

## Isolation and Identification of Patogenic Funges on *Oryza sativa* Plant in Dulomo Utara, Gorontalo City

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### ABSTRAK

Penyakit tanaman yang disebabkan oleh jamur patogen merupakan salah satu faktor pembatas dalam produksi padi (*Oryza sativa*). Identifikasi jamur patogen sangat penting dilakukan sebagai langkah awal dalam pengendalian penyakit tanaman padi. Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi jamur patogen pada tanaman padi yang dibudidayakan di Dulomo Utara, Kecamatan Kota Utara, Kabupaten Kota Gorontalo. Penelitian bersifat deskriptif eksploratif yang dilaksanakan pada September hingga Oktober 2024. Pengambilan sampel dilakukan dengan metode purposive sampling pada bagian tanaman yang menunjukkan gejala serangan penyakit. Isolasi jamur dilakukan menggunakan metode pengenceran dan sebar, dilanjutkan dengan pemurnian dan identifikasi di Laboratorium Agensi Hayati Balai Perlindungan Tanaman Pertanian. Karakterisasi dan identifikasi jamur dilakukan berdasarkan karakter morfologi makroskopik dan mikroskopik mengacu pada Bergey's Manual of Determine Bacteriology 9th Edition (Holt et al., 1994) dan buku kunci identifikasi jamur (Barnett and Hunter, 1972; Pitt and Hocking, 2009). Hasil penelitian berhasil mengidentifikasi 2 jamur patogen tanaman padi yaitu *Aspergillus niger*, dan *Fusarium oxysporum*. Temuan ini memberikan informasi penting untuk pengembangan strategi pengendalian penyakit pada tanaman dan dapat dijadikan pengujian lebih lanjut.

### ABSTRACT

Plant diseases caused by pathogenic fungi are one of the limiting factors in rice (*Oryza sativa*) production. Identification of pathogenic fungi is very important as a first step in controlling rice plant diseases. This study aims to isolate and identify pathogenic fungi in rice plants cultivated in North Dulomo, North Kota Sub-district, Gorontalo City District. The research was descriptive and exploratory, conducted from September to October 2024. Sampling was conducted using purposive sampling method on plant parts that showed symptoms of disease attack. Fungal isolation was carried out using dilution and scattering methods, followed by purification and identification at the Biological Agents Laboratory of the Agricultural Plant Protection Centre. Fungal characterisation and identification were carried out based on macroscopic and microscopic morphological characters referring to Bergey's Manual of Determine Bacteriology 9th Edition (Holt et al., 1994) and fungal identification key book (Barnett and Hunter, 1972; Pitt and Hocking, 2009). The results of the study successfully identified pathogenic fungi of rice plants, namely *Aspergillus niger*, and *Fusarium oxysporum*. These findings provide important information for the development of disease control strategies in plants and can be used as further testing.

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## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important agricultural commodities for food security in many countries, including Indonesia. It is the main source of carbohydrates for most of the population. However, rice production is often threatened by various diseases caused by

microorganisms, including fungi. Diseases caused by fungi can cause significant losses in yield and seed quality (Nurkayah *et al.*, 2024). One of the main challenges in rice cultivation is the attack of fungal diseases that can disrupt plant growth. Several pathogenic fungi, such as *Fusarium*, *Rhizoctonia*, and *Pyricularia*, have been identified as the main causes of disease in rice plants. These disease attacks often occur under erratic weather conditions, such as high humidity and temperatures that favour fungal growth (Lana, 2023).

Common pathogenic fungi that attack seeds include *Fusarium* sp., *Pythium* sp., and *Phomopsis* sp. (Chailani and Djauhari., 2012). Risnawaty *et al.* (2012) mentioned that fungi associated with rice seeds are not only fungi carried by seeds from the field but also fungi from the warehouse, such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*, all four of which are facultative parasitic fungi. These fungi will contaminate the seeds when stored in the warehouse. The damage caused by fungi is the emergence of fungal toxins, decreased nutritional value of seeds, decreased weight of seeds and sprouts.

The pathogenic fungus *Pyricularia grisea* is able to attack rice at various growth stages from seed to panicle growth phase. Winarni (2013) states that blast disease can arise because the seeds used are unhealthy seeds, thus disrupting germination and plant growth, which can then reduce the quality and quantity of production. Disease will arise when pathogenic fungi are associated with plant tissues that live and develop in them. Pathogenic fungi in the plant body secrete enzymes and toxins that can cause disease (Rahayu., 2015). Pathogenic fungi can attack seeds, especially seeds in storage. Seeds are targeted by pathogenic fungi because they are rich in nutritional sources such as carbohydrates, proteins, and fats. Therefore, pathogenic fungi can utilise seeds as a source of nutrition by infecting seeds (Hanif and Susanti, 2019).

Seed health is absolutely necessary both physiologically and pathologically, because seeds can be plant disease-carrying agents. Seeds that carry diseases will cause serious problems, such as reduced seed germination, death in the seedling phase, and increased disease development in the old plant phase. Seeds that have been infected at the time of sowing, the growth of rice plants is uneven so that when transferred to the field, the plants show disease symptoms such as stunting, leaf rust, and broken rice panicles. Seeds that have been infected with pathogens not only inhibit plant growth but can also cause poisoning (Amteme and Tefa., 2018). Another impact felt by farmers is economic loss, because the cost of maintenance and labour becomes greater and reduced production (Ramdan and Kalsum., 2017).

One way to overcome these problems is to prevent the seeds beforeplanting in the field, providing quality seeds that are free from disease (Saylendra, 2010). Therefore, knowing the types of pathogenic fungi that infect seeds and checking the quality of seeds against diseases requires research on "Isolation And Identification of Fungi From Padi (*Oryza Sativa* L.) Plants Suspected of Disease".

## 2. METHOD

This research was conducted on 30 September - 11 October 2024. Rice plant samples were taken from Rice Farm, Dulomo Utara, Kota Utara Sub-district, Gorontalo City District. Isolation and identification of fungi were carried out at the Biological Agents Laboratory, Gorontalo Province Plant Protection Centre.

The tools used in this research are Laminar Air Flow, Oven, Incubator, Bunsen Lamp, Hot Plate Stirrer, Erlenmeyer, Beaker Glass, Ose Needle, Petri Dish, Analytical Scales, Stirring Rod, tweezers, microscope, prep glass, cover glass. The materials used in this study were rice

samples, PDA (*Potato Dextrose Agar*) media, 5% clorox, distilled water, alcohol, aluminium foil, cotton.

### Research Procedures

#### *Isolation of Pathogenic Fungi*

The initial stage of isolation is rice seeds affected by pathogen attack, washed with running water until clean, then washed in 5% Clorox solution for 1 minute repeated twice, followed by putting into 70% alcohol for 1 minute repeated twice. After that, it was rinsed with sterile distilled water for 1 minute and repeated twice and then the leaves were dried on sterile tissue, the leaves were cut approximately 1 cm<sup>2</sup> under aseptic conditions and planted in a 9 cm petri dish containing PDA media. Then the last rinse distilled water was taken 1 ml and poured (isolated) into another new PDA, this treatment served as a control (Ariyanto *et al.*, 2013).

#### *Purification of Pathogenic Fungi*

Purification is carried out on each fungal colony that is considered different based on macroscopic morphology, which includes the colour and shape of the colony. Each microorganism was taken with an ose needle, then regrown on a petri dish containing PDA media (Ariyanto *et al.*, 2013).

#### *Preparation of Fungal Preparations*

Stages for making fungal preparations are preparing object glass, cover glass, and tissue, fungi that have been isolated on PDA media are taken with an ose needle and covered using cover glass. The preparation is placed in a container that has been given a moist tissue base and incubated for 2-3 days (Ariyanto *et al.*, 2013).

#### *Identification of Pathogenic Fungi*

Fungal isolates that have been purified (purification) are then observed, both macroscopically and microscopically which are then identified based on *The Barnett and Hunter Identification Guidebook* (1972). Macroscopic observations include colony colour, colony shape, colony texture and colony growth. While microscopic observations include, among others, the hyphae are concentrated or not concentrated, hyphal growth (branched or unbranched), hyphal colour (dark or transparent hyaline), conidia colour (dark or transparent hyaline), presence or absence of conidia and conidia shape (round, oval, chain or irregular) (Ariyanto *et al.*, 2013).

### Data Analysis

Macroscopic and microscopic data were tabulated, presented in pictures. The isolation results of each pathogenic fungal isolate were identified, documented and presented in the form of descriptions, and the images were analysed using the method of Barnett and Hunter (1972).

## 3. RESULT AND DISCUSSION

The sampling location was in a rice field in North Dulomo Village, North Kota District, Gorontalo Regency. At this location, rice plants infected with the disease were found. Early symptoms observed included brown spots on the leaves and grains, and the presence of empty grains. This condition indicates the possibility of infection by pathogenic fungi that have adapted to the local environment, causing disease in rice plants. Rice samples were taken randomly from one location to ensure a good representation of field conditions. Subsequently, the samples were taken to the biological agency laboratory for further testing to identify the pathogen causing the disease and determine appropriate control measures.

## Observation of Fungal Pathogen Infection Symptoms on Rice Plants in Dulomo Village, North Kota Subdistrict, Gorontalo Regency

Based on the results of fungal culture on rice plant samples, isolation and purification were obtained using the dilution and dispersion method, namely at concentrations of  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ . The medium used was *Potato Dextrose Agar* (PDA), which is an ideal medium for fungal growth. Isolation and purification resulted in three fungal isolates with different colony morphological characteristics.

### 1. *Aspergillus niger*

*Aspergillus* has several distinctive features, including septical hyphae and a branching mycelium that is usually colourless. Vegetative hyphae lie on the surface of the substrate, while fertile hyphae appear above the substrate surface. Colonies of *Aspergillus* are generally compact, with conidiophores that can be scabrous or non-scabrous. These conidiophores grow from "foot cell" structures, which are curved and thickened mycelium. The tips of the conidiophores form vesicles that swell and produce sterigmata, where conidia develop. Sterigmata can be simple, coloured, or colourless. Conidia are arranged in chains, with colours varying from green, brown, to black, depending on the species. Some species can grow well at 37°C or higher (Debby et al., 2003).

*Aspergillus niger* isolates were obtained from rice seeds. At the beginning of its growth, *Aspergillus niger* colonies were blackish brown and at the edges of the cup were greenish white, round in shape, thick mycelium, filling a Petri dish with a diameter of 6 cm at 7 HST. On microscopic observation, the conidium was seen to be brown in colour, round and fused.

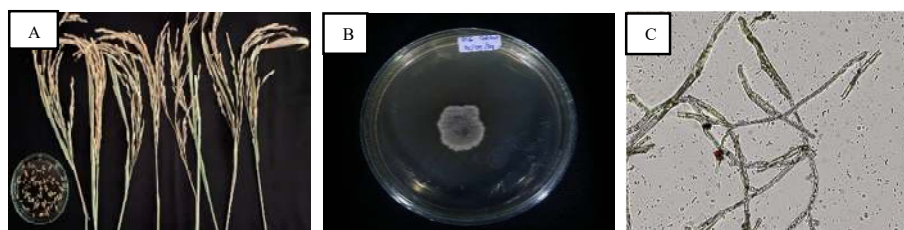


Figure 1: Symptomatic appearance of rice seeds infected with *Aspergillus niger*, as well as microscopic appearance of microconidia of the pathogen at 400x magnification.

### 2. *Fusarium oxysporum*, spp

One of the diseases that attack rice plants is wilt disease caused by the pathogen *Fusarium oxysporum*. According to Agrios (2005), common symptoms include wilting of leaves, discolouration of xylem vessels to brown, and the formation of necrosis on roots and stems. *Fusarium oxysporum* isolates were obtained from rice seeds and leaves (Figure 2). On PDA media, the mycelium is initially white, getting older the colour becomes beige or pale yellow, under certain circumstances it is slightly purple pink. The mycelium is fibrous and forms branching. It filled an 8 cm diameter petri dish at 6 HSI.

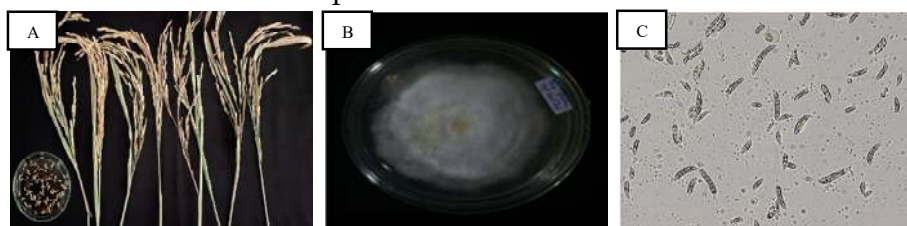


Figure 2: Symptoms of *Fusarium oxysporum* infection on rice seeds and leaves, and microscopic view of microconidia of the pathogen at 400x magnification.

Macroscopic observations of the three isolates showed that although all three had the same surface texture (cotton-like) and irregular edge shape, colony colour was the main differentiator. This colour difference may indicate that the three isolates may be different species of fungi or at least different strains of the same species.

Microscopic observations provide important additional information for fungal identification. Hyphal structure, spore shape and size, and the presence or absence of specialised reproductive structures can be key in determining the genus or even species of the fungus. Although the microscopic images provided in the document are not described in detail, this information is essential for further analyses. For a more accurate identification, further analyses such as more detailed microscopic observations, measurements of the fungal structure, and molecular analyses are required. However, based on the macroscopic and microscopic characteristics that have been observed, it can be concluded that these three fungal isolates have potential as pathogens of rice plants. Further research is needed to determine the specific role of each isolate in the disease that attacks the rice plant.

#### 4. CONCLUSION

Based on the results of the above research, two types of pathogenic fungi that attack rice plants are *Aspergillus niger*. and *Fusarium oxysporium*, spp.

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