New Disease Reports

First report of *Lecanicillium fungicola* var. *aleophilum* infecting white-button mushroom in Vietnam

L.D. Thao*, V.T.P. Binh, T.N. Khanh, H.M. Thanh, L.T. Hien, P.T. Anh, T.T.T. Trang, N.V. Dung and H.T. Hoai

Division of Plant Pathology & Phyto-immonology, Plant Protection Research Institute (PPRI). Duc Thang, Bac Tu Liem, Hanoi, Vietnam

*E-mail: thaoledinh.ppri@mard.gov.vn

Received: *20 Mar 2020.* ***Published:*** *28 Apr 2020.* ***Keywords:*** *Agaricus bisporus, brown spots, Verticillium.*

The white-button mushroom (*Agaricus bisporus*) is an important edible mushroom grown throughout the world (Atila *et al*., 2017). In Vietnam, the mushroom is grown in open fields during the winter season and when the ambient temperature exceeds 20°C, in phytotrons at temperatures ranging from 10-20°C. Surveys in Northern Vietnam from 2018 to 2019 revealed that white-button mushroom crops were infected with a wide range of pathogens. Brown spots were the most serious disease observed in mushroom-growing areas, affected crops having a disease incidence of >50%, leading to a significant decrease in productivity and quality. The disease symptoms were small to large brown, sunken spots, on the mushroom fruiting bodies (Fig. 1).

Diseased samples were collected from Quang Ninh and Hai Phong Province. Causative agents were isolated on quarter-strength potato dextrose agar (PDA), containing 0.02 g/l streptomycin and 0.02 g/l penicillin. The cultures were purified by single spore isolation. The fungal isolates were examined under a light microscope and showed the same morphological characteristics. A representative isolate was chosen for further study and stored in the culture collection of the Plant Protection Research Institute of the Vietnam Academy of Agricultural Sciences (NMQN06.18.12).

Fungal colonies of NMQN06.18.12 on PDA were white, short and depressed, reaching 5 cm in diameter after seven days' incubation at 25°C. The fungus could grow at 30°C, distinguishing it from two other pathogenic fungi on *A. bisporus*: *Lecanicillium fungicola* var. *flavidum* (maximum temperature for growth <27°C), and *L. f.* var. *fungicola* (maximum temperature for growth <30°C) (Zare & Gams, 2008). Conidiophores were hyaline, up to 450 µm, bearing 1-7 whorls, each whorl contained 3 - 5 phialides, often 4 phialides. Phialides were cylindrical, tapering to the apex, (16.4-) 19.1-26.7 (-28) × (1.5-) 1.7-2.1 (-2.2) µm (n=30). Conidia were sub-global to ellipsoidal and cylindrical, (2.2-) 3.7-6.5 (-8.8) × (1.5-) 1.8-2.4 (-3) µm (n=30) (Fig. 2). The fungus was identified as *Lecanicillium fungicola* var. *aleophilum* (syn. *Verticillium fungicola* var. *aleophilum*) according to the description of Zare & Gams (2008). This identification was confirmed by ITS sequence analysis using the ITS1/ITS4 primers (White *et al*., 1990). The amplicon was sequenced and the consensus sequence deposited in GenBank (Accession No. MT176478). A BLAST analysis showed that the ITS sequence from NMQN06.18.12 shared 100% identity with that of *L. f.* var. *aleophilum* (Strain CBS 357.80; NR_111064). Phylogenetic analysis by the maximum likelihood method using RAxML software demonstrated that the isolate in this study grouped in a monophyletic clade with the ex-type strain of *L. f.* var. *aleophilum* (NR_111064) supported by a bootstrap value of 99% (Fig. 3). Isolate NMQN06.18.12 was identified as *L. f.* var. *aleophilum* based on ITS sequence analysis and morphological characteristics.

To fulfil Koch's postulates, isolate NMQN06.18.12 was used to inoculate fresh white-button mushrooms. Twenty milliliters of a spore suspension (10^5 spores per ml) of *L. f.* var. *aleophilum* was dropped onto each of three mushroom caps using a sterile pipette. The same volume of sterile water was used for controls. The white-button mushrooms were placed into an incubator at 25°C for three days and the experiment was repeated three times. Typical symptoms of sunken brown spots were observed three days post-inoculation and the same pathogen was re-isolated from symptomatic tissues. Control mushroom tissues remained healthy and no pathogens were isolated. To our knowledge, this is the first report of *L. f.* var. *aleophilum* causing brown spots on white-button mushrooms in Vietnam. This finding is vital to develop successful disease management strategies.

References

1. Atila F, Owaid MN, Shariati MA, 2017. The nutritional and medical benefits of *Agaricus bisporus*: a review. *Journal of Microbiology, Biotechnology and Food Sciences* **7**, 281-286. http://dx.doi.org/10.15414/jmbfs.2017/18.7.3.281-286
2. White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. San Diego, CA, USA: Academic Press, 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1
3. Zare R, Gams W, 2008. A revision of the *Verticillium fungicola* species complex and its affinity with the genus *Lecanicillium*. *Mycological Research* **112**, 811-824. http://dx.doi.org/10.1016/j.mycres.2008.01.019

Figure 1

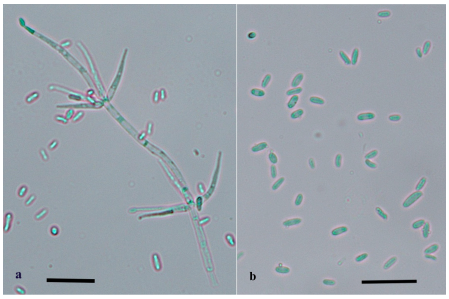

Figure 2

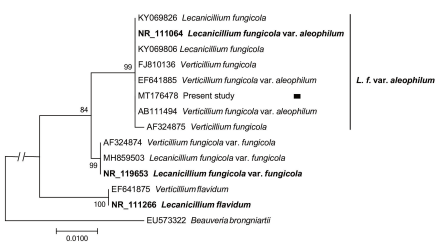

Figure 3

To cite this report: Thao LD, Binh VTP, Khanh TN, Thanh HM, Hien LT, Anh PT, Trang TTT, Dung NV, Hoai HT, 2020. First report of *Lecanicillium fungicola* var. *aleophilum* infecting white-button mushroom in Vietnam. *New Disease Reports* **41**, 23. http://dx.doi.org/10.5197/j.2044-0588.2020.041.023

 This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.