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CHARACTERIZATION OF QUARANTINE PLANT PEST IN IMPORTED POTATO SEEDS AND ITS IMPLICATIONS FOR QUARANTINE MEASURES

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Abstract
The high accessibility of potato imports increases the risk of carrying quarantine plant pest organisms. Characterization of pathogenic fungi on potato seedlings is not only identified visually. Several tests need to be carried out to get accurate results. The blotter test method has become the standard for testing seeds for the Animal, Fish, and Plant Quarantine Center because it is practical, does not require complicated and expensive laboratory equipment, and fungi can be identified effectively. The problem formulation of this research is how the characterization results of potato seed commodities imported from Australia in July 2023 can ensure that the commodities are free from pathogenic fungi. Characterizing imported potato seeds aims to reduce the risk of spreading pathogenic fungi Helminthosporium solani that can harm the agricultural sector. Characterization using the blotter test method goes through 4 stages, 1) sample preparation, 2) sample sterilization, 3) sample incubation, and 4) observation and identification. The characterization results showed that pathogenic fungi were not found in imported potato seed samples from Australia. The fungus found was the species Cephalotrichum brevistipitatum, which is an endophytic fungus, not Helminthosporium solani. The results of this characterization imply that the Animal, Fish, and Plant Quarantine Center released the potato seeds and allowed them to enter Indonesian territory.

Keywords*: Import; Potato Seeds; Blotter Test; Cephalotrichum brevistipitatum.*

INTRODUCTION

Potatoes (*Solanum tuberosum*) are a horticultural vegetable commodity with high economic value in Indonesia (Saptana et al., 2022; Taylor & Dawson, 2021). Potatoes belong to the Solanaceae family within the order Solanales (USDA, 2024). This commodity significantly contributes to food security by supporting the national food diversification program, especially as a staple food alternative to rice (Pradigta, 2021). The nutritional content of potato tubers includes protein, essential amino acids, carbohydrates, minerals (P, Mg, K), and vitamins C and B (Komalasari, 2022). Carbohydrates are the largest component of potato tubers (Saar-Reismaa et al., 2020). Potato consumption has been increasing, reaching 4.69 kg per capita in 2021, while domestic production stands at only 17.46 tons/ha and fluctuates annually (Sahara et al., 2022; Saptana et al., 2022; Eliášova, 2023).

Potato production in Indonesia in 2022 was only 1.3 million tons (Statistics Indonesia, 2023), significantly lower than the world potato production in China and India (**Table 1)** (FAO, 2023). For decades, Indonesia has relied on potato imports due to the government's lack of commitment to developing superior varieties local potato seeds. Despite having vast agricultural land production remains low without high-quality seeds. The low-quality standards, productivity, and limited availability of potato seeds have driven the Indonesian government to import potatoes. The countries exporting potatoes to Indonesia include Germany, Australia, the United States, and Belgium (Paudel et al., 2022).

Potato imports to Indonesia in early 2023 reached 7.16 million kilograms, with the majority imported from Germany, followed by Canada, the United States, the United Kingdom, and Australia (Statistics Indonesia, 2023b). The high accessibility of potato imports from various countries increases the risk of introducing quarantine plant pests. *Helminthosporium solani* is categorized as a quarantine plant pest Group I pest, which cannot be fully eliminated from its carrier medium, according to Minister of Agriculture Regulation No. 25, 2020. Potatoes infected by

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the *Helminthosporium solani* fungus typically exhibit silvery-gray lesions on the periderm of the tuber (Tiwari et al., 2021). Infection by this pathogenic fungus causes damage to various processes and poses a threat to horticultural cultivation areas in Indonesia (Qomar et al., 2022).

Table 1. Total World Potato Production in 2023

No	Country	Total Production
1	China	94,362,175
2	India	54,230,000
3	Ukraine	21,356,320
4	United States	18,582,370
5	Russia	18,295,535
6	Germany	11,312,100
7	Bangladesh	9,887,242
8	France	8,987,220
9	Poland	7,081,460
10	Egypt	6,902,816
11	Netherlands	6,675,590
…	…	…
37	Indonesia	1,361,064

(Source: Food and Agriculture Organization of the United Nations, 2023)

Imported potato seeds must go through the Animal, Fish, and Plant Quarantine Center to minimize the risk of introducing and spreading pathogenic fungal OPTK. Animal, Fish, and Plant Quarantine Center oversees the supervision of commodities transported by land, sea, or air. Quarantine measures, based on Government Regulation of the Republic of Indonesia Number 29 of 2023, include inspection, isolation, observation, treatment, detention, rejection, destruction, and release (Government of Indonesia, 2023). The examination of documents and the health of imported plant commodities is part of the quarantine process to ensure that the commodity is safe to enter Indonesian territory. The treatment determined by Animal, Fish, and Plant Quarantine Center depends on the characterization process conducted.

Characterization of fungal quarantine plant pests on potato seeds cannot be done visually, several tests are needed to obtain accurate results. Identifying the infecting quarantine plant pests requires further analysis, such as isolation, DNA testing, quantitative and qualitative analyses (Dumaria et al., 2023). The blotter test method is one isolation technique that involves growing fungi in a petri dish with moist sterile Whatman filter paper (Ahmed et al., 2021). This method is used in this activity because it is considered more effective for characterizing various types of seed-borne fungi compared to the standard method of sampling fungal colonies with a sterile inoculation loop (Singh et al., 2018; Sobianti et al., 2020; Sardi et al., 2021). The urgency of this research lies in the need to characterize pathogenic fungi in imported Australian potato seeds entering Indonesian territory to ensure that no target pathogens listed in quarantine plant pests are present from Australian potato seeds, as a preventive measure to reduce the risk of spreading diseases and pests that could harm the agricultural sector in Indonesia.

RESEARCH METHODS

Research Type and Design

This study was conducted using a descriptive research design with a laboratory observational. The blotter test method was applied to detect, identify, and analyze the presence of fungi in potato seed samples.

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Location and Time of Research

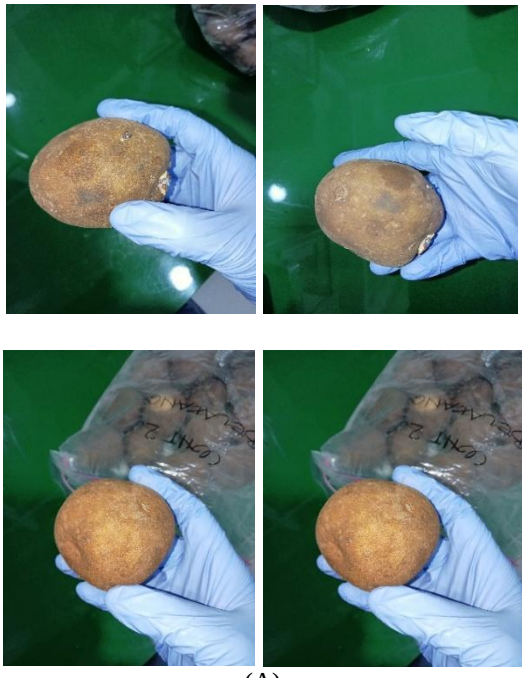
The research was conducted at Animal, Fish, and Plant Quarantine Center- Central Java, located at Jl. Jendral Soedirman No. 81, Semarang, Central Java. Document inspection and sampling took place at the Semarang Container Terminal, located at Pelabuhan Indonesia III, Jalan Coaster No. 10A, North Semarang, Tanjung Mas, North Semarang District, Semarang, Central Java. Blotter tests and fungal characterization were carried out at the mycology laboratory. These activities were conducted over a period of four months, from July 17 to November 17, 2023.

Research Procedure

The characterization of quarantine plant pests in imported potato seeds from Australia using the blotter test method was conducted in four stages: 1) sample preparation, 2) sample sterilization, 3) sample incubation, and 4) observation and identification.

Sample Preparation

Potato seed samples were obtained from inspections of containers 1, 2, and 3, which were automatically sampled through the Indonesian National Single Window System (INSW) Animal, Fish, and Plant Quarantine Center, Central Java system. These potato seeds originated from Australia. Sample preparation was carried out in the laminar air flow of the mycology laboratory. Symptomatic potato seeds were sorted and cut using a sterile scalpel into 1 cm x 1 cm pieces (**Figure 1**).



(A)

(B)

Figure 1. Sample Preparation Stage. a) sorting of symptomatic potato seeds, b) cutting samples
(Source: Personal Documentation, 2023)

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Sterilization of the Washing of Samples

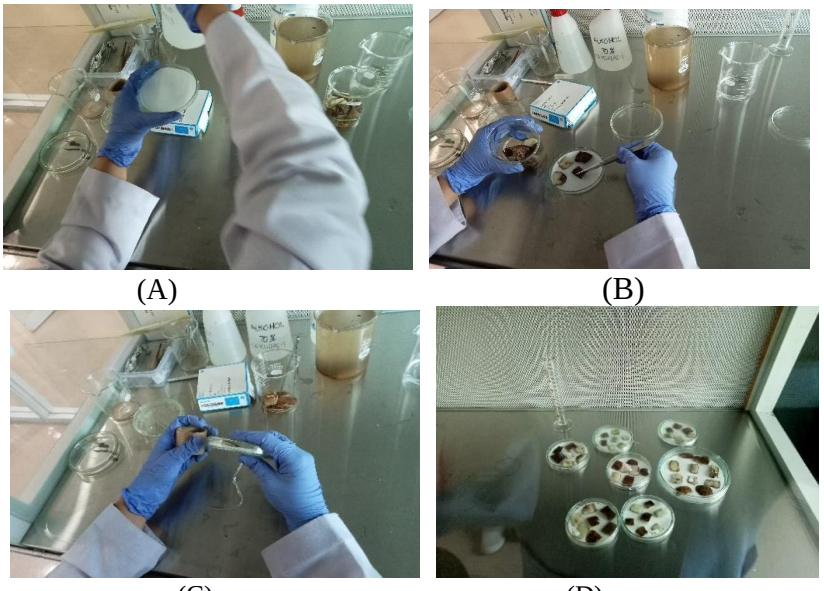
The sterilization of the samples was conducted by immersing pieces of potato seeds in a 5% Clorox solution for 2 minutes. The sample pieces were then washed with sterile distilled water, a process that was repeated three times (**Figure 2**).

(A) (B)

Figure 2. Sterilization and Washing Stages of Potato Seed Samples. (a) Sterilization of samples using a 5% Clorox solution, (b) Washing of samples with sterile distilled water
(Source: Personal Documentation, 2023)

Sample Incubation

The incubation of the potato seed samples began by wetting three sheets of Whatman paper with sufficient sterile distilled water until they were moist, then placing them in a petri dish. The potato seed samples were picked up with sterile tweezers and placed on the Whatman paper, with 10 seeds in each petri dish. The petri dish containing the samples was wrapped in plastic wrap and placed under appropriate incubation conditions, specifically under a 40 W near ultraviolet (NUV) lamp with a lighting cycle of 12 hours of light and 12 hours of darkness alternately. After seven days of incubation, the fungal colonies growing on the samples were observed under a binocular stereo microscope (**Figure 3**).



(A) (B)

(C) (D)

Figure 3. Sample Incubation**.** (A) Preparation of Whatman paper, B) Placement of potato seed samples, (C) Sealing of petri dishes with plastic wrap, (D) Incubation process for 7 days
(Source: Personal Documentation, 2023)

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Observation and Identification

The fungi observed under the binocular stereo microscope are then prepared for further observation of spores and other fungal structures using a compound microscope (**Figure 4a**). To prepare the slides, the object glass and cover glass were sterilized with 70% alcohol and dried with tissue paper. A drop of Hoyer's solution was placed on the object glass, and fungal spores growing on the surface of the potato seeds were carefully transferred into the solution using a

preparation needle. The specimen on the object glass was then covered with a cover glass and sealed with transparent nail polish applied to all four edges of the cover glass. The prepared slides were observed under a compound microscope, and the images were documented using a computer and NIS-Elements software. The observations were documented and analyzed (**Figure 4b).**

(A) (B)

Figure 4. Observation and Identification. (A) Observation using a binocular stereo microscope, (B) Observation using a compound microscope.
(Source: Personal Documentation, 2023)

Data Analysis Technique

Macroscopic observations were first performed using a binocular stereo microscope to examine the general characteristics of fungal growth on the potato samples. Subsequently, microscopic observations were carried out using a compound microscope to identify fungal structures, including spores and other morphological characteristics using a computer integrated with NIS-Elements software. The identification of fungi was conducted by analyzing morphological characteristics such as colony appearance, spore shape, size, septation, and other distinguishing structures. The collected data were analyzed descriptively by interpreting the observed morphological characteristics and comparing them with relevant identification references to determine the fungal genera or species present in the samples.

RESULTS AND DISCUSSION

Observations of the characterization of OPTK potato seeds imported from Australia are presented in **Figure 5**. The results of the observations using a stereo microscope showed no similarities in the characteristics of the *Helminthosporium solani* fungus, which was the target of the quarantine plant pests potato seed testing, in the samples. Further analysis of the spores and other parts of the fungus was conducted using a compound microscope, as presented in **Figure 6.**

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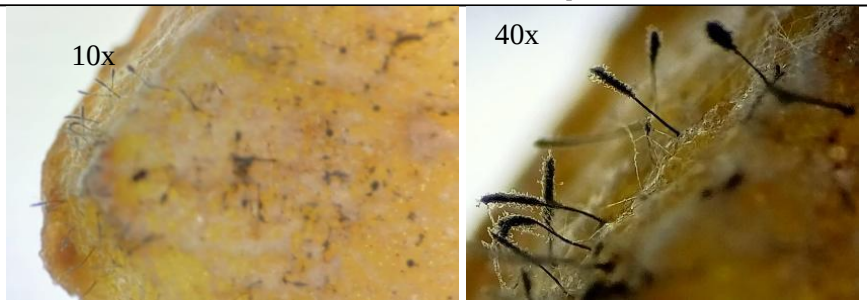

(A) (B)

Figure 5. Observation Results Under a Stereo Microscope. (A) 10x magnification, (B) 40x magnification.
(Source: Personal Documentation, 2023)

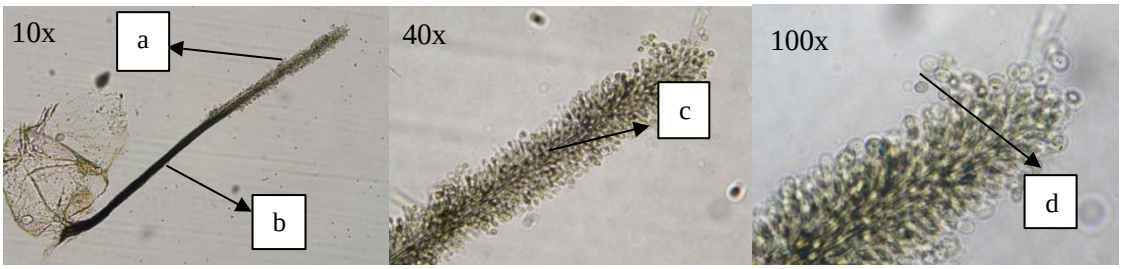

Figure 6. Morphological Characterization Using a Compound Microscope**.** (a) Sporangium, (b) Sporangiophore, (c) Conidiogenous cells, (d) Conidia.
(Source: Personal Documentation, 2023)

The characteristics of the observed fungal colonies include dark hyphae, dark conidiophores, and conidiogenous cells that vary in shape, ranging from cylindrical to round and elongated cylindrical. The colonies are synematous and have a dark brown to blackish coloration. The identification results indicate that the observed fungi belong to the genus *Cephalotrichum*. *Cephalotrichum brevistipitatum* is characterized by septate hyphae, brown to hyaline coloration, smooth and thin walls, and unbranched or slightly branched conidiophores that form synnemata. The conidiolar heads are blackish brown, and the conidia are ellipsoidal or short clavate (Sandoval-Denis et al., 2016). While several species of *Cephalotrichum* are not considered human pathogens and are not known to produce mycotoxins (Das et al., 2020), some species within the genus, including *Doratomyces* (synonym: *Cephalotrichum*), have been reported as pathogens in potatoes, causing spot rot disease (Mamaghani et al., 2022). Research by Tuchkov et al., (2023) indicates that several species of *Cephalotrichum* exhibit a parasitic effect on potato tubers, leading to brown necrosis following leaf inoculation. The pathogenicity of *Cephalotrichum* fungi warrants further study and monitoring. There is a need for clarification of its phylogenetic specialization, evaluation of the disease hazard to different potato varieties, and the development of protective measures to mitigate potential damage.

The characterization results are detailed in the supporting document of the Report on the Results of the Implementation of Health Inspection of Carrier Media/Wooden Packaging/Identity Inspection and Safety Testing of Fresh Food of Plant Origin (DP-5). The results confirm that the imported potato seed samples from Australia are free of the quarantine plant pests targets. *C.brevistipitatum* is not an quarantine plant pests target and is not classified as a pathogenic fungus, indicating that fumigation of the potato seed commodities is unnecessary. Fumigation is a quarantine measure used to eliminate pest organisms with toxic gases or chemicals. Common fumigants include Chloropicrin (CP), Methyl Bromide (MBr), Dazomet (DZ), 1,3-Dichloropropene (1,3-D), and Phosphine (PH3) (Castellano-Hinojosa et al., 2022). Among these, Phosphine fumigation is considered more effective than Methyl Bromide (Hasan et al., 2020).

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Quarantine actions based on the characterization results of imported potato seed samples from Australia involve the issuance of releases. According to Government Regulation of the Republic of Indonesia Number 29 of 2023, releases are granted based on the results of testing and characterization that confirm the absence of pathogenic fungi (Government of Indonesia, 2023). This is formalized by issuing a Plant Quarantine Release Certificate/Fresh Food Safety of Plant Origin (KT-9). The potato seed commodities are declared healthy, allowing for their transportation, and service users who have paid for quarantine services receive the KT-9 certificate, as indicated on the receipt. Quarantine authorities are required to attach a sticker or seal to the carrier media (potato seeds) to indicate that it has been inspected. The KT-9 certificate is a necessary document for the entry of these commodities into Indonesian territory.

CONCLUSIONS AND SUGGESTIONS

The characterization of quarantine plant pest in potato seed commodities from Australia at Animal, Fish, and Plant Quarantine Center-Central Java identified the fungus *Cephalotrichum brevistipitatum*. This species is not a target quarantine plant pests and does not pose a risk of causing economic or environmental damage, as it is non-pathogenic. The results of the characterization indicate that the potato seeds from Australia are cleared for entry into Indonesian territory.

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