 06, pp. 47-54+64.

12. Hua XF, Chen X, Huang MQ, Chen X, Zhu CH & Chen G (2021), "Pathogen Identification of Strawberry Root Rot in Fujian Province", *China Vegetables*(in Chinese), No. 12, pp. 79-84.

13. Huang TZ (1983), "The first report of top blight of Cunninghamia lanceolata", *Journal of Northeast Forestry University*(in Chinese), No. 03, pp. 45-50.

14. Jayawardena RS, Zhang W, Liu M, Maharachchikumbura SSN, Zhou Y, Huang JB, Nithong S, Wang ZY, Li XH, Yan JY & Hyde KD (2015), "Identification and characterization of Pestalotiopsis-like fungi related to grapevine diseases in China", *Fungal Biology*, Vol. 119 No. 5, pp. 348-61.

15. Jeewon R, Liew E & Hyde K (2004), "Phylogenetic evaluation of species nomenclature of Pestalotiopsis in relation to host association", *Fungal diversity*, Vol. 1739-55.

16. Keith LM (2008), "First Report of Pestalotiopsis virgatula Causing Pestalotiopsis Fruit Rot on Rambutan in Hawaii", *Plant Disease*, Vol. 92 No. 5, pp. 835.

17. Keith LM, Velasquez ME & Zee FT (2006), "Identification and Characterization of Pestalotiopsis spp. Causing Scab Disease of Guava, Psidium guajava , in Hawaii", *Plant Disease*, Vol. 90 No. 1, pp. 16-23.

18. Kumar S, Stecher G & Tamura K (2016), "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets", *Molecular biology and evolution*, Vol. 33 No. 7, pp. 1870-1874.

19. Lan YF, Cong QQ, Wang QW, Tang LN, Li XM, Yu QW, Cui X, An XR, Yu CX, Kong FH & Li XD (2019), "First Report of Cladobotryum protrusum Causing Cobweb Disease on Cultivated Morchella importuna", *Plant Disease*, Vol. 104 No. 3, pp. 977.

20. Li L, Pan H, Chen MY & Zhong CH (2016), "First Report of Pestalotiopsis microspora Causing Postharvest Rot of Kiwifruit in Hubei Province, China", *Plant Disease*, Vol. 100 No. 10, pp. 2161.

21. Li SJ, Li SY, Zhang ZY, Zhu HMY, Qiao TM & Han S (2018), "First Report of Pestalotiopsis trachicarpicola, a Causal Agent of Leaf Spot of Eucommia ulmoides in Southwest China", *Plant Disease*, Vol. 102 No. 12, pp. 2656-2656.

22. Liu AR, Shuang CC, Shang YW, Tong X, Guo LD, Jeewon R & Wei JG (2010), "Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in Pestalotiopsis taxonomy", *Molecular Phylogenetics and Evolution*, Vol. 57 No. 2, pp. 528-535.

23. Liu Q, Ma H, Zhang Y & Dong C (2018), "Artificial cultivation of true morels: current state, issues and perspectives", *Critical Reviews in Biotechnology*, Vol. 38 No. 2, pp. 259-271.

24. Liu W, Cai YL, He PX, Ma XL & Bian YB (2019), "Occurrence and Control of Pests and Diseases in Field Cultivation of Morchella Mushrooms", *Acta Edulis Fungi*(in Chinese), Vol. 26 No. 02, pp. 128-134+3-5.
25. Liu YJ, Huang B & Wu ZH (2014), "First Report of Pestalotiopsis vismiae Causing Trunk Disease of Chinese Hickory (Carya cathayensis) in China.", Plant Disease, Vol. 98 No. 11, pp. 1588-1588.

26. Lu QY, Liu J, Tu CH, Di FA, Zheng Q, Guo QL, Zhang ZZ & Qin W (2019), "Isolation and Identification of Morchella Pathogenic Fungi and Determination of In Vitro Antibacterial Activity of 30 Plant Essential Oils", E3S Web of Conferences, Vol. 13606005.

27. Maharachchikumbura SSN, Guo LD, Cai L, Chukeatirote E, Wu WP, Sun X, Crous PW, Bhat DJ, McKenzie EHC, Bahkali AH & Hyde KD (2012), "A multi-locus backbone tree for Pestalotiopsis, with a polyphasic characterization of 14 new species", Fungal Diversity, Vol. 56 No. 1, pp. 95-129.

28. Maharachchikumbura SSN, Guo LD, Chukeatirote E, Bahkali AH & Hyde KD (2011), "Pestalotiopsis—morphology, phylogeny, biochemistry and diversity", Fungal Diversity, Vol. 50 No. 1, pp. 167-187.

29. Maharachchikumbura SSN, Guo LD, Liu ZY & Hyde KD (2016), "Pseudopestalotiopsis ignota and Ps. camelliae spp. nov. associated with grey blight disease of tea in China", Mycological Progress, Vol. 15 No. 3, pp. 22-28.

30. Morales-Mora LA, Martínez-Salgado SJ, Valencia De Ita M, Andrade-Hoyos P, Silva-Rojas H. V & Romero-Arenas O (2019), "First Report of Leaf Spot and Anthrancnosis Caused by Pestalotiopsis sp. on Strawberry in Puebla, Mexico", Plant Disease, Vol. 103 No. 10, pp. 2668-2668.

31. Palou L, Montesinos-Herrero C, Tarazona I, Besada C & Taberner V (2015), "Incidence and Etiology of Postharvest Fungal Diseases of Persimmon (Diospyros kaki Thunb. cv. Rojo Brillante) in Spain", Plant Disease, Vol. 99 No. 10, pp. 1416-1425.

32. PEIXIN, H., CONGCONG, L., YINGLI, C., YA, Z., YINBING, B. & WEI, L. (2018), "First report of pileus rot disease on cultivated Morchella importuna caused by Diploöspora longispora in China", Journal of General Plant Pathology, Vol. 84 No. 1, pp.

33. Qi M, Xie CX, Chen QW & Yu ZD (2021), "Pestalotiopsis trachicarpicola, a novel pathogen causes twig blight of Pinus bungeana (Pinaceae: Pinoideae) in China", Antonie Van Leeuwenhoek, Vol. 114 No. 1, pp. 1-9.

34. Ruíz-Herrera J & Osorio E (1974), "Isolation and chemical analysis of the cell wall of Morchella sp.", Antonie van Leeuwenhoek, Vol. 40 No. 1, pp. 57-64.

35. Sambyal K & Singh RV (2021), "A comprehensive review on Morchella importuna: cultivation aspects, phytochemistry, and other significant applications", Folia microbiologica, Vol. 66 No. 2, pp. 147-157.

36. Shu J, Yu ZH, Sun WX, Zhao J, Li QL, Tang LH, Guo TX, Huang SP, Mo JY, Hsiang T & Luo SM (2019), "Identification and Characterization of Pestalotioid Fungi Causing Leaf Spots on Mango in Southern China", Plant Disease, Vol. 104 No. 4, pp. 1207-1213.

37. Steyaert RL (1949), "Contribution a l'étude monographique de Pestalotia de Not et Monochaetia Sacc. (Truncatella gen. nov. et Pestalotiopsis gen. nov.)", Bull. Jard. Bot. Brux, Vol. 19285-358.

38. Tang XB, Ni YJ, Hu YC, Bai JH, Yu L, Chen QX & Wen ZF (2020), "Identification of the fungal pathogen causing Vitis davidii leaf spot and screening of fungicides for control of the disease", Plant Protection(in Chinese), Vol. 46 No. 04, pp. 110-115+154.
39. Tietel Z & Masaphy S (2018), "True morels (Morchella)-nutritional and phytochemical composition, health benefits and flavor: A review", *Critical reviews in food science and nutrition*, Vol. 58 No. 11, pp. 1888-1901.

40. Wang YC, Xiong F, Lu QH, Hao XY, Zheng MG, Wang L, Li NN, Ding CQ, Wang XC & Yang YJ (2019), "Diversity of Pestalotiopsis-Like Species Causing Gray Blight Disease of Tea Plants (Camellia sinensis) in China, Including two Novel Pestalotiopsis Species, and Analysis of Their Pathogenicity", *Plant Disease*, Vol. 103 No. 10, pp. 2548-2558.

41. White TJ, Bruns S, Lee S & Taylor J (1990), "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics", pp. 315-322.

42. Xing LJ, Li MC & Yu QL (2021), "General Mycology", pp. 428-430.

43. Xu XL, Liu SY, Lv YC, Zeng Q, Liu YG & Yang CL (2021), "Leaf blight on Photinia x fraseri caused by Pestalotiopsis trachicarpica in China", *Plant Disease*.

44. Zeng SH (1998), "Electronic Microscope Observations on the Cellular Structure Changes of Cunninghamia lanceolata Cone Scales after Infected by Pestalotiopsis apiculatus", *Journal of Fujian Forestry Science and Technology (in Chinese)*, No. 04, pp. 31-33.

45. Zhang YC, Lu QY, Wang YM, Tu CH, Liu L, Xie YD & Liu J (2020), "Isolation and identification of Morchella pathogenic bacteria and determination of antibacterial activity of 22 essential oils", *IOP Conference Series Earth and Environmental Science*, Vol. 615 No. 1, pp. 012098.

46. Zhi CY, Qiao J, Zhao JG, Du YQ & Zhang XZ (2021), "Research Progress In Artificial Cultivation of Morchella ", *Northern Horticulture (in Chinese)*, No. 15, pp. 143-150.

47. Zhu YH, Liu YJ, Bi FH, Jiang H, Zhao BW, Lu LQ & Chen MJ (2021), "Identification and biological characteristics of the pathogen causing leaf spot disease on Photinia x fraseri", *Plant Protection (in Chinese)*, Vol. 47 No. 05, pp. 210-215+235.

**Table**

Table 1 is only available as a download in the Supplemental Files section.

**Figures**
Figure 1

Please See image above for figure legend.
**Figure 2**

Morphological characteristics of pathogen M-5 (a, b) Colony on PDA, (a) from above, (b) from below, (c) Conidiomata, (d-g) Conidia with concolorous and versicolorous median cells

Please See image above for figure legend.
Figure 3

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Supplementary Files

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