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MORPHOLOGICAL IDENTIFICATION OF FUNGAL PATHOGENS ASSOCIATED WITH POST-HARVEST DISEASES

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ABSTRACT
Post-harvest products are perishable and vulnerable to diseases that lead to quality deterioration and yield loss. One of the primary diseases found in most post-harvest products is caused by fungal pathogens. This study identified fungal pathogens associated with post-harvest products through morphological characterization. Fruit and vegetable samples were collected from traditional markets and fruit stores in Central Java. The results of fungal pathogens identification causing disease on post-harvest products showed that *Pestalotiopsis* sp and *Neopestalotiopsis* sp. were found on guava with white blackish mycelium, present concentric ring and blackspot, *Aspergillus* sp. on tomato with yellow-greenish mycelium, present concentric ring only, *Botrytis* sp. with grey mycelium and *Rhizoctonia solani* with white greyish mycelium and present of a concentric ring on apple, *Rhizoctonia solani* with greyish black and present of blackspot on mango, and *Colletotrichum* sp. with white greyish mycelium and present of a concentric ring, conidiomata and blackspot on citrus. This study concluded that the most fungal pathogens on post-harvest that we found were *Pestaloptia* sp., *Rhizoctonia solani, Aspergillus* sp., *Botrytis* sp., and *Colletotrichum* sp.

Keywords:, fungal pathogens, horticultural commodities, identification, post-harvest diseases.

INTRODUCTION
Horticultural products, such as fruits and vegetables, play a significant role in Indonesia's economy. However, most of the production of horticultural commodities has decreased from 2021 to 2022 (Statistics Indonesia, 2023). Since post-harvest products are perishable, the diseases may occur during handling, from harvesting to consumption (Zhang *et al*., 2019). Losses in yield caused by post-harvest diseases are more significant than generally realized because the value of fresh fruits increases severalfold while passing from the field to the consumers, reaching 30-50% of the total production. It is crucial to consider fruit quantity and quality reductions when assessing post-harvest disease losses because post-harvest diseases may not make a fruit unsalable but still lower its value. (Strano *et al*., 2022). One of the main factors causing yield loss and decreased product quality was causing pathogens threat

damage. Post-harvest pathological damage to horticultural products is damage to organs, tissues, cells, and fluids due to pathogen infections supported by environmental conditions in storage (Zhang *et al*., 2019).

Fruits are threatened by different microorganisms, such as fungi, bacteria, and, to a lesser extent, viruses, which are the agents of various diseases. Most losses of fruit diseases of commercial importance result from pre- or post-harvest infections with fungal pathogens because fungal highly adaptive characteristic allows them to grow and develop under storage conditions (Pétriacq *et al*., 2018). Post-harvest fungal decay results mainly from preharvest latent infection or wound infection that can occur before or after harvest (Wenneker & Thomma, 2020). Development of symptom expression would show during storage. Most fungal pathogens post-harvest are able to infect fruits through natural openings such as lenticel, stomata, and wounded fruits (Wenneker, 2019).

Major fungi threatening post-harvest products are molds, mildews, and rots that have the capacity to infect a wide range of plant species (Pétriacq *et al*., 2018). However, it is well-known fungal pathogens causing post-harvest losses are *Colletotrichum* spp., *Aspergillus* spp., *Botrytis* sp., *Rhizoctonia solani*, *Alternaria*, *Diplodia*, *Monilinia*, *Penicillium*, *Phomopsis, Rhizopus, Mucor*, and *Sclerotini* (Ewekeye & Odebode, 2021; Singh & Sharma, 2018; Zhafarina *et al*., 2021; Wani & Wani, 2011; Singh *et al*., 2018).

Many threats challenge production at a post-harvest level. Some of these pathogens, even when causing their most considerable damage after fruit harvesting, are known to be already present in the plants during cultivation. Some post-harvest commodities, such as tomato (*Lycopersicum esculentum*), guava (*Psidium guayava*), citrus (*Citrus sinensis* L), apple (*Malus domestica*), and mango (*Mangifera indica*), are potential horticultural commodities in Indonesia. However, if post-harvest diseases infest, it will reduce the quality and quantity of these commodities and subsequently will be unfit for consumption. The importance of identification accurately reflects fungi's unique morphological and functional traits. Image-based identification is a common method for identifying an object based on its attributes. The most often utilized features in identification based on a microbiological picture are morphological and textural characteristics (Pramesti *et al*., 2022). Therefore, it was necessary to carry out research to identify fungal pathogens associated with post-harvest products through morphological characterization in some horticultural commodities such as mangoes, guava, apple, citrus, and tomato. It can be an early warning system to reduce or eliminate the effects of plant diseases and biological invasions.

MATERIAL AND METHODS

1. Sample Collection

Fruits and vegetable samples were using purposive sampling with blackspot and rot symptoms criteria that were collected from Central Java, Indonesia.

2. Isolation of fungal associated with post-harvest diseases

Fruits and vegetable samples with blackspot and rot symptoms were disinfected by rubbing the surface with 70% alcohol. A 1 cm × 1 cm section between the healthy and affected areas was cut and placed on a Petri dish containing Potato Dextrose Agar (PDA) medium. The cultures were then incubated at room temperature for 4-7 days. The isolation of fungi followed the research of Zhafarina *et al.* (2021).

3. **Purification of fungal isolates with single-spore isolation**

Suspension of fungal isolates were prepared by cutting 1 cm × 1 cm of 7 days culture on PDA medium. The culture was then added to 500 µL sterile water in a 1.5 mL tube. The suspension was homogenized by the vortex. The suspension was then scratched on a new PDA medium and incubated at room temperature for 1-2 days. The germinated conidium was taken under a microscope using a sterile needle and replaced to a new PDA medium. Incubation was conducted at room temperature. The single spore isolation of fungi followed the research of Zhafarina *et al.* (2021).

4. **Morphological analysis**

Morphological identification of fungal isolates was carried out based on macroscopic and microscopic characteristics and with the help of the identification key of mycology. Pure cultures were identified by visual examinations (macroscopic) and observed under a light microscope (microscopic). The pathogens were identified based on their cultural and microscopic morphological characters. The culture of morphological characters were analyzed through their colony's color, including aerial and reverse views, texture, concentric ring, conidiomata, blackspot, and growth rate. The observation of macroscopic was recorded after 14 days of incubation. The growth rate was calculated as the 14-day average of mean daily growth (mm per day). A fast growth rate grew within one week, and a slow one may require a longer incubation time (Fodnes, 2022). Small PDA pieces (2 × 2 × 2 mm) were squeezed on object glass with cover glass for the microscopic study. The microscopic was observed under an optical microscope with a digital camera at 40x and 100x magnification (OLYMPUS CX23 Biological Microscope, Optilab PT. Miconos.

RESULTS AND DISCUSSIONS

The result of the sample collection is shown in Table 1. The fungal pathogens are well known to affect fruit and vegetable production quality and quantity. Different levels of degradation can occur depending on the pathogens that attack the commodities. Both fungal pathogens and post-harvest products were living tissues, and for fungi to infiltrate these tissues, they must get past the numerous defenses that plants have developed over time to ward against them. This indicates that a particular commodity and the fungal species that have surmounted these obstacles to cause overt spoiling are distinct to one another. Post-harvest spoilage of Amla fruits is one of the major problems that reduces the economic quality of the fruits. The study was to identify the post-harvest fungal pathogens associated with fruit and vegetables, which were common based on morphological characteristics to distinguish the differences (Sultana *et al.,* 2023). The result of the identification of post-harvest commodities is shown in Table 2.

1. ***Pestaloptiosis* sp. and *Neopestaloptiosis* sp. on Guava**

Pestalotiopsis sp. and *Neopestalotiopsis* sp. cause symptoms of dry rot on crystal guava fruit, which are sunken brownish and blackish spots (**Fig. 1-A**)**.** The symptoms were caused by *Pestalotiopsis* sp. and *Neopestalotiopsis* sp. The spot was darkened in color and expanded to discrete, circular, and dark brown into black spots. Additionally, the lesion formed a scabbed appearance. The symptoms were in line with the state of Solarte *et al*. (2018), who stated that the first visible symptoms of guava scab are small brown (coffee-bean colored) spots that develop into corky scabs on fruit surfaces. The scabs develop heads that resemble oxidized nails. Large lesions are created when the little lesions combine to cover a portion of the fruit, sink into the flesh, and ultimately distort the entire fruit.

Macroscopic characteristics of isolates *Pestalotiopsis* (G1) sp. and *Neopestalotiopsis* sp. (G2) are shown in **Fig.1-B.** The characteristic of the G1 isolate was a white-blackish mycelium with a granular colony texture and a formed concentric ring with a black spot. Also, the G2 isolate had white-blackish mycelium with a velvety colony texture and formed a concentric ring, but it was not as clear as the G1 isolate. Both G1 and G2 isolates performed at a fast growth rate (**Table 2**).

Meanwhile, microscopic characteristics of *Pestalotiopsis* sp. and *Neopestalotiopsis* sp. were indicated by their fusiform conidia with straight or curved cell edges and flagellum at both ends. Setula was the flagellum in the apical cell, while the pedicel was the flagellum in the basal cell. The median cells were yellow to brown, while the apical and basal were hyaline (Herliyana *et al*., 2022). The difference in conidia between *Pestalotiopsis* sp. and *Neopestalotiosis* sp. is that the typical conidia of *Pestalotiopsis* sp. show concolorous median cells while *Neopestalotiopsis* sp. shows versicolored median cells (Solarte *et al*., 2018).

2. **Aspergillus sp. on tomato**

Fungal associated with the symptoms of tomato was *Aspergillus* sp. According to Kator *et al*. (2018), *Aspergillus* sp. was reported as a fungal associated with diseases on tomatoes and living as a saprophyte on decaying fruit. The symptom in this observation was dry brown spots, shown in **Fig.2-A.** The isolate was yellow-greenish in aerial view and yellow-flattering in reverse view. Its powdery colony texture formed a concentric ring (**Fig.2-B**). The surface color of the colony was greyish green with all the margins, and hyaline was found on the reverse. The colony growth is moderate to rapid (Kator *et al*., (2018).

The microscopic of *Aspergillus* sp. was specific, as shown in **Fig.2-C.** The conidiophores originated from the basal foot cell on the supporting hyphae and terminated in a vesicle apex. The conidiophore was upright, straightforward, long, hyaline, and terminating in a globose or clavate swelling (Barnett & Hunter, 1995). The genus *Aspergillus* had a typical formation called vesical. Phialides were hyaline, flask-shaped, biseriate, and attached to the vesicle via a supporting cell metula. Conidia, which had a round shape, was located over the phialides.

3. **Botrytis sp. and Rhizoctonia solani. on Apple**

The rotting fruit on the apple indicated the symptoms. The rotting fruit was brown spot and pale-mid brown (**Fig.3-A**). The symptoms were caused by *Botrytis* sp., with specific symptoms of gray mold. The early stage of gray mold on fruit-to-fruit spreading resulted in the necrotic brown spot (**Fig.3-A**). Based on the same symptoms, cultural, and morphological appearance shown in **Fig.3,** *R. solani* was responsible for causing *Rhizoctonia* brown rot on apples (Wani & Wani, 2011). Affected tissues became soft, dark brown, or black, which might ultimately become completely girdled and collapsed.

Macroscopic characteristics of isolates of *Botrytis* sp. (AGS-1) and *R. solani* (AGS-2) are shown in **Fig. 3-B.** *Botrytis* sp. isolates showed grey mycelium on aerial view and black on reverse view with cottony on colony texture. Seven days after incubation, the white color of mycelium changed into grey. On day 7, the mycelium grew entirely on PDA and the growth rate was included in the fast group. On the other hand, *R. solani* isolates performed white greyish on aerial view and white greyish flat on reverse view with velvety on colony texture. The growth rate was fast. This finding was similar to Wani and Wani (2011), who stated that *R. solani* produced light grey mycelium on PDA medium, which turned into a dark grey color with age.

Microscopic appearances of AGS-1 and AGS-2 isolates are shown in **Fig.3-C-D.** The AGS-1 isolate was considered to have the same characteristics as *Botrytis* sp. According to Barnett and Hunter (1995), this isolate had long, slender, often pigmented conidiophores with septate hyphae. Based on the result, the conidia were globose near the apex of the conidiophore, as obtained by Komalaningrat *et al*. (2018). AGS-2 isolate was *R. solani*, which had typical hyphae forming right-angle branching (**Fig.3-C**). Swollen hyphae formed small sclerotia; its color was brown to black variable in form, with septate hyphae.

4. **Rhizoctonia solani on Mango**

R. solani was found on the mango with fruit rot symptoms. Characteristics of mango that was infected by *R. solani* showed brown to black spots on the surface of the fruits. Pratiwi *et al*. (2016) reported small to large necrotic and slightly concave in the surface of the fruits. The symptoms of mango infected by *R. solani* (**Fig.4-A**) were indicated by brown to black necrotic spots, which enlarged into fruit rotting.

The mycelium of the isolate in the PDA medium appeared white, later turned greyish, produced long thread-like hyphae, and showed fast and abundant growth, which attained 90mm within four days after incubation. The color of the mycelium (**Fig.4-B**) was greyish black on aerial view and black flat on reverse view with cottony on colony texture. Sclerotia was formed during the observation 14 days after incubation. The sclerotia had a greenish color that spread randomly on Petri dish culture.

Typical hyphae formed from the isolate (**Fig.4-C**)**.** The margin of the colony was circular and smooth, with the hyaline hyphae gradually turning brown and branching at 90° below the septa with distinct constrictions (Vijayaraghavan, 2019). These findings were aligned with our observation that the hyphae were hyaline and septate and had typical hyphae form right-angle branching. Based on these characteristics, the isolate was identified as *R. solani*.

5. **Colletotrichum sp. on citrus**

According to Zhafarina *et al*. (2021), the symptoms found on citrus were necrotic, light brown to dark lesion sunken areas. Based on the observation, the symptoms of affected citrus were light brown to dark brown spots that kept enlarging. There was a sunken lesion on the citrus. The centers of the slightly larger spots were cracked, exposing the flesh of the fruit.

ORG isolated from citrus was shown white greyish on aerial view and greenish grey flat on reverse view with velvety conidia texture. The conidiomata were formed randomly with a black color. The ORG isolate had concentric rings and black spots on the reverse view. From the macroscopic characteristics, the isolate belonged to the type of *Colletotrichum* sp. *Colletotrichum* was known to have many species, and the taxonomic of the genus Colletotrichum changed frequently, so there would be many appearance characteristics of *Colletotrichum* sp.

Conidia produced by the isolate was cylindrical with two ends acute or one end slightly obtuse and rounded on both ends (**Fig. 5-C**)**.** The conidia produced by the isolate were identical to the genus *Colletotrichum*. The genus *Colletotrichum* had acervuli disc-shaped or cushion-shaped and waxy. Its conidiophore was simple, closely grouped, or compacted, arising from a stroma-like base, sometimes almost appearing as a sporodochium. The conidia were hyaline (Barnett & Hunter, 1995). Based on these cultural and morphological characteristics, the isolate was identified as *Colletotrichum* sp*.*

For genus-level identification, morphology-based identification is a valuable technique, and molecular data are necessary to identify species level accurately. Accurate identification of these infections is essential for developing effective control strategies since the scientific name of a fungal pathogen connects the knowledge about the species, including its biology, host range, distribution, and possible risk (Bhunjun *et al*., 2021). Molecular data is necessary for the following research to determine the specific species of fungal pathogens associated with post-harvest products.

CONCLUSION

The results of the identification of fungal pathogens associated with several post-harvest products using morphological analysis methods based on macroscopic and microscopic appearance showed that the fungus *Pestalotiopsis* sp and *Neopestalotiopsis* sp. were found on guava with white blackish mycelium, present concentric ring, and blackspot, Aspergillus sp. on tomato with yellow-greenish mycelium, present concentric ring only, Botrytis sp. with grey mycelium and Rhizoctonia solani with white greyish mycelium and present of the concentric ring on apple, Rhizoctonia solani with greyish black and present of blackspot on mango, and Colletotrichum sp. with white greyish mycelium and present of concentric ring, conidiomata and blackspot on citrus.

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Table 1. The origin of fruit and vegetable samples

Commodities	Origin
Guava	Field Fruit in Wonogiri, Central Java
Tomato	Traditional Market in Klaten, Central Java
Mango	Fruit Store in Klaten, Central Java
Citrus	Fruit Store in Klaten, Central Java
Apple	Fruit Store in Klaten, Central Java

Table 2. Morphological Characteristics of Fungal Isolates Associated with Post Harvest Products.

No	**Isolates**	**Morphological Characterization**						**Growth Rate**
		Aerial Views	**Reverse Views**	**Colony Texture**	**Concentric Ring**	**Conidiomata**	**Black Spot**	
1	G-1	white blackish	white yellowish flat	granular	v	-	v	fast
2	G-2	white blackish	whiteyellownish flat	velvety	v	-	v	fast
3	TMT-1	yellow-greenish	yellow flat	powdery	v	-	-	fast
4	AGS-1	grey	black	cottony	-	-	-	fast
5	AGS-2	whitegreyish	whitegreyish flat	velvety	v	-	-	fast
6	MIDR	greyish black	black flat	cottony	-	-	v	fast
7	ORG	white greyish	greenish grey flat	velvety	v	v	v	fast

Additional information : G-1; guava isolate-1, G-2; guava isolates-2, TMT-1; tomato isolate, AGS-1; apple isolate-1, AGS-2; apple isolate-2, MIDR; mango isolate, ORG; citurs isolate.

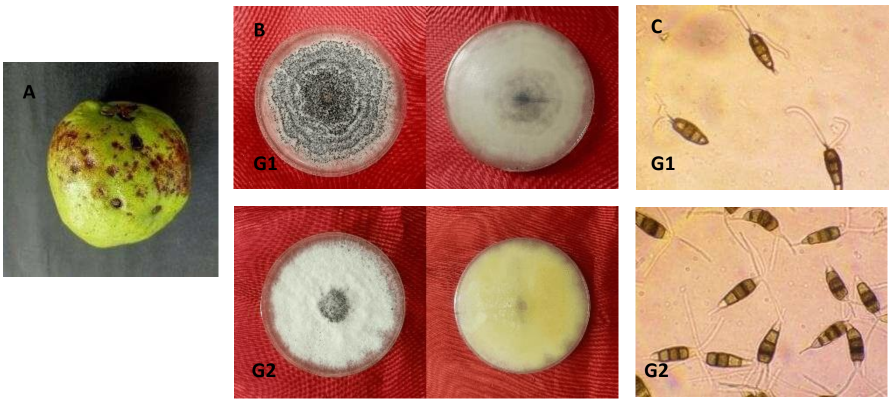

Figure 1. Symptoms of necrotic black spot on guava (A), Macroscopic of *Pestaloptiosis* sp.(B; G1) *Neopestaloptiosis* sp. (B; G2), Conidia of *Pestaloptia* sp. at 100x magnification (C;G1) *Neopestaloptiosis* sp. at 100x magnification(C; G2)

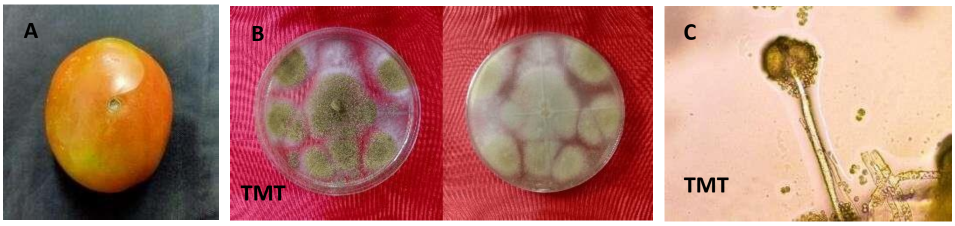

Figure 2. Symptoms of brown spot on tomato (A), Macroscopic of *Aspergillus* sp. on PDA (B), Conidiophore of *Aspergillus* sp. at 100x magnification (C)

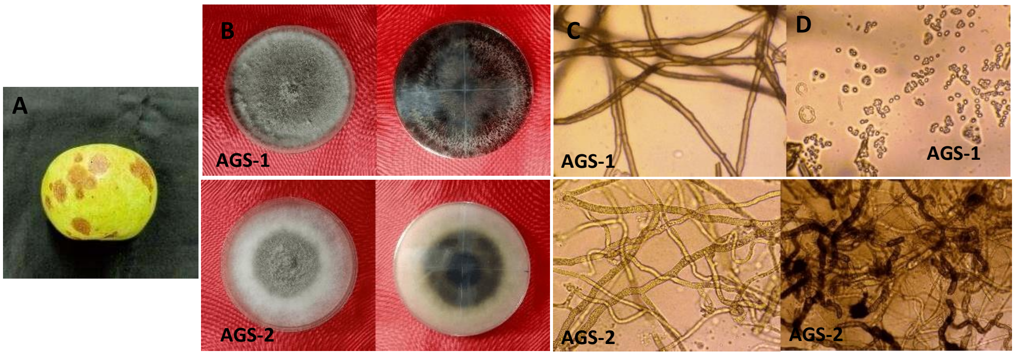

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Figure 3. Symptoms of brown spot on apple (A), Macroscopic of *Botrytis* sp. (causing grey mold) (B; AGS-1) *Rhizoctonia* sp. (B; AGS-2) on PDA, Hyphae of *Botrytis* sp. at 100x magnification(C; AGS-1) *Rhizoctonia* sp. at 40x magnification (C; AGS-2), Conidia of *Botrytis* sp. at 40x magnification (D; AGS-1), Sclerotia appearance of *Rhizoctonia* sp. at 40x magnification (D; AGS-2)

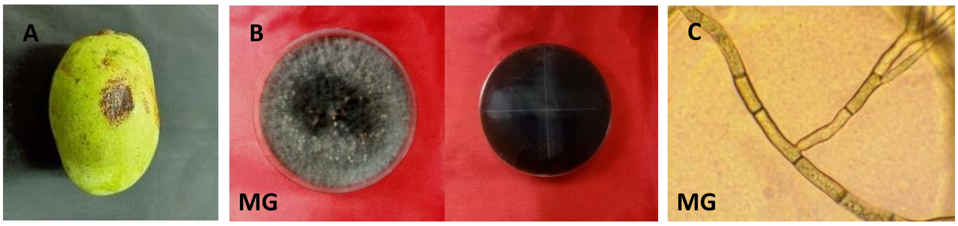

Figure 4. Symptoms of brown spot on mango (A), Macroscopic of *Rhizoctonia solani* on PDA with sclerotium (B), Typical hyphae of *Rhizoctonia solani* at 100x magnification (C)

Figure 5. Symptoms of brown spot on citrus (A), Macroscopic of *Colletotrichum* sp. on PDA with conidiomata (B), Conidia of *Colletotrichum* sp. at 100x magnification(C)