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Early Detection of Pathogenic Fungi *Curvularia* sp. on Rice Seeds (*Oryza sativa*) Based on Modified Infrared Image Analysis

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Abstract

Background: *Curvularia* sp. is a seed-borne pathogenic fungus that can reduce rice plant productivity. At the same time, conventional seed health testing methods still rely on visual observation and require a relatively long incubation time. This study aims to evaluate the potential of infrared image analysis based on a modified imaging system for early detection of *Curvularia* sp. infection in rice seeds. **Methods:** Seed health testing was performed using the blotter test method. At the same time, image acquisition was performed with a digital microscope equipped with an infrared light source, and images were analyzed using pseudocoloring and RGB-based color segmentation. **Results:** The results showed differences in infrared signal intensity patterns in *Curvularia* sp.-inoculated seeds, which could be identified on the fourth day after inoculation, earlier than visual observation, which showed symptoms on the fifth day. Detection accuracy was calculated using a confusion matrix based on visual observation as the reference method, with a sample size of 50 seeds per observation day, yielding an average detection accuracy of 91% over seven days of observation. **Conclusions:** The modified infrared image analysis method has the potential to serve as an early detection method for Curvularia sp. infection in rice seeds, although its performance depends on the limitations of the imaging system and the validation method used.

Keywords: *Curvularia* sp.; Early detection; Infrared image; Rice seeds

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Introduction

Rice (*Oryza sativa* L.) is a significant food commodity that plays a crucial role in meeting the community's food needs (Harahap et al., 2015). Efforts to increase rice productivity include using high-quality, healthy seeds, as they serve as the initial source of plant growth in the field. Seeds infected with seed-borne pathogens can become a source of inoculum and significantly reduce yields. One seed-borne fungal pathogen frequently reported to attack rice plants is *Curvularia* sp., which can cause leaf spot disease and reduce rice yields by up to 50% (Wibawa et al., 2018).

Seed health testing is a crucial step to ensure seeds are free of pathogens before they are used in cultivation. The blotter test is a commonly used conventional method for detecting pathogenic fungi in seeds (Lestari et al., 2014). This method still has limitations because it relies on visual observation of fungal mycelium growth, requiring a relatively long incubation time and the observer's expertise. Accurate and precise results from conventional seed pathogen detection require several resources, including time, labor, and implementation costs (Das et al., 2015). Latent infections in seeds are often not visually detectable in the early stages, potentially evading seed selection.

With advances in technology, imaging methods are increasingly used in agriculture to detect plant diseases early. One approach with potential for development is infrared imaging, which is non-destructive and allows observation without direct contact with the object (Rego et al., 2020). Infrared imaging has been reported to be capable of detecting physiological and structural changes in pathogen-infected plants before the appearance of visual symptoms, such as the detection of Pepper Yellow Leaf Curl Virus (PYLCV) in chili plants (Dumaria et al., 2023) and Cucumber Mosaic Virus (CMV) in tomato plants (Zhu et al., 2018). These research results indicate that differences in infrared signal response can serve as an early indicator of pathogen infection and reflect variations in seed surface reflectance resulting from changes in tissue structure induced by fungal infection.

Most previous studies have used spectrally calibrated infrared imaging systems or thermal cameras, focusing primarily on vegetative organs such as leaves. The application of infrared image analysis to rice seeds to detect seed-borne fungal infections before visual symptoms appear is still limited. The use of modified, uncalibrated infrared imaging systems as an exploratory approach for early detection of seed infections has not been widely reported.

Based on this background, this study aims to evaluate the potential of qualitative infrared image analysis using a modified imaging system for early detection of *Curvularia* sp. infection in rice seeds. This research is expected to provide initial information regarding the use of infrared imagery as a supporting method in seed health testing, particularly in detecting early indications of fungal infection before visual symptoms appear.

Methods

Characteristics of Curvularia sp. Mushroom

The *Curvularia* sp. fungal isolate used was isolated from rice plants. Fungal identification was performed to the genus level based on morphological characteristics. Macroscopic observations showed dark brown colonies with a cotton-like surface (Figure 3.a). Based on the Munsell Color Chart classification, *Curvularia* sp. colonies have a Hue value of 10YR, a Value of 2, and a Chroma of 2 (Very Dark Brown) (Munsell, 1975). This is supported by Marbun et al.'s (2023) observation that Curvularia sp. colonies are blackish-brown, with a cotton-like surface and uneven edges.

Microscopic observations showed that *Curvularia* sp. fungi have septate hyphae, brown conidia, and a water droplet shape with curved tips (Figure 3.b). This is supported by Sobianti et al.'s (2020) statement that the microscopic characteristics of *Curvularia* sp. include upright conidiophores and curved brown to black conidia. *Curvularia* sp. conidia are approximately 20.4 μm long. This is supported by the research results of Leiwakabessy et al. (2020), which found that *Curvularia* sp. conidia have 3-4 septa measuring 18.5-21.1 μm.

(a) (b)

Figure 3. *Curvularia* sp. morphology. (a) macroscopic form on PDA media, (b) microscopic

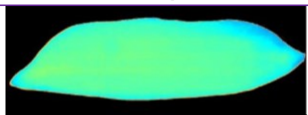
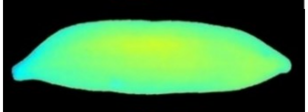
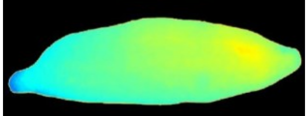
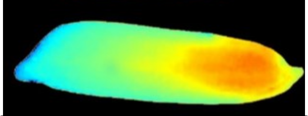
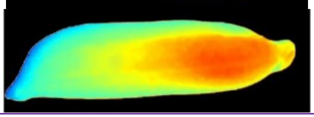
Observation Results of the Detection of Curvularia sp. Fungus.

The results of *Curvularia* sp. detection in rice seeds using the blotter test method and infrared image analysis over seven days of observation are presented in Table 1. From the first to the third day, the pseudo-staining images showed blue to yellowish-green colors, indicating that no infection symptoms were detected. These results are consistent with visual observations (RGB images), which showed no infection symptoms during the same period.

Changes in the color pattern in the infrared images began to appear on the fourth day after inoculation, as indicated by orange to red colors on some seeds. Meanwhile, visual observations only revealed infection symptoms on the fifth day after inoculation. Visually, infected seeds exhibited blackish-brown mycelial growth with a cotton-like texture on the seed surface, a typical symptom of *Curvularia* sp. infection (Sobianti et al., 2020). Ramdan & Ummu (2017) stated that seeds infected with fungi generally have a surface covered by fungal mycelium.

The ability of modified infrared images to detect infection earlier is thought to be related to changes in seed tissue structure caused by fungal infection. The differences in infrared intensity observed in the image are due to variations in the seed surface's infrared reflectance. Fungal infection causes changes in tissue structure, mycelial accumulation, and water content, thereby affecting the infrared reflectance characteristics of the seed surface (Vresak et al., 2016). These changes result in contrasting color differences between healthy and infected seeds in pseudocoloring images. This finding is in line with the results of research (El Masry et al., 2020), which showed that differences in infrared reflectance are an early indicator of pathogen infection before visual symptoms appear in wheat seeds infected with Fusarium sp.

Table 1. Observation Result for Detecting *Curvularia* sp. Fungus

Day Of Observation	RGB Image	Pseudo Coloring Result Image
1		
2		
3		
4		
5		

Validation Test

A validation test was conducted to assess the effectiveness of the infrared image-based detection method for *Curvularia* sp. fungal infections in rice seeds. The test used a confusion matrix, a table consisting of four main components: (a) True Positive (TP): diseased seeds predicted as diseased by the infrared image; (b) True Negative (TN): healthy seeds predicted as healthy by the infrared image; (c) False Positive (FP): healthy seeds predicted as diseased by the infrared image; (d) False Negative (FN): diseased seeds predicted as healthy by the infrared image. This validation was conducted by comparing the infrared image detection results with visual observations as a reference.

The results of the validation test for *Curvularia* sp. detection in rice seeds (Table 2) demonstrate the performance of the detection method over 7 days of observation. From the first to the third day, all seed samples (50 grains per observation day) were classified as true negatives, achieving 100% accuracy. This indicates that no changes indicative of infection were detected during the early incubation phase, either through infrared image analysis or visual observation.

Early signs of infection were detected on the fourth day through infrared image analysis, even though visual symptoms were not yet visible to the naked eye. This was evidenced by the increasing number of seeds classified as false positives: 12 were detected, while 38 were classified as true negatives, resulting in a decrease in accuracy to 76%. The emergence of false positives at this stage is thought to be related to the ability of infrared images to detect changes in seed surface reflectance that occur early in infection, before the development of visually observable morphological symptoms. Therefore, some false positives may represent latent infections that were not detected by visual observation.

Detection accuracy began to increase on the fifth and sixth days, reaching 80% and 82%, respectively. This indicated the development of a *fungal infection caused by Curvularia sp*. Accuracy further increased to 100% on the seventh day, with 25 healthy seeds correctly identified as true negatives and 25 infected seeds correctly identified as true positives. The average detection accuracy over the seven days of observation was 91%.

Table 2. Validation Test Result for Detection of *Curvularia* sp. on Rice Seeds

Observation Day-	Number of True Detection		Number of False Detections		Detection Accuracy
	True Positive	True Negative	False Positive	False Negative	
1	0	50	0	0	100%
2	0	50	0	0	100%
3	0	50	0	0	100%
4	0	38	12	0	76%
5	8	32	6	4	80%
6	12	29	8	1	82%
7	25	25	0	0	100%
				Average	91%

The graph in Figure 4 shows the detection accuracy of *Curvularia* sp. in rice seeds using the infrared method over a seven-day observation period. During this period, fluctuations occurred, with a decrease in detection accuracy on the fourth day due to the appearance of false positives (healthy seeds detected as diseased by the infrared method).

The infrared imaging system used in this study was a modified camera that had not been spectrally calibrated. Therefore, the analysis was limited to differences in infrared reflectance on the seed surface and did not represent absolute temperature measurements. Environmental factors during image acquisition, such as room conditions and lighting, may affect the infrared reflectance of the seed surface. Visual observation was used as a reference in the validation test, but it has limitations for detecting latent infections at early stages. These limitations can contribute to variations in accuracy and to the occurrence of false-positive results in the early stages of observation.

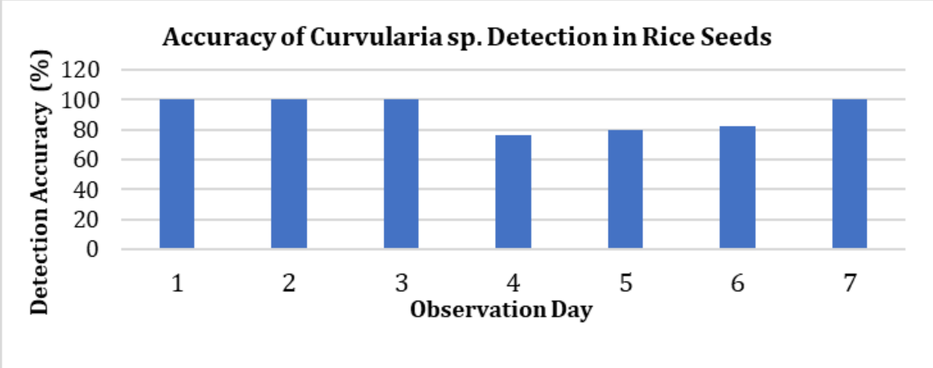


Figure 4. Accuracy Chart of *Curvularia* sp. Detection of Rice Seeds

Conclusions

Based on the research results, pseudo-coloring-based infrared image analysis shows potential for detecting early symptoms of Curvularia sp. infection in rice seeds before visual observation. This early detection is based on differences in the infrared reflectance characteristics of the seed surface, which appear before morphological symptoms are visually observed. Validation test results show that detection performance varies throughout the observation period, with an average accuracy of 91%. However, the detection results in this study are still influenced by the device's technical limitations, environmental factors during image acquisition, and differences in sensitivity between the infrared and visual observation methods. Therefore, the infrared method in this study has the potential to serve as a supporting approach for early detection of fungal infections in rice seeds, but it still requires further development.

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Declaration statement

The authors reported no potential conflict of interest.

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