



Pathogenecity and *in-vitro* Assessment of Some Fungicides on the Mycelial Growth of Rice Blast Pathogen (*Magnaporthe oryzae* B. Cauch) in Jigawa State, Nigeria

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Abstract

It is beyond any reasonable doubt that rice (*Oryza sativa* L.) ranks first among its counterparts cereal food crops in the world, being unmatched in both worldwide demand and economic significance. Serving as a staple food to about two-thirds of the world population, the cultivation of this crop faces various constraints due to numerous infections, among which rice blast stands out as a significant issue affecting rice production in Jigawa state, Nigeria. In order to avert the reported situation, an *in-vitro* experiment was conducted using five selected fungicides through food poisoning technique at 10,000, 1,000 and 100 ppm. Results of *in-vitro* test showed that among the five tested fungicides, Mancozeb and Hexaconazole appeared to have higher fungicidal activity against *M. oryzae* by completely inhibiting the fungal growth at 1,000 and 10,000 ppm of Z-force (Mancozeb) and the growth was restricted at 10,000 ppm of Hexacal (50 g Hexaconazole litre⁻¹). The fungal mycelium's expansion of *M. oryzae* at 10,000 ppm of Dress-force (Imidacloprid 20% + Metalaxyl-M 20% + Tebuconazole 2% WS) was 9.33 mm, Seed care (Imidacloprid 10% + Thiram 10% WS) was 14.67 mm and for the Blast force (Isoprothiolane 40% WP) was 16.00 mm. The plates used as control exhibited the most extensive mycelial growth of the tested pathogen (measuring 59.33 mm). This connotes that of the five fungicides tested, Mancozeb and Hexaconazole could best be used for the eradication of *M. oryzae* infection on rice pending further research.

Keywords: Fungicides, in vitro, Mycelial growth, Pathogenecity, Rice blast

Introduction

Rice blast, a pervasive issue in rice cultivation, poses a significant threat due to its potential to cause complete yield loss, reaching up to 100% in optimal environmental condition. This disease is widely acknowledged as the foremost global affliction affecting rice production, spanning various regions worldwide and has been documented in over 102 countries across the globe. Given that more than three billion individuals rely on rice as a staple food, it is not surprising that this disease has garnered numerous appellations (Chakraborty *et al.*, 2023). In a previous report, the NRC (1996) emphasized the importance of rice in improving nutrition as it supplies almost 60% of the energy

from diet and protein from plant sources. This contributes to food security, rural development, and sustainable land management. However, the need for rice is projected to witness a sustained growth in the forthcoming years, specifically until the year 2035. As per an all-encompassing investigation carried out, it can be anticipated that the global requirement for processed rice will escalate to 496 million tons by 2020, in comparison to the 439 million tons recorded in 2010 (Samal *et al.*, 2022).

Coming to Nigerian context, the Federal Government had since 2018 banned the Rice importation into the country. As a result, Nigerians now have to increase rice production to meet the demands of the country's teeming population

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of over 200 million persons. However, rice is a special crop as it does not thrive in many agricultural fields. It does best in swamp, Wet lands or waterlogged soils free from biotic and abiotic stresses. This has actually made its production a little bit difficult. And even in regions where it is produced under irrigation in northern Nigeria, the crop suffers from the emergence of some deadly diseases which if not properly checked, may reduce the production of this crop commercially (Hadiza et al., 2022). One major disease reported on rice in Jigawa state of Nigeria is rice blast which was found to be associated with other diseases in the area notably sheath blight, spot and others (Hadiza et al., 2022). Although there seem to be no any report of this nature from Kano and Katsina states, some pockets of the pathogens might be there in isolated cases or has not been exploited yet from these areas.

Rice blast, a widespread challenge in rice farming worldwide, represents a considerable threat because it has the capacity to lead to a complete loss of yield, reaching up to 100% under favorable circumstances (Chakraborty et al., 2023). This disease is widely acknowledged as the most crucial worldwide disease in rice-producing regions, having been observed in over 85 countries (Wang et al., 2022). As a result, it stands out as the predominant fungal infection affecting rice cultivation (Sun et al., 2020).

Blast infection is capable of infiltrating rice plants at various stages, from seedling to maturity and can result in complete seedling loss in seedbeds as well as epidemics in the field. The effects of the infection can be observed through the presence of lesions on different areas of the plant such as leaves, leaf collars, stems, and nodes, internodal sections of culms, panicles, and even grains; while *M. oryzae* has the ability to infect all leaf tissues, infection of the panicle can lead to complete loss of grains. Depending on the specific part of the rice plant that is affected, this disease may be referred to as leaf blast, collar rot, node blast, panicle blast, or rotten neck blast (Paul et al., 2022). Symptoms manifest on all plant parts located above ground. The prevailing symptom typically consists of lesions or spots, measuring approximately 1-1.5 cm in length and 0.3-0.5 cm in width (Mandal et al., 2023).

Rice blast outbreaks can be highly intense in an ecosystem with temperate and sub-tropical climate, particularly when there is inadequate or ineffective control practice. The blast disease caused by *M. oryzae*, which this pathogen also named as *Pyricularia oryzae*, remained a crucial threat to rice cultivation worldwide (Mynbayeva et al., 2023). Blast-induced losses manifest through notable declines in crop yield, milling and the financial burden incurred from fungicide application. Unlike the majority of rice diseases, blast demonstrates an explosive nature, capable of swiftly obliterating an entire harvest. The extent of the resulting detriment hinges upon the specific plant part affected and the variety in question. Infection of the leaf results in a reduction in photosynthetic area, potentially leading to the death of the plant. Panicle infections have decline yield, thereby resulting in significant economic detriments (Paul et al., 2022).

Blast epidemics primarily rely on climatic factors, agricultural practices including nitrogen application and water availability and the susceptibility of specific cultivars. Although nutrition plays a beneficial role in disease management, certain farming practices can potentially disrupt nutritional balance, leading to the development of diseases (Borer et al., 2022). Similarly, Sharma (2020) stated that an excess of nitrogen fosters disease development, thereby promoting an increase in inoculum levels. Conversely, it has been noted that a deficiency in nitrogen also leads to an increase in disease incidence, particularly observed in weakened plants lacking adequate defense mechanisms against diseases (Easterday et al., 2022; Yan et al., 2023).

Materials and Methods

Source of Inoculum

Leaf and panicle samples exhibiting symptoms of blast disease were gathered from each farmer's field where the disease had been identified during a survey of rice fields carried out in 2020 preceding this study. The afflicted samples (both leaves and panicles) were carefully inserted in paper bags and appropriately labeled with the collector's name, sample location, and date of collection (Amayo et al., 2020; Kutama et al., 2012). The samples were transported to the Federal University Dutse Biology Laboratory for additional analysis.

Isolation and Identification of *M. oryzae*

The infected plant materials collected from the field during 2020 dry and wet seasons were used for isolation of the blast disease causal pathogen (*Magnaporthe oryzae*) using standard tissue isolation technique. Infected neck and leaf sections were incised longitudinally using a sterilized scalpel and subsequently rinsed thoroughly with flowing tap water before being divided into smaller fragments. Using 3.5% Sodium hypochlorite (NaHCl) solution, these samples were sterilized for a period of 30 seconds and then washed thrice in sterile deionized water, followed by inoculation on to Potato Dextrose Agar (PDA) plates and allowed to growth at temperature of 28 ± 1 °C for ten days until fungal growth with *Magnaporthe oryzae* were appeared. The Fungal identity was confirmed based on cultural characteristics and conidial morphology. Observation was conducted on the radial expansion of colonies at the point of maximum growth in a particular isolate plate during the incubation process. Additional cultural characteristics, including growth rate, margin type, colony color, and spore production were documented. Media formulation and preparation method utilized in this study was according to the protocol used by Kutama et al. (2013) and Mustapha et al. (2022).

Monoconidial Isolation

Since *M. oryzae* is heterokaryotic, monoconidial isolation (hyphal tip) was used to purify the isolate and maintain the pure culture on 2% water agar plates. Lesions that observed to have well-developed were identified, extracted, then thoroughly rinsed in flowing tap water for a duration of 2 hours. The leaf fragments underwent surface sterilization with 3.5% Sodium hypochlorite, followed by sequential

washing with sterilized distilled water. They were then incubated to sporulate on sterile glass slides through placing in a humid chamber at a temperature of 28 °C for a period of 48 hours. Once sporulation is adequate, lesions were transferred to test tubes containing double distilled water and agitated for 1 minute using a vortex. Approximately 1 ml of the resulting spore suspension was aseptically inoculated onto a clean plates supplemented with 2% agar. Individual spores were subsequently identified and collected under microscopic examination. Following identification, spore was carefully placed onto PDA slants. The slants were allowed to incubate at 28 °C for 48 hours before being stored at 4 °C. The culture was refreshed every two months to maintain pure isolates of *M. oryzae* obtained through the hyphal tip isolation method (Al Noman and Shamsi, 2021; Teli *et al.*, 2016).

Sporulation Variation

Microscopic examinations were employed to evaluate the isolate's sporulation ability on the media. A small amount of culture was applied to a clean, grease-free slide and mixed thoroughly with lactophenol before covering it with a slip. The criteria for indexing sporulation frequency were applied according to the established technique outlined by Henry and Andersen (1948). Thus, more than 30 spores were regarded as excellent sporulation, 20-30 as good, 10-20 as fair and less than 10 spores as poor sporulation and indexed as 4, 3, 2, 1 and 0 where there was no sporulation observed respectively.

Pathogenicity Test

This test was conducted to verify the potential of the cultured fungus to induce blast disease under controlled conditions on healthy rice leaves. Faro-44, a widely recognized rice variety, was planted in sterilized soil. When the seedlings reached the appropriate stage, they were treated with a spore suspension prepared by submerging a 15-day-old culture in 10 ml of distilled water, then vigorously agitating followed by filtering for 90 seconds to get rid of mycelial fragments. Spore density was adjusted to 1×10^5 spores per milliliter using a hemocytometer before inoculation. A control group consisted of non-inoculated plants, while inoculated plants were protected with transparent nylon bags to maintain required air moisture, and symptoms' development was periodically monitored. Infected plants were subjected to re-isolation, and isolate obtained were compared with the one used during inoculation for the confirmation of the pathogen identity (Hasan *et al.*, 2016; Teli *et al.*, 2016).

In-vitro Assay on the Effect of Fungicides on Mycelial Growth of *M. oryzae*

Five fungicides, namely Z-force (Mancozeb 80% WP), Dress force (Imidacloprid 20%, Metalaxyl-M 20%, and Tebuconazole 2%), Seed care (Imidacloprid 10%, Thiram 10% WS), Blast force (Isoprothiolane 40% WP), and Hexacal (50 g Hexaconazole L⁻¹), were evaluated using the food poisoning method at concentrations of 100 ppm, 1,000 ppm, and 10,000 ppm. These fungicides were added to

potato dextrose agar (PDA) medium immediately after sterilization. PDA medium lacking any fungicide served as the control. To prevent bacterial contamination, the sterilized PDA was supplemented with Streptomycin Sulphate at a concentration of 1 ml L⁻¹ and Penicillin at 1,000,000 units L⁻¹. Once the PDA medium solidified, a 1 cm disc of pure culture of *M. oryzae* was inoculated at the radius of each agar plates and incubated at 30 °C. Each treatment was replicated four times. Radial growth of the test fungus was recorded at every 24h until the mycelial growth fully covered the upper surface in the control treatment. This experimental design was based on the methodology described by Hajano *et al.* (2012) and Kulkarni and Peshwe (2019).

Determination of Disease Parameters

Two weeks after the second/ final spray (2 WAFS), observations on the incidence of leaf blast were observed and expressed as Percent Disease Incidence (PDI) according to Mustapha *et al.* (2022). Disease Severity Rating (DSR) was also according to the rating scale of 0-5 scale according to Turaidar *et al.* (2018). Thus, 1 = No visible symptoms, 2 = 1-10% (slightly severe), 3 = 11-25% (moderately severe), 4 = 26-59% (severe infection) and 5 = 60≤-90% (highly susceptible, necrosis and complete death of plant occur) were considered as the disease rating scale.

Statistical Analysis

Data obtained from the determination of the *in-vitro* susceptibility, precisely the mycelial growth was subjected to single factor ANOVA at P<0.05. Least Significant Difference (LSD) was used as *post hoc* test where statistics was found to be significant. All statistics was carried out using IBM-SPSS statistical software.

Results and Discussion

Pathogenicity Test

A pathogenicity assessment (Table 1) was conducted on rice blast pathogen (*M. oryzae*) isolated from infected rice sampled from three different sampling sites within the study area. Characteristic blast symptoms were observed on the 14th day after inoculation, corresponding to two weeks post-inoculation (2 WAI). Initially, symptoms of leaf blast was observed as reduced, water-soaked and grey-green colored spots with a dark-green margin, that quickly expand to several millimeters wide. Eventually, they developed into spindle-shaped lesion with a greyish-white center, surrounded by a brown margin and some necrotic areas (Zewdu, 2021). In instances of neck blast, infected panicles became darkened and shrunken, resulting in a chaffy ear head during the early stages. Later, the panicle drooped downward at the neck, while the infected nodes turned black. The observed symptoms in this study aligned with the description provided by Kulmitra *et al.* (2017).

Effects of the Selected Fungicides on Mycelial Growth of *M. oryzae*

The *M. oryzae* mycelial growth exhibited varying responses to distinct fungicides employed in the experiment. Significant differences in the inhibition of growth of mycelial in *M.*

oryzae were observed across different concentrations tested of each fungicide, as determined by ANOVA (LSD = 5.991; d.f. = 30; $p < 0.05$).

Table 1: Pathogenicity of isolated pathogen (*M. oryzae*) on healthy rice (FARO 44)

Sample Isolate	Disease Parameters		
	PDI (%)	DSR	Status
Auyo Isolate	65.40	4	Severe (susceptible host)
Hadejia Isolate	70.20	4	Severe (susceptible host)
Kaugama Isolate	68.40	4	Severe (susceptible host)

[Legends: PDI = Percent Disease Incidence, DSR = Disease severity rating]

As the fungicides concentrations increased, mycelial growth of *M. oryzae* progressively diminished. Notably, Mancozeb demonstrated complete inhibition of the test fungus's growth. It emerged as the most efficacious fungicide, preventing *M. oryzae* growth at concentrations of 10,000 and 1000 ppm, respectively (Sumaya et al., 2023). Various

studies have explored a broad spectrum of fungicides and several have proven effective at inhibiting rice blast fungus, *M. oryzae* (Lori et al., 2021; Song et al., 2022; Balol et al., 2022). Nevertheless, Mancozeb demonstrated the most potent fungicidal efficacy in inhibiting the growth of *M. oryzae*, displaying an EC_{50} of 0.25 ppm (Kongcharoen et al., 2020). Thus, it can be deduced and corroborates the findings of this research that Mancozeb serves as an effective agent in managing rice blast disease induced by *M. oryzae*.

However, fungal growth inhibition observed in Mancozeb could be due to its potentiality to inhibit fungal growth through multiple mechanisms. Studies have demonstrated that it induces cytological abnormalities in yeast, suggesting its pro-oxidant and apoptotic impacts. Alternatively, its high compatibility with thiol groups in proteins leads to oxidative reactions, degradation of protein, and disruption of energy synthesis in yeast (Scariot et al., 2016). Additionally, this effects could be linked to its ability to elevates mitochondrial Reactive Oxygen Species levels and hyper-polarizes mitochondrial membranes, ultimately leading to apoptosis (Obiazikwor and Ojeile, 2021). Nonetheless, the fungicide also hampers enzyme function in fungi by creating a compound with metal-containing enzymes crucial in ATP synthesis (Patel et al., 2014).

Table 2: *In-vitro* Efficacy of Different Fungicides on Mycelial Growth of *M. oryzae*

Treatment	Zone of mycelial growth (mm)		
	100	1000	10000
Mancozeb 80% WP	24.00 ^c	0.0 ^e	0.00 ^e
Imidacloprid 20%, Metalaxyl-M 20% and Tebuconazole	35.33 ^b	16.67 ^d	9.33 ^d
Imidacloprid 10% and Thiram 10% WS	40.67 ^b	22.67 ^c	14.67 ^d
Isoprothiolane 40% WP	38.00 ^b	27.33 ^c	16.00 ^d
50 g Hexaconazole L ⁻¹	34.00 ^b	14.00	0.00 ^e
Control		59.33 ^a	
LSD		5.991	

As it showed in the table 2 above, among the five tested fungicides, Mancozeb and Hexaconazole appeared to be the fungicides with higher inhibitory effect against the *M. oryzae*, inducing growth restriction at 1000 and 10,000 ppm of Mancozeb and the growth was restricted at 10,000 ppm of Hexacal (50 g Hexaconazole L⁻¹). All other fungicides were observed to have lower effect of inhibiting the growth of mycelia of the pathogen completely. The growth of *M. oryzae* at 10,000 ppm of Dress-force (Imidacloprid 20% + Metalaxyl-M 20% + Tebuconazole 2% WS) was 9.33 mm, Seed care (Imidacloprid 10% + Thiram 10% WS) was 14.67 mm and for the Blast force (Isoprothiolane 40% WP) was 16.00 mm. The plates used as control exhibited the most extensive mycelial growth of the tested fungus, measuring 59.33 mm.

Conclusion

The *in-vitro* assessment of the five fungicides revealed that Mancozeb and Hexaconazole exhibited significant effectiveness against *M. oryzae*. The fungus's growth was entirely suppressed at concentrations of 1000 and 10,000 ppm for Mancozeb, and growth was limited at 10,000 ppm

for Hexacal (50 g Hexaconazole L⁻¹). However, all other fungicides were deemed ineffective.

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