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Short Communication: Molecular identification of *Colletotrichum gloeosporioides* causing anthracnose on shallot in Bantul, Yogyakarta, Indonesia

LUTHFIANA M. SYAFITRI[1], ARIF WIBOWO[1], ANI WIDIASTUTI[1,♥], SITI SUBANDIYAH[1], STEPHEN HARPER[2]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Karang Malang, Caturtunggal, Depok, Sleman 55281, Yogyakarta, Indonesia. ♥email: aniwidiastuti@ugm.ac.id

[2]School of Agriculture and Food Sciences, Faculty of Science, The University of Queensland. St Lucia QLD 4072, Australia

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Abstract. *Syafitri LM, Wibowo A, Widiastuti A, Subandiyah S, Harper S. 2023. Molecular identification of* Colletotrichum gloeosporioides *causing anthracnose on shallot in Bantul, Yogyakarta, Indonesia. Biodiversitas 24: 4530-4534.* Anthracnose is one of the most common diseases in shallot cultivation which contributes to cause a significant production loss in Indonesia. Morphological identification of *C. gloeosporioides* causing anthracnose in Indonesia had been described, however there is no report on the molecular identification to confirm the pathogen species. This report is conducted to reveal species of *Colletotrichum* causing shallot anthracnose based on specific primer for special complex of *Colletotrichum*. The method used in this study was sample isolation, morphological observation and molecular identification. The symptomatic leaf sample was collected from Bantul, Yogyakarta, one of Indonesia's shallot productions, to be cultured on potato dextrose agar (PDA) medium. Morphological identification was carried out by using macroscopic and microscopic examinations. Molecular identification of the isolated fungi was amplified using specific primers for *Colletotrichum gloeosporioides* species complex, CgInt and ITS4. Based on molecular identification, the pathogens were identified as *C. gloeosporioides* Penz, which showed 98.07% percent identity with *C. gloeosporioides* AJ311884.1 isolate. This study elucidated that CgInt and ITS4 primers showed as a reliable primer set to be used for *Colletotrichum* species complex identification. The primer set was able to differ the conserved region among some *Colletotrichum* spp. Therefore, it is potential to be used for molecular identification. To our best knowledge, this is the first novel report on molecular identification of *C. gloeosporioides* causing anthracnose in shallot in Indonesia.

Keywords: CgInt, *Colletotrichum gloeosporioides* species complex, ITS4, specific primer

INTRODUCTION

Shallot (*Allium cepa* L. Aggregatum group), family of Alliaceae, is the second most widely cultivated horticultural crop in the world after tomatoes (FAOSTAT 2018). Shallot is renowned and most important vegetable spices in many Asian nations, they are consumed as a spice, medicinal herb, salad ingredient, and vegetables. Several shallot production centers in Indonesia are located in West Java, Central Java, Special Region of Yogyakarta, East Java, and Banten, contributing to 78.1% of the total national shallot production (Lawalata et al. 2015). Bantul Regency is one of the largest shallot production areas in Special Region of Yogyakarta, with 79,045 quintals of production and 770 ha of harvested area in 2016, with Kretek district being the largest harvested area and production in Bantul Regency (Statistics Indonesia of Yogyakarta 2018).

In 2022, the national shallot production was 1 982 360,00 tons, decreased from production in 2021 which reached 2 004 590,00 tons in 2019 (Statistics Indonesia 2023). Due to the existence of infectious diseases, the shallot production has not been able to keep up with the rising consumer demand. One of the main shallot diseases is anthracnose, which causes 80% to 100% of yield losses and causing the rise in market price. Dutta et al. (2022) reported that anthracnose disease was also main disease in Onion (*A. cepa* L.), which was caused by *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*). Based on the research, Lopes et al. (2021) noted that the species of fungal pathogens mostly belong to *C. gloeosporioides* species complex. This species complex is very famous for having wide range of host in horticultural crops and industrial tree plants (Hassan et al. 2018; Hattori et al. 2021). Anthracnose symptoms in onion mainly showed small, pale, sunken and water-soaked lesions. These lesions gradually develop into dark, elliptical-shaped lesions that cause chlorosis and necrotic areas along the leaf axis (Duta et al. 2022). CABI (2023) noted that in Indonesia, the disease was first reported in 1993 in Lembang, Bandung, West Java, especially during the rainy season reported in Onion Newsletter for the Tropics. Since then, the disease spread in many areas of central shallot in Indonesia, such as in South Kalimantan (Safitri et al. 2019), Aceh (Sarianti and Subandar 2022) and Yogyakarta (Budiarti et al. 2022). Currently, this also becomes a significant problem that many shallot farmers in Bantul are dealing with.

Besides being known as the causative agent of anthracnose, *C. gloeosporioides* is also known to cause leaf

spots with a risk of yield loss up to 100%, tuber deterioration, and bulb rot during the storage (Salunkhe et al. 2022). Sharma and Kulshrestha (2015) described the morphology and spores of *C. gloeosporioides*, which were irregular and appeared in the form of brown to black dots. Acervuli have various sizes, and release masses of conidia when ripe in humid conditions. Conidia are straight, cylindrical and oval. The size of the conidia varies from 11-16 x 4-6 m and 13.8 x 4.8 m. Colletotrichum's conidia are spread by wind or heavy rain (Penet et al. 2014). This fungus can also cause foliar twisting and curling symptoms when infecting the onion plants in complex infection with *Fusarium* spp., which is so called as anthracnose - twister disease complex (Perez and Alberto 2020; Dutta et al. 2022; Reecha et al. 2022). Morphological identification of *C. gloeosporioides* causing anthracnose in Indonesia has been reported (Budiarti et al. 2022; Safitri et al. 2019), however more accurate pathogen based on molecular identification is needed to comprehend the disease infection and management.

Colletotrichum gloeosporioides species complex on shallot has not been studied molecularly yet in Indonesia. The primer pair of CgInt and ITS4 has been reported in several publications for molecular identification of *C. gloeosporioides* species complex causing anthracnose in onion (Gyempeh et al. 2016), avocado (Silva-Rojas and Vila-Quezada 2011; Honger et al. 2016), apple (Oo et al. 2018), strawberry (Gan et al. 2017), fruit rot (Li and Zhang 2007) and mango (Silva Neto et al. 2022). Therefore, refore, this study aims to do molecular identification of anthracnose causing pathogen in Indonesia based on the primer set. This is the first and novel report on molecular identification of *Colletotrichum* causing shallot anthracnose in Indonesia based on CgInt and ITS4 primer set as a specific primer for *C. gloeosporioides* species complex.

MATERIALS AND METHODS

Fungal isolation from symptomatic shallot's leaves

Sample of shallot leaf showing the symptoms of anthracnose was taken from shallot plantation in Kretek district, Bantul Regency, Yogyakarta Special Province, Indonesia and brought to the laboratory for fungal isolation. Sample was washed with tap water to remove soil debris, then soaked in 0.5% sodium hypochlorite (NaOCl) for around 30 sec. The sample was then transferred to sterile distilled water for 30 sec, then dried on sterile filter paper. The leaves were cut into small pieces about 2-3 mm aseptically, and placed on potato dextrose agar (PDA) plates. The plates were stored at room temperature for 4-7 days. Mycelial tip from the growing fungi was sub-cultured to a new media as a pure culture.

Identification of fungal morphology

The macroscopic and microscopic properties of the seven-day isolate were used to undertake fungal morphology observations. The pattern and color of the fungal colony were characterized through macroscopic observation. While microscopy observations included hyphae and spore forms. The observations were conducted under a binocular microscope (Olympus X32). Preparation was made from the colony parts then put on an object glass with lactophenol cotton blue to make observation easier, followed by a cover glass.

Molecular identification

DNA extraction of fungal isolates was carried out using the Genomic DNA Mini Kit (Plant) (Geneaid, Taiwan). The mycelium and spores were ground using glass beads to destroy the tissue. The sample was carried out according to the Geneaid protocol. The DNA obtained was stored at -20ºC. DNA amplification was performed using specific primers of CgInt (forward): 5'-GGCCTCCCGCCTCCGGGCGG-3' and ITS 4 (reverse): 5'-TCCTCCGCTTATTGATATGC-3', with a fragment length of about 480 bp (Mills et al. 1992; Gyempeh et al. 2016; Patil et al. 2016) for *C. gloeosporioides* species complex. The genomic template was amplified with the BIORAD T100 Thermal Cycler PCR machine using a PCR program: Initial denaturation at 95ºC for 3 min. Thirty-four cycles of denaturation at 94ºC for 1 min, annealing at 52ºC for 30 sec, and elongation at 72ºC for 1 min. Final elongation at 72ºC for 5 min.

The PCR product was visualized with the PowerPac Universal BIORAD electrophoresis machine using 1% agarose gel with DNA florosafe staining at 100 V for 30 min. The DNA bands on the agarose gel were then observed using a High-Performance UV Transilluminator. The amplicon then sent to 1st BASE Pte Ltd Singapore for sequencing.

Phylogenetic tree analysis

The phylogenetic tree analysis was carried out using the MEGA X program (Kumar et al. 2018), using the Neighbor-Joining method with a bootstrap of 1,000x (Cruz-Lagunas et al. 2020).

RESULTS AND DISCUSSION

Morphological and microscopic observations of fungal isolate

Fungal isolate was obtained from shallot with anthracnose symptoms in Bantul, Yogyakarta. The symptoms were grey lesions on the edges of leaf and an orange mass of acervuli in the center (Figure 1). Perez and Alberto (2020), explained the symptoms of *C. gloeosporioides* infection in the field, starting with the appearance of a whitish lesion with an orange dot-like mass in the middle, the orange mass would then form concentric rings. In severe conditions, the leaves will dry, and the lesion can be obviously seen on bulb, leading to bulbs rotting and death of the whole plant (Dutta et al. 2022). The colony originally produced white to gray mycelia that darkened with the age (Figure 2). Reecha et al. (2022) also explained the same thing, that *C. gloeosporioides* colonies isolated from shallot plants were initially gray in color, which later became dark brown. Microscopic observation of fungal isolates showed that the fungi produce abundant conidia with oval elongated shape and size of 13 µm in length and

3.52-3.89 µm in width. *Colletotrichum gloeosporioides* group typically has one type of conidia with no septate, as it is shown in Figure 3.

Research conducted by Hekmawati et al. (2018) showed that *C. gloeosporioides* was able to infect various shallot varieties in Indonesia, including Tajuk, Bauji, Bima Curut, Bima Rajat, Bali Lancur, and Bali Karet. In addition, it causes a decrease in tuber weight. Study on shallot anthracnose globally was still limited, as not massive as study in onion anthracnose. Both plant species have the same name species (*Allium cepa*), although they are in different groups and mainly have different geographic centers of cultivation. Onion anthracnose is a devastating disease that reduces the quality and quantity of bulb production (Dutta et al. 2022; Reecha et al. 2022). Infected onion tubers have a lower content of protein, sugar, phenolic compounds (Ghangaonkar 2013; Alberto 2014), and antioxidants (Srivastava and Kumar 2013). This will be important prior knowledge for further study of shallot anthracnose.

Molecular analysis

Identification of *C. gloeosporioides* found in Indonesia is mainly based on morphological characters, which could lead to bias information as many species of *Colletotrichum* could have similar morphology. Therefore, in this research, *C. gloeosporioides* species complex was detected molecularly from sample isolated in Bantul shallot plantation, Yogyakarta.

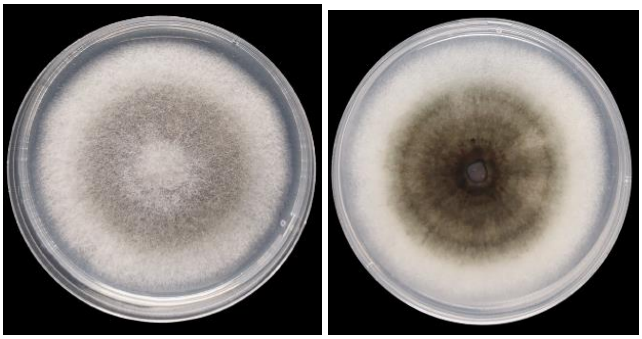
Amplification was performed using specific primers, CgInt and ITS4, with a fragment length of 480 bp (Mills et al. 1992), as shown in the figure below (Figure 4). Amplification using specific primers, CgInt and ITS4 was confirmed to be used in C. *gloeosporioides* species complex detection (Gyempeh et al. 2016). The CgInt primer, an oligonucleotide primer, was synthesized from the Internal Transcribed Spacer (ITS) 1 region of ribosomal DNA (rDNA) (Mills et al. 1992), combined with the ITS 4 primer, a conserved sequence of rDNA (Liu et al. 2012).

DNA sequence analysis

The results of sequence analysis using NCBI BLAST showed that the fungal isolate had the greatest similarity 98.07% with the C. *gloeosporioides* (Table 1). The percent identity indicates that *C. gloeosporioides* is the same species or closely related to the tested fungal isolate, *Colletotrichum* SF22, based on amplification results with specific primers CgInt and ITS4 for *C. gloeosporioides* species complex. The set of primer has been studied in many reports to identify the species in a clade of *C. gloeosporioides* species complex (Mills et al. 1992; Gyempeh et al. 2016).

The alignment result of *Colletotrichum SF22* in Table 1 is confirmed by Phylogenetic tree analysis (Figure 5). This analysis is used for observing the phylogenetic relationships which are generally described in a branching line using molecular data such as DNA or proteins (Zhang et al. 2018). The results of phylogenetic tree reconstruction showed that *Colletotrichum* SF22 had a closer kinship with *Colletotrichum gloeosporioides* (AJ311884.1 and AB710144.1) compared to *C. boninense* isolate LH49, *C. costaricense* isolate RB184, *C. scovillei* LD242, *C. acutatum* voucher Rbf-26, while *Botrytis aclada* strain OnionBC-18 was used as an outgroup (Figure 5).

Figure 1. Symptom of anthracnose on shallot leaf in Bantul, Yogyakarta. The center of lesion is covered with orange mass of spores



A **B**

Figure 2. Morphological characteristics of fungal isolate from shallot leaf with anthracnose symptoms collected from the field. Fungal isolate had a white mycelium that darkened in the center over time. Mycelial growth after culturing for 7 days covering 7.5 cm of Petri plate. (A) Front view; (B) Back view

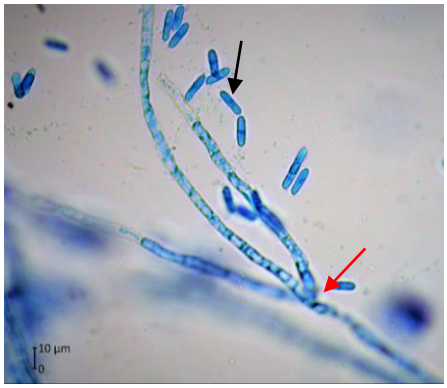


Figure 3. Microscopic observation of fungal isolates using a microscope with a magnification of 400x. a Black arrow: conidia about 13 µm in length and 3.52-3.89 µm in width; Red arrow: Cellular and branched hyphae

Taken together from sequence alignment and phylogenetic tree analysis, it is confirmed that *Colletotrichum* SF22 isolated from anthracnose shallot in Bantul is *C. gloeosporioides.* Based on result on this study, a set primer of CgInt and ITS4 showed reliable to be used for *Colletotrichum* species complex identification. The sequencing results showed that the aligned sequences could also hit different species complex of *Colletotrichum*, such as *C. boninense* (*C. boninense* species complex*), C. scovillei* (*C. scovillei* species complex), *C. acutatum (C. acutatum* species complex*)* in different percent identity. Moreover, it is also confirmed by the phylogeny tree that those species are obviously separated in different branches line, which showed that *C. gloeosporioides* is more closely related to *C. boninense*, compared to *C. scovillei*, *C. acutatum*, and *C. costaricense. Colletotrichum* spp. is a big family of fungal genera, and CgInt and ITS4 primer set showed to be able to differ the conserved region among some *Colletotrichum* spp., therefore it is possible to be chosen for molecular identification.

Colletotrichum gloeosporioides is often reported to cause shallot's disease in several production centers, such as in Java; Bantul (Budiarti et al. 2022), Aceh (Sarianti and Subandar 2022) and South Kalimantan (Safitri et al. 2019) but none for the molecular identification. The specific primer set (CgInt and ITS4) is a reliable primer to be considered in studying *C. gloeosporioides* species complex molecularly so that disease management could be developed more accurately.

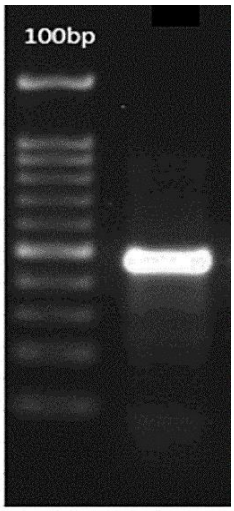


Figure 4. Visualization of the PCR product of fungal isolates isolated from shallot with anthracnose symptoms using specific primers CgInt and ITS4 for *C. gloeosporioides* species complex with target fragment length at 480 bp

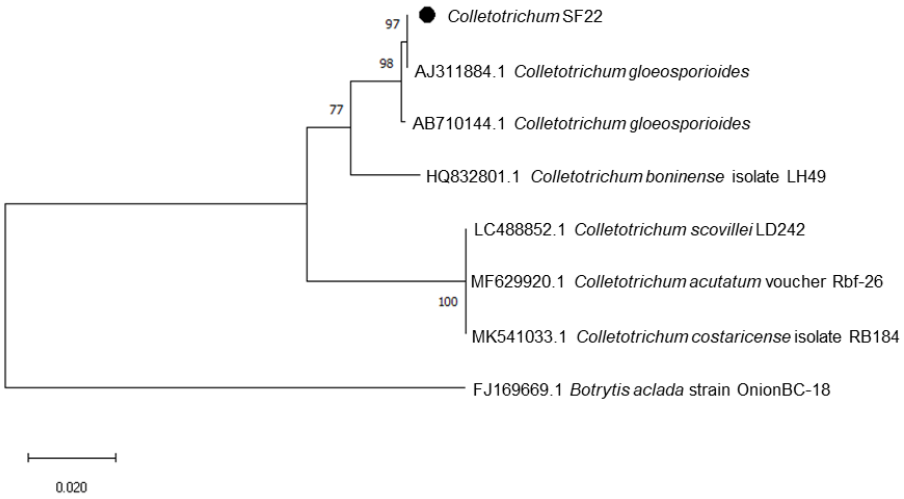


Figure 5. Phylogenetic tree *Colletotrichum* SF22 isolate using Neighbor-Joining Tree method with a bootstrap of 1,000x

Table 1. Sequence analysis results using the BLAST-N program

Isolate	Accession Number	Relationship nearby fungi	Percent Identity
Colletotrichum SF22	AJ311884.1	*Colletotrichum gloeosporioides*	98.07%
	AB710144.1	*Colletotrichum gloeosporioides*	97.86%
	HQ832801.1	*Colletotrichum boninense* isolate LH49	95.94%
	MK541033.1	*Colletotrichum costaricense* isolate RB184	93.21%
	LC488852.1	*Colletotrichum scovillei* LD242	92.60%
	MF629920.1	*Colletotrichum acutatum* voucher Rbf-26	92.60%
	FJ169669.1	Botrytis aclada strain OnionBC-18	82.38%

In conclusion, the present study revealed that shallot anthracnose in Bantul, Yogyakarta was caused by *C. gloeosporioides*, based on specific primers on *C. gloeosporioides* species complex (CgInt and ITS4). This finding confirmed previous reports on morphological analysis of *Colletotrichum* causing shallot anthracnose, furthermore, this is the first report on molecular identification of *C. gloeosporioides* on shallot in Indonesia. This report is basic important data for further comprehensive research on shallot anthracnose in Indonesia caused by *C. gloeosporioides*. Further research in molecular identification and genetic diversity of *Colletotrichum* spp. causing shallot anthracnose and its pathogenesis is significant to contribute extensive approach in managing shallot plant health to gain sustainable increased production.

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REFERENCES

Alberto RT. 2014. Pathological response and biochemical changes in *Allium cepa* L. (bulb onions) infected with anthracnose-twister disease. Plant Pathol Quarantine 4 (1): 23-31. DOI: 10.5943/ppq/4/1/4.

Budiarti SB, Cahyaningrum H, Nugroho MAS. 2022. Disease inventory of shallots (*Allium ascalonicum* L.) variety of lokananta from seed (true shallot seed). AgriHealth: J Agrifood Nutr Public Health 3 (2): 143-153. DOI: 10.20961/agrihealth.v3i2.64617. [Indonesian]

CABI. 2023. Anthracnose on shallot (*Allium cepa* group aggregatum) in Java. https://www.cabdirect.org/cabdirect/abstract/19942303386.

Cruz-Lagunas B, Ortega-Acosta SÁ, Reyes-García G, Toribio-Jiménez J, Juárez-López P, Guillén-Sánchez D, Damián-Nava A, Romero-Ramírez Y, Palemón-Alberto F. 2020. *Colletotrichum gloeosporioides* causes anthracnose on grapefruit (*Citrus paradisi*) in Mexico. Australas Plant Dis Notes 15: 31. DOI: 10.1007/s13314-020-00401-z.

Dutta RKJ, Nadig SM, Manjunathagowda DC, Gurav VS, Singh M. 2022. Anthracnose of onion (*Allium cepa* L.): A twister disease. Pathogens 11: 884. DOI: 10.3390/pathogens11080884.

FAOSTAT. 2018. Food and Agriculture Organization Corporate Statistical Database. [Accessed August 17, 2022].

Gan P, Nakata N, Suzuki T, Shirasu T. 2017. Markers to differentiate species of anthracnose fungi identify *Colletotrichum fructicola* as the predominant virulent species in strawberry plants in Chiba Prefecture of Japan. J Gen Plant Pathol 83: 14-22. DOI: 10.1007/s10327-016-0689-0.

Ghangaonkar NM. 2013. Studies on biochemical changes in infected onion bulbs. J Sci 2 (1): 7-8.

Gyempeh N, Offei SK, Cornelius EW, Honger JO. 2016. Importance of the onion leaf twister disease in Ghana and the effect of *Trichoderma asperellium* on the mycelial growth and sporulation on the causal agent. Ghana J Sci 55: 51-65.

Hassan O, Jeon, JY, Chang T, Shin JS, Oh NK, Lee YS. 2018. Molecular and morphological characterization of *Colletotrichum* Species in the *Colletotrichum gloeosporioides* Complex associated with persimmon anthracnose in South Korea. Plant Dis 102 (5): 1015-1024 DOI: 10.1094/PDIS-10-17-1564-RE.

Hattori Y, Nakashima C, Kitabata S, Naito K, Hieno A, Alvarez LV, Motohashi K. 2021. Identification of the *Colletotrichum* species associated with mango diseases and a universal LAMP detection method for C. gloeosporioides species complex. Plant Fungal Res 4 (1): 2-13. DOI: 10.30546/2664-5297.2021.1.49.

Hekmawati, Poromarto SH, Widono S. 2018. Resistance of some shallot varieties against *Colletotrichum gloeosporioides*. Agrosains 20 (2): 40-44. DOI: 10.20961/agsjpa.v20i2.26342. [Indonesian]

Honger JO, Offei SK, Oduro KA, Odamtten GT, Nyaku ST. 2016. Identification and molecular characterisation of *Colletotrichum* species from avocado, citrus and pawpaw in Ghana. South Afr J Plant Soil 33 (3): 177-185. DOI: 10.1080/02571862.2015.1125958.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547-1549. DOI: 10.1093/molbev/msy096.

Lawalata M, Darwanto DH, Hartono S. 2015. Relative efficiency of red onion farming in Bantul Regency with data envelopment analysis (DEA) Approach. Ilmu Pertanian 18 (1): 1-8. DOI: 10.22146/ipas.6169. [Indonesian]

Liu B, Louws FJ, Sutton TB, Correl LJ. 2012. A Rapid qualitative molecular method for the identification of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Eur J Plant Pathol 132: 593-607. DOI: 10.1007/s10658-011-9904-1.

Lopes LH, Boiteux LS, Rossato M, Aguiar FM, Fonseca ME, Oliveira VR, Reis A. 2021. Diversity of *Colletotrichum* species causing onion anthracnose in Brazil. Eur J Plant Pathol 159: 339-357. DOI: 10.1007/s10658-020-02166-8.

Mills PR, Sreenivasaprasad S, Brown AE. 1992. Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. FEMS Microbiol Lett 98 (1-3): 137-144. DOI: 10.1111/j.1574-6968.1992.tb05503.x.

Oo MM, Yoon HY, Jang HA Oh SK. 2018. Identification and characterization of *Colletotrichum* species associated with bitter rot disease of apple in South Korea. Plant Pathol J 34 (6): 480-489. DOI: 10.5423/PPJ.FT.10.2018.0201.

Penet L, Guyader S, Pétro D, Salles M, Bussière F. 2014. Direct splash dispersal prevails over indirect and subsequent spread during rains in *Colletotrichum gloeosporioides* infecting yams. PLoS One 9(12): e115757. DOI: 10.1371/journal.pone.0115757.

Perez PM, Alberto RT. 2020. Chemical management of anthracnose-twister (*Colletotrichum gloeosporioides* and *Fusarium fujikuroi*) disease of Onion (*Allium cepa*). Plant Pathol Quarantine 10: 198-216. DOI: 10.5943/ppq/10/1/19.

Reecha J, Thiruvudainambi S, Mareeswari P, Oviya R, Vellaikumar S. 2022. Morphological characterization of *Colletotrichum gloeosporioides* causing leaf twister blight disease in *Allium cepa* (onion). Pharm Innov J 11 (7): 26-30.

Salunkhe VN, Gedam P, Pradhan A, Gaikwad B, Kale R, Gawande S. 2022. Concurrent waterlogging and anthracnose-twister disease in rainy-season onions (*Allium cepa*): Impact and management. Front Microbiol 13: 1063472. DOI: 10.3389/fmicb.2022.1063472.

Safitri YA, Hasanah U, Salamiah, Samharianto, Pramudi MI. 2019. Distribution of major diseases of shallot in South Kalimantan, Indonesia. Asian J Agric 3 (2): 33-40. DOI: 10.13057/asianjagric/g030201.

Sarianti, Subandar I. 2022. The incidence and severity of anthrachnose disease in onion Plant in Kampong Tanah Bara, Gunung Meriah District, Aceh Singkil Regency. J Pertanian Agros 24 (1): 202-210.

Sharma M, Kulshrestha S. 2015. *Colletotrichum gloeosporioides*: An anthracnose causing pathogen of fruits and vegetables. Biosci Biotechnol Res Asia 12 (2): 1233-1246. DOI: 10.13005/bbra/1776.

Silva Neto JAD, Ambrósio MMDQ, Araújo MBM, Silva RMD, Pinto PSL, Holanda ISA. 2022. Morphological, molecular and pathogenic characterization of *Colletotrichum gloeosporioides* isolated from mango. Agron Rev Caatinga 35 (3): 514-27. DOI: 10.1590/1983-21252022V35N302RC.

Silva-Rojas HV, Ávila-Quezada GD. 2011. Phylogenetic and morphological identification of *Colletotrichum boninense*: a novel causal agent of anthracnose in avocado. Plant Pathol 60 (5): 899-908. DOI: 10.1111/j.1365-3059.2011.02452.x.

Statistics Indonesia of Yogyakarta. 2018. Kretek Subdistrict in Figures 2018. https://bantulkab.bps.go.id/publication/2018/09/26/1cf9dd95fe200fee1274e1ec/kecamatan-kretek-dalam-angka-2018.html. [Indonesia]

Statistics Indonesia. 2023. Production of Vegetables 2022. https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.html. [Indonesia]

Srivastava A, Kumar S. 2013. Biochemical changes in post harvested *Allium cepa* (Onion) and *Capsicum annuum* (Capsicum) under the influence of pathogens. IOSR J Agric Vet Sci 5 (4): 18-21. DOI: 10.9790/2380-0541821.

Zhang D, Kan X, Huss SE, Jiang L, Chen LQ, Hu Y. 2018. Using phylogenetic analysis to investigate eukaryotic gene origin. J Vis Exp 138: 56684. DOI: 10.3791/56684.