

# Inhibitory potential of certain fungicides on *Pyricularia oryzae* Cavara

## Abstract

Tricyclazole has been a successful solution for managing rice blast for over three decades. However, the continuous use of this fungicide has led to the development of resistance and the accumulation of tricyclazole residues within rice grains, prompting a ban on its usage in India. In response to this pressing issue, the present study aims to evaluate the efficacy of alternative fungicides in controlling the growth of *Pyricularia oryzae*, the causal agent of rice blast. The study employed a comprehensive approach, assessing the performance of various fungicides, including picoxystrobin, hexaconazole, isoprothiolane, kresoxim methyl, pyraclostrobin, tebuconazole, kitazin, and zineb, at varying dosage levels (100%, 75%, and 50% of the recommended dose). The findings of this investigation indicated that hexaconazole, isoprothiolane, kitazin, and zineb exhibited a 100% inhibition of *P. oryzae* growth across all tested doses. Pyraclostrobin also demonstrated a 100% inhibition at the highest dose, although its effectiveness decreased slightly at lower doses. In contrast, picoxystrobin and kresoxim methyl did not exhibit significant effectiveness in controlling the growth of the rice blast pathogen. These results provide valuable insights into the potential alternatives to tricyclazole for managing rice blast when used either alone or in combination at reduced doses.

Keywords: Efficacy, Fungicides, Hexaconazole, Mycelium, *Pyricularia* and Rice blast

## Introduction

Rice (*Oryza sativa*) is a staple food for more than half of the world's population, with a productivity of 4.3 tons per hectare (USDA-IPAD, 2023). However, rice productivity is being hampered by various insects and diseases, leading to qualitative and quantitative losses (Law *et al.*, 2017; Wilson and Talbot, 2009). Among these, the 'blast' disease caused by the hemibiotrophic recalcitrant fungus *Pyricularia oryzae* Cavara is the most worrisome, as it can infect the rice crop at all stages of growth and cause up to 100% yield losses under favorable conditions (Netam *et al.*, 2011).

Current disease management strategies involve the use of resistant varieties, biocontrol agents, botanicals, plant extracts, and fungicides (Hazano *et al.*, 2012; Akhilesh *et al.*, 2017; Nazifa *et al.*, 2021). However, the rapid evolution of novel virulent races of *P. oryzae* due to genetic mutations can quickly overcome the resistance in varieties (Wang and Valent, 2017; Gladieux *et al.*, 2018). While biological control approaches using beneficial microbes are environmentally friendly, their effectiveness and persistence under inundated conditions of rice fields remain uncertain (Kumar *et al.*, 2024). Furthermore, the active principles and modes of action behind the *in vitro* efficacy of plant-based extracts are still unknown (Nazifa *et al.*, 2021).

Given these limitations, the use of fungicides for rice blast management remains a viable strategy for farmers. Several fungicides, such as tricyclazole, carbendazim, and mancozeb, have been found to be effective, but their long-term use has led to the development of resistance and residual effects in grains, resulting in their usage ban in some regions (Kongcharoen *et al.*, 2020; Mohiddin *et al.*, 2021). Therefore, the discovery of alternate fungicides for efficient rice blast management is of utmost importance (Fei *et al.*, 2024).

This study aims to evaluate the *in vitro* efficacy of certain fungicides on the growth of *P. oryzae*, the causative agent of rice blast disease. The findings of this research will contribute to the development of more effective and sustainable strategies for managing this devastating disease, ensuring the continued productivity and food security of rice-growing regions.

## Material and Methods

The present experiment was conducted at the Department of Plant Pathology, Agricultural College, Bapatla, with the objective of evaluating the *in vitro* efficacy of certain fungicides on the radial growth of *Pyricularia oryzae*, the causal agent of rice blast disease.

### *Isolation of the Pathogen*

The pathogen, *P. oryzae*, was isolated from infected leaf tissue using the spore drop method with slight modifications (Choi *et al.*, 1999). Rice blast lesions on infected leaves were cut into halves, with each half containing a section of healthy tissue. The tissue bits were surface-sterilized with 1% sodium hypochlorite and rinsed with sterile distilled water. The surface-sterilized tissue bits were then attached to the upper lid of a Petri dish containing 2% water agar, with the adaxial part facing the medium. The plates were incubated at room temperature, and single spore colonies of *P. oryzae* were observed under a light microscope. Each single spore that fell on the water agar was marked and transferred to Petri plates containing potato dextrose agar (PDA). The obtained pathogen culture was then transferred to PDA slants and stored at 4°C for further use.

### *In vitro* Evaluation of Fungicide Efficacy

The efficacy of the selected CIB&RC, India recommended fungicides on the growth of *P. oryzae* was tested *in vitro* using the poison food technique (Nene and Thapliyal, 1973). The fungicides evaluated in the present study were picoxystrobin, hexaconazole, isoprothiolane, kresoxim methyl, pyraclostrobin, tebuconazole, kitazin, and zineb, as described in Table 1.

A fungicide stock solution of 100,000 ppm was prepared by dissolving 1 g of each fungicide in 9 ml of sterile distilled water. Working standards of the desired concentrations of the poisoned media were then made using the formula  $C_1V_1 = C_2V_2$ . The poisoned media were poured into separate Petri dishes under aseptic conditions and allowed to solidify. Mycelial plugs of 7 mm diameter were cut using a sterile cork borer and placed in the center of the Petri plates containing the poisoned medium at 100%, 75% and 50% of the recommended dose, designated as dose 1, dose 2 and dose 3, respectively. The PDA plate without any fungicide amendment was considered as the negative control. The inoculated plates were incubated at  $26 \pm 2^\circ\text{C}$  in an incubator. Data on the radial growth of the mycelium was recorded when full mycelial growth was achieved in the control plates. The inhibition percentage was calculated using the formula given by Vincent (1947): Inhibition percent (%) = ((radial growth in control – radial growth in treatment)/ radial growth in control) x 100.

### Statistical analysis

The experiment was conducted by using a completely randomized design with four replicates. Box-Cox transformation was employed to the radial growth values by using R software and were later subjected to one way analysis of variance. Duncan's multiple Range Test is used for means comparison.

## Results

The *in-vitro* efficacy of various fungicides on the growth of *P. oryzae*, a significant pathogen responsible for causing rice blast, was thoroughly investigated. The study evaluated the fungicidal activity at three different dosage levels, namely dose 1 (100%), dose 2 (75%), and dose 3 (50%), all of which were within the recommended range. From the results obtained it is pertinent that hexaconazole, isoprothiolane, kitazin and zineb exhibited complete (100%) inhibition of *P. oryzae* growth across all three dose levels. Pyraclostrobin also demonstrated a 100% inhibition rate at the lowest dose (dose 1), but its effectiveness slightly decreased in the higher doses, with 90.6% and 90.1% inhibition observed in dose 2 and dose 3, respectively (Table 2 and Figure1). Tebuconazole, another fungicide tested, showed promising results, achieving 100% inhibition in both dose 1 and dose 2. Even in the highest dose (dose 3), tebuconazole maintained a high inhibition rate of 98.3%. Regardless of the mycelial growth in dose 3 of tebuconazole, there is significant difference in the inhibition when compared to hexaconazole, isoprothiolane, kitazin and zineb. Interestingly, the study revealed that picoxystrobin and kresoxim-methyl exhibited relatively lower inhibition rates compared to the other fungicides tested. Significant inhibition was not observed in dose 1, dose 2 and dose 3 of picoxystrobin (84.8%, 80.1% and 71.6 %) and kresoxim methyl (70.9%, 65.0% and 52.6%). These findings provide valuable insights into the *in-vitro* efficacy of various fungicides against the growth of *P. oryzae*, a critical step in the development of effective disease management strategies for rice crops. The study's results highlight the superior performance of hexaconazole,

isoprothiolane, kitazin and zineb, particularly in situations where resistance to other fungicides may be a concern.

## Discussion

Rice blast, caused by the hemibiotrophic filamentous pathogen *P. oryzae*, poses a significant threat to rice production, with the potential for complete yield loss under favorable conditions. Despite the development of blast-resistant varieties through breeding programs and the utilization of biocontrol agents and botanicals, the application of fungicides remains a pivotal aspect of rice blast management. Tricyclazole, a melanin biosynthesis inhibitor, has been a highly effective fungicide for managing rice blast for over three decades (Peterson, 1990). However, its extensive usage has led to the development of resistance, prompting the need for alternative fungicides (Mohiddin *et al.*, 2021).

The present study aimed to evaluate the *in-vitro* efficacy of various fungicides against the growth of *P. oryzae*. The results demonstrate that the fungicides hexaconazole, isoprothiolane, zineb, and kitazin exhibited 100% inhibition of *P. oryzae* at all the tested concentrations (100%, 75%, and 50%). These findings align with previous studies that have reported the effectiveness of these fungicides in managing rice blast. Yadav *et al* (2022) ascertained the cent percent inhibition of *P. oryzae* on usage of kitazin at 500 ppm, 1000 ppm and 1500 ppm. Horo and Gudisa (2021) successfully managed rice blast by using hexaconazole @ 1 L ha<sup>-1</sup>.

Conversely, the inhibition percentages of picoxystrobin and kresoxim methyl decreased with the reduction in fungicide concentration, contrary to the documented efficacy of these compounds in controlling *P. oryzae* (Balol *et al.*, 2022; Neelakanth *et al.*, 2017 and Chen *et al.*, 2015). Additionally, Mohammad *et al.* (2018). found that tricyclazole, when applied alone or in combination with hexaconazole, remains an effective fungicide for rice blast management.

The results of the current study underscore the need for alternative fungicides to combat the growing resistance to tricyclazole. Among the tested fungicides, hexaconazole emerges as a promising alternative, demonstrating 100% inhibition of *P. oryzae* across all concentrations. This finding suggests that hexaconazole could be a viable option for the management of rice blast, particularly in areas where resistance to tricyclazole has been reported.

Conclusion : the present study provides valuable insights into the *in-vitro* efficacy of various fungicides against the rice blast pathogen, *P. oryzae*. The identification of effective alternative fungicides, such as hexaconazole, isoprothiolane, zineb, and kitazin, offers promising prospects for the sustainable management of this devastating disease, ensuring food security and supporting the livelihoods of rice farmers.

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**Table 1: *In-vitro* Efficacy of fungicides on the growth of *Pyricularia oryzae***

Tr.No.	Treatments	Dose (100 %)		Dose (75 %)		Dose (50 %)	
		Radial growth (in mm)	inhibition over control (%)	Radial growth (in mm)	inhibition over control (%)	Radial growth (in mm)	inhibition over control (%)
T1	Picoxystrobin	13.5 <sup>b</sup> (1.164)	84.8	17.5 <sup>c</sup> (1.687)	80.1	25.2 <sup>d</sup> (1.974)	71.6
T2	Hexaconazole	0.0 <sup>a</sup> (0.541)	100.0	0.0 <sup>a</sup> (0.601)	100.0	0.0 <sup>a</sup> (0.617)	100.0
T3	Isoprothiolane	0.0 <sup>a</sup> (0.541)	100.0	0.0 <sup>a</sup> (0.601)	100.0	0.0 <sup>a</sup> (0.617)	100.0
T4	Kresoxim methyl	25.7 <sup>c</sup> (1.226)	70.9	31 <sup>d</sup> (1.821)	65.0	42.2 <sup>e</sup> (2.118)	52.6
T5	Tebuconazole	0.0 <sup>a</sup> (0.541)	100.0	0.0 <sup>a</sup> (0.601)	100.0	1.5 <sup>b</sup> (1.009)	98.3
T6	Pyraclostrobin	0.0 <sup>a</sup> (0.541)	100.0	8.25 <sup>b</sup> (1.477)	90.6	8.7 <sup>c</sup> (1.623)	90.1
T7	Zineb	0.0 <sup>a</sup> (0.541)	100.0	0.0 <sup>a</sup> (0.601)	100.0	0.0 <sup>a</sup> (0.617)	100.0
T8	Kitazin	0.0 <sup>a</sup> (0.541)	100.0	0.0 <sup>a</sup> (0.601)	100.0	0.0 <sup>a</sup> (0.617)	100.0
T9	Control	88.7 <sup>d</sup> (1.292)		88.5 <sup>e</sup> (2.008)		89.2 <sup>f</sup> (2.294)	
	CD (P≤ 0.01)	0.007		0.025		0.058	
	SE(m) ±	0.003		0.009		0.020	
	CV (%)	0.651		1.571		3.128	

Figures in the parenthesis are Box-Cox transformed values. Dose (100%)-  $\lambda < -0.7474747$ ; Dose (75%)-  $\lambda < -0.4242424$ ; Dose (50%)-  $\lambda < -0.3434343$ .

**Table 2: Fungicides used for *In-vitro* efficacy evaluation**

Tr.No	Treatments	Trade name	Dose 1	Dose 2	Dose 3
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T1	Picoxystrobin 22.52% SC (0.12%)	Galileo	1200 ppm	900 ppm	600 ppm
T2	Hexaconazole 5% EC (0.2%)	Contaf	2000 ppm	1500 ppm	1000 ppm
T3	Isoprothiolane 40% EC (0.15%)	Fujione	1500 ppm	1125 ppm	750 ppm
T4	Kresoxim methyl 44.3% SC (0.1%)	Ergon	1000 ppm	750 ppm	500 ppm
T5	Tebuconazole 25.9% EC (0.15%)	Folicur	1500 ppm	1125 ppm	750 ppm
T6	Pyraclostrobin 100g/l CS (0.2%)	Seltima	2000 ppm	1500 ppm	1000 ppm
T7	Zineb 75% WP (0.2%)	Indofil Z-78	2000 ppm	1500 ppm	1000 ppm
T8	Iprobenfos 48% EC (0.2%)	Kitazin	2000 ppm	1500 ppm	1000 ppm

Dose 1- recommended dose, Dose 2- 75% of recommended dose and Dose 3- 50% of the recommended dose

