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Study of Thermal Imaging Potential for Early Detection of *Fusarium* sp. Pathogen on Rice Seeds (*Oryza sativa* L.)

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ABSTRACT

The early detection of Fusarium sp. infection in rice seeds is crucial for improving agricultural productivity and food security. Traditional methods like the Blotter Test, while effective, are time-consuming and require specialized personnel. This study explores the potential of thermal imaging technology to detect Fusarium sp. infections on rice seeds quickly and non-destructively. Rice seeds were inoculated with Fusarium sp. and incubated for seven days, during which surface temperatures were measured daily using the Fluke iSee TC01A thermal camera. The results showed that infected seeds exhibited significantly higher surface temperatures compared to control seeds, particularly from days 3 to 6 of incubation. Scatterplot analyses revealed clear temperature differences between infected and uninfected seeds, supporting the hypothesis that thermal imaging can serve as an early indicator of Fusarium infection. The study also demonstrated the high sensitivity and specificity of thermal imaging, particularly on days 2 to 4 of the incubation period. Logistic regression analysis confirmed the significant relationship between seed temperature and infection status, with prediction accuracy up to 91%. This research suggests that thermal imaging technology could replace traditional methods, offering a faster, more efficient approach for seed health monitoring in the agricultural industry.

1. INTRODUCTION

Early detection of pathogens in rice seeds is becoming increasingly important as the challenges of pathogens that can harm agricultural yields increase (Li *et al*., 2023; Wu *et al*., 2024). One of the pathogens that often attack rice seeds is *Fusarium* sp., which is known to cause various diseases in rice plants, including bakanae disease, which has the potential to significantly reduce the productivity of rice plants (Song *et al*., 2023; Zhang *et al*., 2024). One of the commonly used methods to detect this disease is the Blotter Test, which has become the standard of the International Seed Testing Association (ISTA) (Camargo *et al*., 2017). Although effective, this method requires a long incubation time, microscopic observations, and expert personnel, which of course takes a lot of time and resources (ISTA, 2024).

Various previous studies have examined the potential use of thermal technology in the detection of seed quality and pathogenic activity in plants (Singh *et al*., 2020). For example, thermal imaging technology has been used to detect *Erysiphe graminis* fungal infections in wheat and *Alternaria* sp. on radish seeds, as well as to identify the physical qualities of seeds such as in Norwegian cypress seeds and rice (Awad *et al*., 2015; Chelladurai *et al*., 2010; Vishunavat *et al*., 2023). This technology has advantages such as speed, non-contact, and the ability to detect temperature changes at the pixel level, which allows detailed spatial temperature mapping (Rippa *et al*., 2023). However, although thermal imaging has been shown to be effective in detecting infections in plants, research on its application to rice seeds is limited (ElMasry *et al*., 2020; Lydia *et al*., 2020; Upadhyay *et al*., 2025). Therefore, this research will fill the knowledge gap by focusing on the detection of *Fusarium* sp. on rice seeds using thermal imaging technology.

As the threat of *Fusarium* disease in rice crops increases, it is crucial to develop rapid and efficient detection methods, which can assist farmers in taking preventive measures before the infection progresses further. This study aims to explore the potential of thermal imaging technology in detecting *Fusarium* sp. In rice seeds, a faster approach compared to conventional methods. Thermal imaging technology can provide visual information regarding temperature changes occurring in infected seeds, which are produced by biological reactions due to pathogenic infections (Akhter *et al*., 2022; Zhang *et al*., 2024). The hypothesis put forward in this study is that thermal imaging technology can detect the presence of *Fusarium* sp. on rice seeds with adequate accuracy before visual symptoms appear and that the sensitivity and specificity generated by this technology are better compared to conventional detection methods, such as the Blotter Test.

The purpose of this study is to test the ability of thermal imaging technology in detecting *Fusarium* sp. on rice seeds and to compare their sensitivity and specificity with conventional detection methods. This research is expected to provide a faster, more efficient, and non-destructive alternative to detecting infections in rice seeds, which will be of great benefit to the seed industry and the agricultural sector as a whole. The expected benefit of this research is the creation of more efficient and resource-efficient early detection methods, so as to speed up the seed health testing process and reduce losses caused by pathogenic infections. In addition, this research also aims to provide scientific data that can support the development of thermal imaging technology in agriculture, with the hope that this technology can be widely adopted to improve food security and support the economic stability of farmers. Thus, this research has the potential to be an important contribution in advancing sustainable agriculture through innovations in pathogen detection technology.

2. MATERIALS AND METHODS

This study is a laboratory experiment with a quantitative approach that aims to examine the potential use of thermal imaging in the early detection of *Fusarium* sp. infection in rice seeds (Almoujahed *et al*., 2024; Jiang *et al*., 2020; Sanna *et al*., 2021). Rice seeds were sterilized using 1% NaOCl and then inoculated with *Fusarium* sp. suspension at two concentrations (10^6/ml and 10^7/ml). After inoculation, the seeds are incubated for 7 days and the surface temperature of the seeds is observed daily using the Fluke iSee TC01A thermal camera. Observations were made at the midpoint of the seeds with a distance of ±12 cm and controlled lighting. On day 7, the seeds are checked for infection status using a microscope (blotter test). The research design used a Complete Random Design (RAL) with five replicates for each treatment and variety. The treatment using several rice varieties, observation variables of surface temperature the seeds and infection status.

2.1. Materials

This study used three varieties of rice seeds, namely Ciherang, IR 64, and Mekonga. These three varieties were selected based on the consideration that they are commonly cultivated rice varieties in the study area and are susceptible to *Fusarium* sp. infection. The selection of these varieties is expected to provide a comprehensive overview of the differences in the potential for detecting *Fusarium* sp. infection using thermal imaging in rice seeds.

2.2. Experimental Design

The experimental design used was a completely randomized design (CRD) with a total of 60 experimental units. The factors tested in this study were seed varieties (Ciherang, IR 64, and Mekonga) and treatment consisting of four different treatment groups, with five replicates for each treatment. The following are the details of the treatments applied:

1. DB < 95: Seeds that were not inoculated with *Fusarium* sp. suspension, but left in environmental conditions with temperature and humidity that did not reach 95% of the optimal conditions for seed growth.
2. DB ≥ 95: Seeds not inoculated with *Fusarium* sp. suspension, but left under environmental conditions with temperature and humidity reaching 95% of optimal conditions.
3. F10E6: Seeds inoculated with *Fusarium* sp. suspension at a concentration of 10^6/ml.
4. F10E7: Seeds inoculated with *Fusarium* sp. suspension at a concentration of 10^7/ml.

2.3. Experimental Procedure

After the rice seeds were sterilized with NaOCl 1%, they were inoculated with a suspension of *Fusarium* sp. at two different concentrations (10^6 CFU/ml and 10^7 CFU/ml). After inoculation, the seeds were incubated for 7 days at controlled temperature and humidity. Every day, the surface temperature of the seeds is measured using a Fluke iSee TC01A thermal camera. Temperature measurements are taken at the center of the seeds with a distance of ±12 cm between the camera and the seeds, and the lighting in the experimental room is kept consistent.

On the 7th day, the seeds are examined for infection status using the blotter test method performed with a microscope to confirm the presence of *Fusarium* sp. infection.

2.4. Data Analysis

The temperature data obtained from the thermal camera measurements were analyzed descriptively using the mean and standard deviation to describe the temperature distribution between treatment groups. Then, inferential analysis using ANOVA (Analysis of Variance) was performed to determine the temperature differences between treatment groups and varieties. Additionally, boxplots will be used to visualize the temperature data distribution.

In addition to descriptive analysis and ANOVA, a Receiver Operating Characteristic (ROC) analysis was conducted to assess the potential detection ability of *Fusarium* sp. infection in rice seeds during the early days of incubation based on seed surface temperature measurements.

2.5. Measurements with a Thermal Camera

The Fluke iSee TC01A thermal camera was positioned perpendicular to the rice seeds at a distance of ±12 cm from the center of the seeds. The camera position was kept stable throughout the experiment, and temperature measurements were taken on each seed under controlled lighting in the experimental area. A schematic diagram of the thermal camera setup, including the distance and position of the camera relative to the seeds, is provided to help readers understand the layout of this experiment.

In the graphs and data visualizations, abbreviations are used to clarify the treatments performed, namely DB < 95, DB ≥ 95, F10E6, and F10E7, which are further explained in the image or graph captions. For visual clarity, the X-axis and Y-axis lines will be thickened to distinguish them from the thinner grid lines, enabling readers to easily identify the intended axis in the graph.

3. RESULTS AND DISCUSSION

3.1. Seed Temperature Observation with Thermal Camera

Based on Figure 1. The findings of the study show results of observation rice seeds both visually and through thermal imaging on day 0 and day 7 of the incubation period. Figures (a) and (b) show the condition of the seeds in the petri dish on day 0 and day 7, respectively, while figures (c) and (d) show the results of imaging the surface temperature of the seeds using the Fluke iSee TC01A thermal camera on the same day. On day 0 (figures a and c), the seeds appeared clean with no signs of infection and the surface temperature was relatively low, while on day 7 (figures b and d), the fungi olony of *Fusarium* sp. begin to appear on the surface of the seed, which is visible as a grayish-white colony in figure (b). The results of the thermal image in figure (d) show an increase in the surface temperature of the seeds which is characterized by a red-yellow color, which indicates higher metabolic activity due to infection. This increase in temperature is a physiological indicator of infection, considering *Fusarium* sp. generates heat through metabolic activity and stimulates the host's physiological response, such as increased respiration (Borokini *et al*., 2025; Gianinetti, 2022; Lipińska *et al*., 2022).

3.2. Visualization of Seed Temperature During the Incubation Period

Based on Figure 2, in general, during the 7-day incubation period (H0 to H7), the seed temperature of the three varieties showed a fairly consistent pattern, both for the Ciherang, IR 64, and Mekongga varieties. In Part A, Part B,

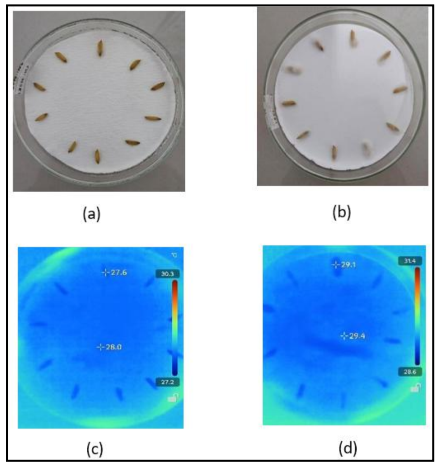


Figure 1. Non-thermal camera visual rice seeds and thermal cameras: (a) and (c) day 0 observation, (b) and (d) day 7 observation

and Part C, it can be seen that on day 0 to day 2, the seed temperature was relatively uniform across all treatments for each variety, reflecting the initial conditions before the development of significant biological activity. However, on days 3 to 5, seeds inoculated with *Fusarium* sp. showed a higher temperature rise pattern compared to controls, especially at inoculation treatment with high concentrations (10^7 conidia/ml), reflecting the metabolic activity of *Fusarium* sp. which began to actively infect seed tissues, causing decreased seed energy reserves, increased respiration, and impaired metabolism (Lipińska *et al*., 2022; Tahmasebi *et al*., 2023). All three graphs also show that the seed temperature peaked on the 4th day of incubation. On the 6th and 7th days, the seed temperature tends to decrease or stabilize, which can be caused by sporulation activity from the fungus that causes a decrease in temperature, in addition to which the structure of the mycelium also affects thermal emissions (Lipińska *et al*., 2022). This temperature drop can also occur due to the formation of defense mechanisms in seed tissues (Chang *et al*., 2022).

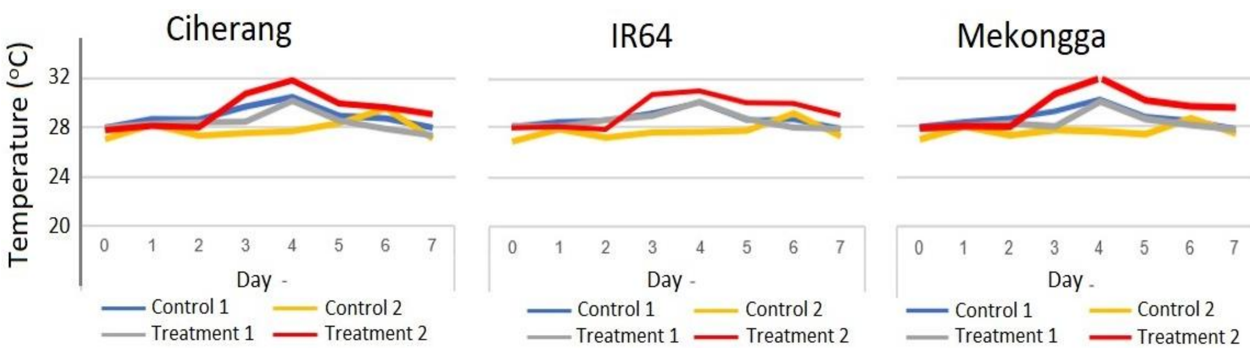


Figure 2. Daily temperature of rice seeds from various varieties during incubation: (a) Ciherang, (b) IR64, and (c) Mekongga

3.3. Daily Temperature Boxplot Visualization

Based on Figure 3, visualization of the seed temperature boxplot per day showed differences in temperature distribution between days and varieties, with higher temperature variations, especially in the inoculation treatment of *Fusarium* sp. compared to controls, especially on days 4 to 6. This indicates the presence of biological activity due to fungal infections that cause an increase in metabolic temperature (Gámez-Arjona *et al*., 2022; Lipińska *et al*., 2022; Tahmasebi *et al*., 2023). In the Ciherang variety, an increase in temperature variation was seen on day 3 to day 5, with a wider interquartile range (IQR) on day 5 in seeds inoculated with *Fusarium* sp. 10^7 conidias/ml, which indicates greater temperature variation between seeds. In addition, there are some outliers (2 dots below the box) that indicate seeds with much lower temperatures, which may be caused by seed damage or seeds that are no longer physiologically active (Liu *et al*., 2020; Španić & Šarčević, 2024).

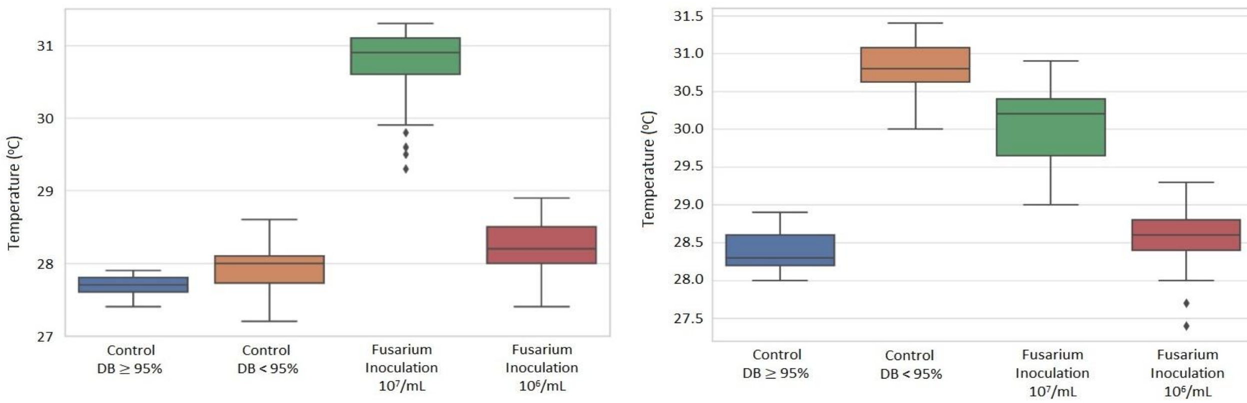


Figure 3. Boxplot of seed temperature for the Ciherang variety: (a) on the third day; and (b) on the fifth day

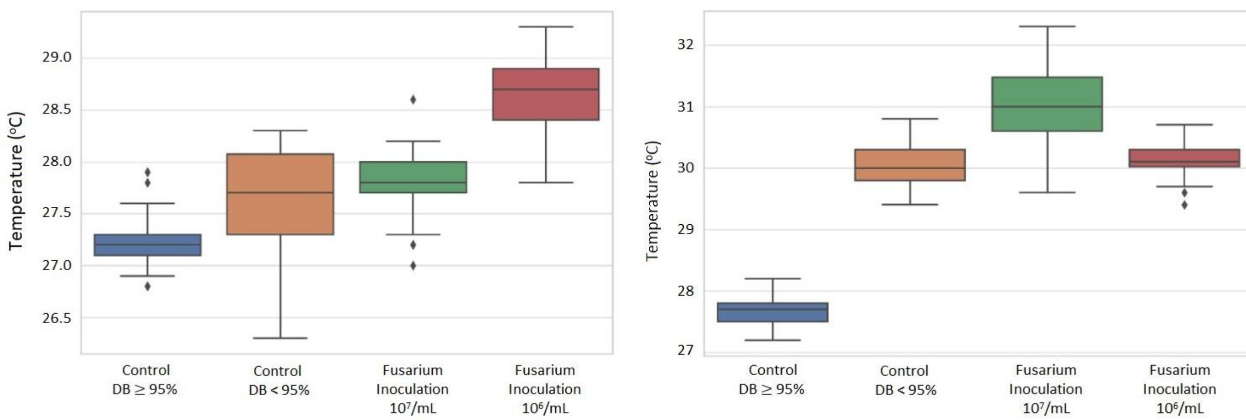


Figure 4. Boxplot of seed temperature for the IR64 variety: (a) on the third day; and (b) on the fifth day

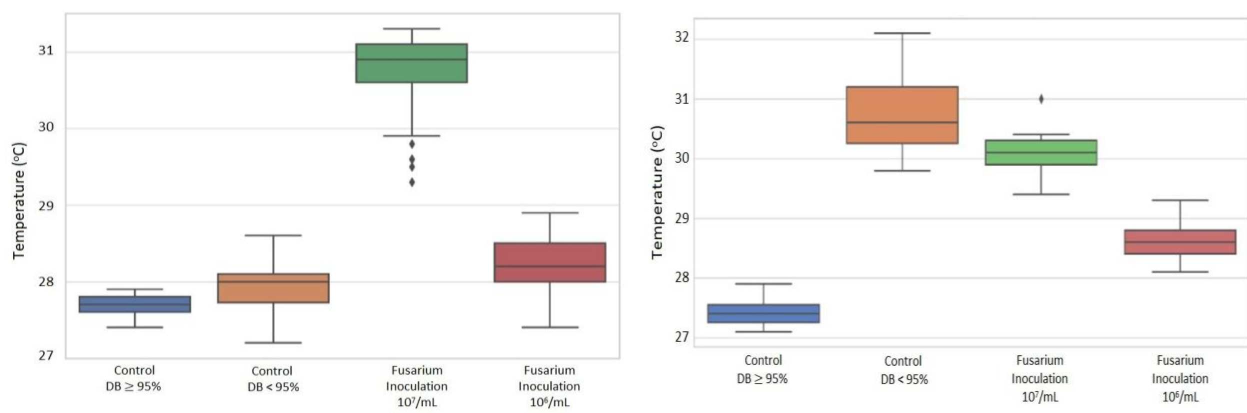


Figure 5. Boxplot of seed temperature for the Mekongga variety: (a) on the third day; and (b) on the fifth day

In the IR64 variety, the observation results showed a significant increase in median temperature in seeds inoculated with *Fusarium* sp. with a concentration of 10^7 conidia/ml, which began to be detected from day 2 until it peaked on day 4. On day 2, temperature outliers began to appear indicating greater variation in the inoculated seeds compared to the controls (Figure 4a). On day 4, the temperature of the infected seeds showed a higher spike, but with greater variation between the individuals of the seeds tested, when compared to control and other treatments (Figure 4b). This significant increase in temperature reflects an increase in metabolic activity caused by *Fusarium* sp. pathogenic infection, which indicates the physiological response of plants to biotic stress induced by fungal infections.

In the Mekongga variety, observations showed a more gradual increase in temperature compared to the IR64 variety, with a temperature spread in the seeds inoculated with *Fusarium* sp. 10^7 conidia/ml widened further into the 3rd day, which also recorded its peak temperature of the day. The peak of temperature variability that occurs on day 3 reflects a slightly slower, but still significant, infection response, which can be seen in Figures 5a and 5b. The boxplot showed a difference in seed temperature distribution between control and treatment, as well as between the beginning and end days of incubation. These findings further strengthen the assumption that *Fusarium* sp. causes an increase in metabolic temperature in infected seeds, as well as shows variability in response between seeds (Lipińska *et al*., 2022; Rippa *et al*., 2023).

3.4. Day 7 Seed Temperature Analysis vs Infection Status

In the analysis of seed temperature on day 7 against infection status, the scatterplots showed a clear distribution pattern in each variety, with temperature as a relevant predictor variable. In the Ciherang variety (Figure 6), most of the uninfected seeds were in the temperature range of 26.0–28.5°C, while the infected seeds were concentrated in the temperature range of 28.5–30.5°C, with a fairly firm separation between these two groups, supporting temperature as an indicator of *Fusarium sp* infection. In the IR64 variety (Figure 7), uninfected seeds were in the temperature range of 26.8–28.8°C, whereas more infected seeds were in the temperature range of 29.0–30.5°C, with inoculation treatments of 10^6/ml and 10^7/ml showing a higher temperature pattern than controls, indicating an earlier detected physiological response. Similarly, in the Mekongga variety (Figure 8), where healthy seeds dominate the temperature of 26.5–28.5°C and infected seeds are in the temperature range of 28.5–30.8°C, with a clear separation between the temperature groups of healthy and infected seeds. These patterns support temperature as a non-destructive indicator of *Fusarium* sp. infection (Ali *et al*., 2020; Tahmasebi *et al*., 2023).

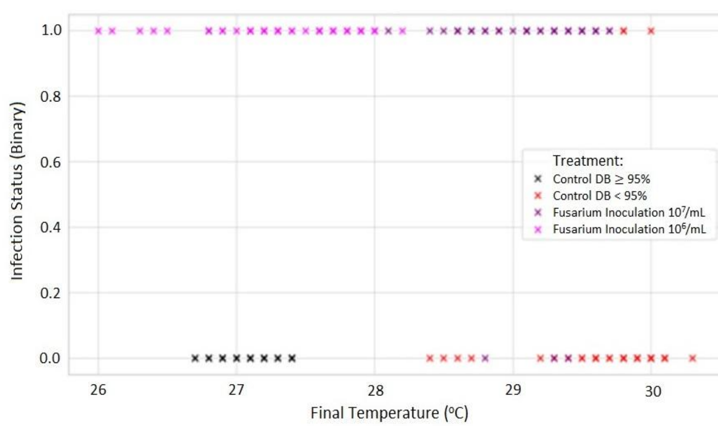


Figure 6. Scatterplot seed temperature vs. infection status of Ciherang variety on day 7

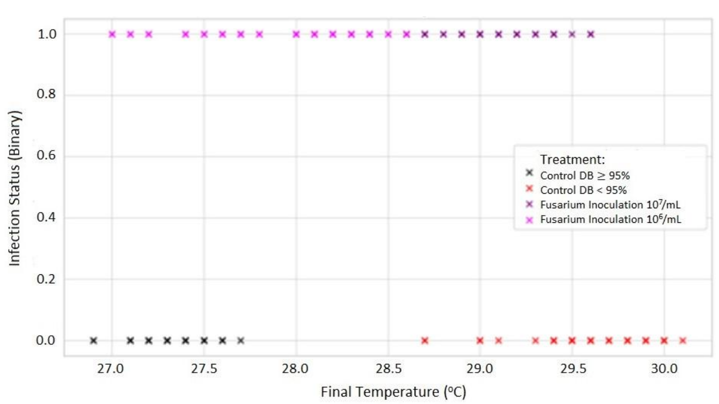


Figure 7. Scatterplot seed temperature vs. infection status of IR64 variety on day 7

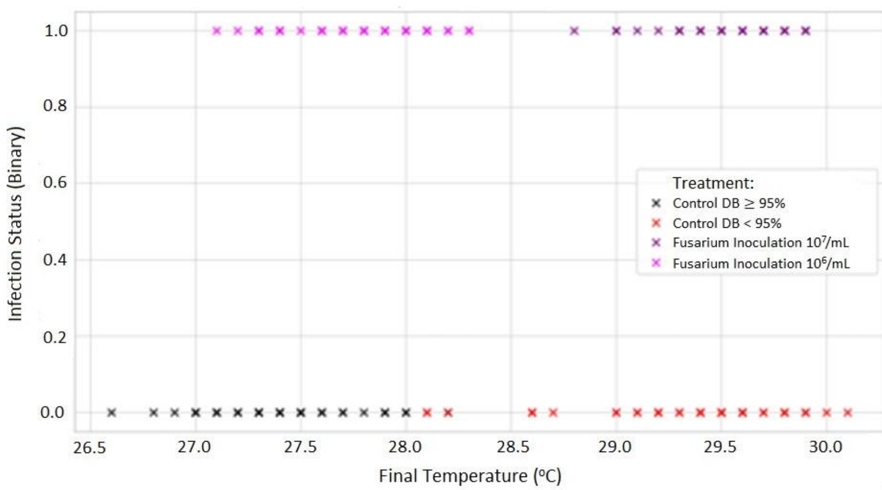
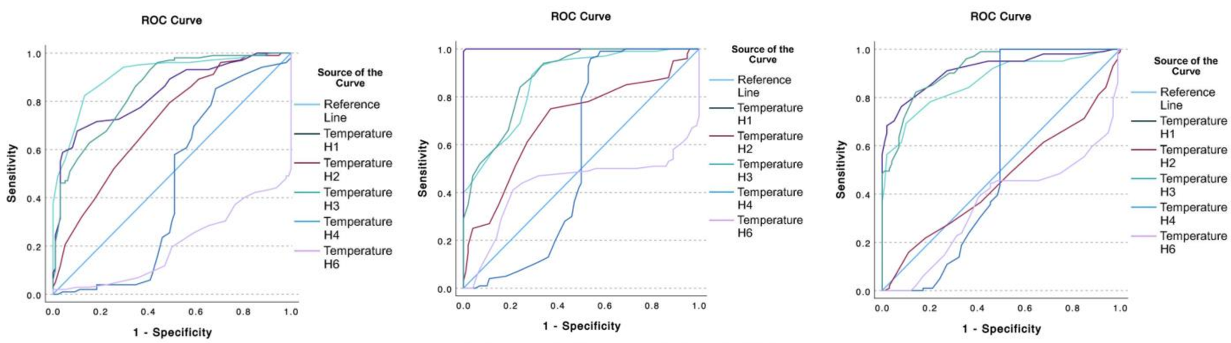


Figure 8. Scatterplot seed temperature vs. infection status of Mekongga variety on day 7



(a) (b) (c)

Figure 9. ROC curve of the three rice varieties: (a) Ciherang, (b) IR64, and (c) Mekongga

3.5. ROC Curve and Logistic Regression Analysis

ROC (Receiver Operating Characteristic) analysis is performed to determine the day with the best classification performance based on the AUC (Area Under Curve) value, which refers to the time-dependent ROC approach (Kamarudin *et al*., 2017; Nahm, 2022). This approach allows the identification of the most physiologically relevant time in the seed infection process. The results of the analysis showed that in the Ciherang variety (Figure 9a), the highest AUC was recorded on day 2 with a value of 0.909, indicating that the temperature on that day had high sensitivity and specificity in predicting infection. For the IR64 variety (Figure 9b), the highest AUC was recorded on day 3 with a value of 1,000, indicating a perfect classification ability of the infection status, in line with the pattern of early temperature increase in this variety. Meanwhile, in the Mekongga variety (Figure 9c), the highest AUC was recorded on the 4th day with a value of 0.920, indicating that the temperature on that day had excellent classification accuracy in predicting infection. Based on the best AUC, a logistic regression analysis was performed, which showed that temperature had a significant relationship with the likelihood of infection, with a regression coefficient of positive value, which indicated that the higher the surface temperature of the seeds, the greater the chance of infection. This regression model yields a p-value of 0.05, which confirms that temperature is statistically significant in predicting infection, and the full results are presented in Table 1, including coefficient (β), standard error (SE), p-value, and odds ratio (OR).

Based on Table 1 presents the results of the seed temperature logistic regression analysis on the infection status of three rice varieties (Ciherang, IR64, and Mekongga). For Ciherang, the coefficient (β) is 5.012 with a p-value of <0.001, indicating a strong significant relationship between seed temperature and infection status. The odds ratio of

150.279 suggests that the likelihood of infection increases significantly with changes in seed temperature for this variety. The prediction accuracy for Ciherang is 83.5%, which indicates the high accuracy of the model in predicting infection status for this variety. IR64, on the other hand, has a very large coefficient (71.952) and a very high standard error (3719.818), which leads to a *p*-value of 0.985, indicating no significant relationship between seed temperature and infection status. Additionally, the model for IR64 experienced perfect separation, meaning that the data points could be perfectly classified, but the model couldn't provide a meaningful odds ratio. Finally, Mekongga has a coefficient of 0.992 and a *p*-value of 0.022, which indicates a statistically significant but weaker relationship between seed temperature and infection status. The odds ratio of 2.698 shows that the likelihood of infection for Mekongga is 2.7 times higher with changes in seed temperature. Its prediction accuracy is 91.0%, which suggests it has the highest prediction reliability among the three varieties.

Table 1. Results of seed temperature logistic regression analysis on infection status

Varieties	Coefficient (β)	Std. Error	*p*-value	Odds Ratio	Prediction Accuracy
Ciherang	5.012	0.693	<0.001	150.279	83.5%
IR64	71.952	3719.818	0.985	-	100% (model has perfect separation)
Mekongga	0.992	0.434	0.022	2.698	91.0%

3.6. ANOVA and Non-Parametric Tests

The results of the ANOVA test and the non-parametric test for each variety showed that the surface temperature of the seeds differed significantly between treatments, especially on days 3 to 6. In the Ciherang variety, the ANOVA test showed $p < 0.05$ from day 3, which was supported by the t and Mann-Whitney tests, which indicated a real difference between infected and uninfected seeds. A similar pattern was found in the IR64 and Mekongga varieties, although in IR64, significance values appeared from day 2. These findings are consistent with trend graphs and scatterplot visualizations, which show that the temperature of infected seeds is higher than that of control seeds. The Mann-Whitney test, which was used on data that did not meet the assumptions of normality and homogeneity, corroborated these findings, so the statistical data support the interpretation that seed surface temperature can significantly differentiate the infection conditions in all three varieties (Darma, 2021; Hardani *et al*., 2020; Ramadhany, 2024).

3.7. Combined Analysis of the Three Varieties

Table 2 presents the combined daily average temperatures of three rice varieties (Control 1, Control 2, Treatment 1, and Treatment 2) measured across seven incubation days. The temperatures were recorded with specific details for each variety and day, showing slight variation across the days. Control varieties generally exhibited lower temperatures than the treatment varieties, with Treatment 2 having the highest temperatures starting from Day 3 onward, particularly on Day 4 where it reached 31.66°C. The data also shows minor fluctuations in the standard deviations (represented as ±) for each variety, suggesting consistent temperature patterns across the incubation days.

Table 3 outlines the results of an ANOVA analysis conducted on the combined seed temperatures of the three rice varieties, focusing on the variation between groups across each incubation day. Sum of squares (between) indicates the total variation explained by the differences between the groups for each day, while mean square (between) represents

Table 2. Result recapitulation of combined daily average temperature of 3 rice varieties

Incubation Day	Control 1 (DB < 95%)	Control 2 (DB ≥ 95%)	Treatment 1 (Inoculation* 10^6/mL)	Treatment 2 (Inoculation* 10^7/mL)
Day 0	27.62±0.36	26.96±0.37	28.01±0.39	27.87±0.42
Day 1	27.83±0.44	28.04±0.33	28.16±0.50	28.08±0.24
Day 2	27.67±0.38	27.28±0.28	28.46±0.47	27.94±0.31
Day 3	27.96±0.35	27.64±0.19	28.51±0.49	30.75±0.38
Day 4	30.03±0.42	27.66±0.27	30.16±0.31	31.66±0.65
Day 5	30.67±0.50	27.83±0.54	28.65±0.33	30.09±0.44
Day 6	29.86±0.91	29.15±0.56	28.03±0.44	29.79±0.43
Day 7	29.55±0.42	27.27±0.26	27.70±0.49	29.23±0.41

Table 3. ANOVA results combined seed temperature of 3 rice varieties

Incubation Day	Sum of Squares (Between)	df	Mean Square (Between)	F-Value	*p*-Value
Day 0	98.353	3	32.784	219.149	< 0.001
Day 1	8.880	3	2.960	19.419	< 0.001
Day 2	110.734	3	36.911	274.866	< 0.001
Day 3	889.712	3	296.571	2165.223	< 0.001
Day 4	1227.597	3	409.199	2117.878	< 0.001
Day 5	761.064	3	253.688	1205.733	< 0.001
Day 6	323.926	3	107.975	285.379	< 0.001
Day 7	566768	3	188.923	1151.632	< 0.001

the average variation. The *F*-values show the ratio of variance between the groups to the variance within the groups, with all *p*-values being significantly less than 0.001. This indicates that the differences in daily average temperatures between the four treatments were statistically significant across all days, confirming that the experimental treatments had a notable impact on temperature changes.

3.8. Research Implications

This study shows that thermal imaging technology can be an efficient and non-destructive tool in detecting *Fusarium* sp. On the Seeds. A key implication of this study is the potential to replace conventional detection methods such as the Blotter Test, which require greater time and resources. This technology can speed up the infection detection process, provide significant advantages in improving disease resistance, and support sustainability and efficiency in the seed and agriculture industries as a whole. The results of this study also open up opportunities for the development of thermal imaging technology for further applications in the agricultural sector, which can help improve food security and economic stability of farmers.

3.9. Research Limitations

A major limitation of this study is the use of thermal imaging technology that relies heavily on expensive hardware, such as thermal cameras, which may not be easily accessible to farmers or small industries. In addition, the study was limited to three specific rice varieties, which means that the results obtained cannot necessarily be generalized to all existing rice varieties. Further research is also needed to explore possible external factors, such as humidity and light, that could affect detection accuracy using thermal imaging technology.

4. CONCLUSION

The conclusion of this study is that thermal imaging technology can be used effectively to detect *Fusarium* sp. on rice seeds with better accuracy compared to conventional methods. An increase in the surface temperature of infected seeds can be a useful indicator in detecting infection at an early stage. The suggestion for further research is to extend the application of this technology to other rice varieties and to test the application of this technique in more diverse field conditions. In addition, further research can focus on developing thermal imaging devices that are more affordable and easily accessible to farmers to increase the widespread adoption of this technology

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
AA	✓	✓	✓		✓	✓	✓		✓	✓	✓			
HN				✓				✓		✓		✓		
Mar								✓		✓		✓		

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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