Journal of Advances in Biology & Biotechnology

Volume 27, Issue 10, Page 1405-1411, 2024; Article no.JABB.120442
ISSN: 2394-1081

Evaluating Alternative Fungicides for Effective Management of Rice Blast Disease: Inhibitory Effects on *Pyricularia oryzae* Cavara

N. Kiran Kumar [a*], P. Anil Kumar [a], P.Madhusudhan [b], Ch. Sreenivasa Rao [c] and B.Srikanth [c]

[a] *Department of Plant Pathology, ANGRAU-Agricultural College, Bapatla, India.*
[b] *Department of Plant Pathology, Agricultural Research Station, Nellore, India.*
[c] *Regional Agricultural Research Station, Lam, Guntur, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i101560

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120442

Original Research Article

Received: 04/06/2024
Accepted: 06/08/2024
Published: 23/10/2024

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

**Corresponding author: E-mail: n.kirankumar@angrau.ac.in;*

Cite as: *Kumar, N. Kiran, P. Anil Kumar, P.Madhusudhan, Ch. Sreenivasa Rao, and B.Srikanth. 2024. "Evaluating Alternative Fungicides for Effective Management of Rice Blast Disease: Inhibitory Effects on Pyricularia Oryzae Cavara". Journal of Advances in Biology & Biotechnology 27 (10):1405-11. https://doi.org/10.9734/jabb/2024/v27i101560.*

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; rice blast.

1. 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 [1,2]. Among these, the 'blast' disease caused by the hemi-biotrophic 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 favourable conditions [3].

Current disease management strategies involve resistant varieties, biocontrol agents, botanicals, plant extracts, and fungicides Hazano et al*.,* 2012; Akhilesh et al*.,* [4] Nazifa et al*.,* [5]. However, the rapid evolution of novel virulent races of *P. oryzae* due to genetic mutations can quickly overcome the resistance in varieties [6,7]. While biological control approaches using beneficial microbes are environmentally friendly, their effectiveness and persistence under inundated conditions of rice fields remain uncertain [8]. Furthermore, the active principles and modes of action behind the *in vitro* efficacy of plant-based extracts are still unknown [5].

Given these limitations, using fungicides for rice blast management remains a viable strategy for farmers. Several fungicides, such as tricyclazole, carbendazim, and mancozeb, are 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 [9,10]. Therefore, discovering alternate fungicides for efficient rice blast management is of utmost importance [11].

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.

2. MATERIALS AND METHODS

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

2.1 Isolation of the Pathogen

The pathogen, *P. oryzae*, was isolated from infected leaf tissue using the spore drop method with slight modifications [12]. 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.

2.2 *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 [13]. 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 ± 2°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 [14]. Inhibition percent (%) = ((radial growth in control – radial growth in treatment)/ radial growth in control) x 100.

2.3 Statistical Analysis

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

3. 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 Fig. 1). 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 a 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.

4. 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 favourable 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 blasts for over three decades [15]. However, its extensive usage has led to the development of resistance, prompting the need for alternative fungicides [10].

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. [16] ascertained the cent percent inhibition of *P. oryzae* on usage of kitazin at 500 ppm, 1000 ppm and 1500 ppm. Horo and Gudisa [17] successfully managed rice blast by using hexaconazole @ 1 L ha^{-1}.

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[b] (1.164)	84.8	17.5[c] (1.687)	80.1	25.2[d] (1.974)	71.6
T2	Hexaconazole	0.0[a] (0.541)	100.0	0.0[a] (0.601)	100.0	0.0[a] (0.617)	100.0
T3	Isoprothiolane	0.0[a] (0.541)	100.0	0.0[a] (0.601)	100.0	0.0[a] (0.617)	100.0
T4	Kresoxim methyl	25.7[c] (1.226)	70.9	31[d] (1.821)	65.0	42.2[e] (2.118)	52.6
T5	Tebuconazole	0.0[a] (0.541)	100.0	0.0[a] (0.601)	100.0	1.5[b] (1.009)	98.3
T6	Pyraclostrobin	0.0[a] (0.541)	100.0	8.25[b] (1.477)	90.6	8.7[c] (1.623)	90.1
T7	Zineb	0.0[a] (0.541)	100.0	0.0[a] (0.601)	100.0	0.0[a] (0.617)	100.0
T8	Kitazin	0.0[a] (0.541)	100.0	0.0[a] (0.601)	100.0	0.0[a] (0.617)	100.0
T9	Control	88.7[d] (1.292)		88.5[e] (2.008)		89.2[f] (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%)- λ<--0.7474747; Dose (75%)- λ<--0.4242424; Dose (50%)- λ<--0.3434343.

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

Tr.No	Treatments	Trade name	Dose 1	Dose 2	Dose 3
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

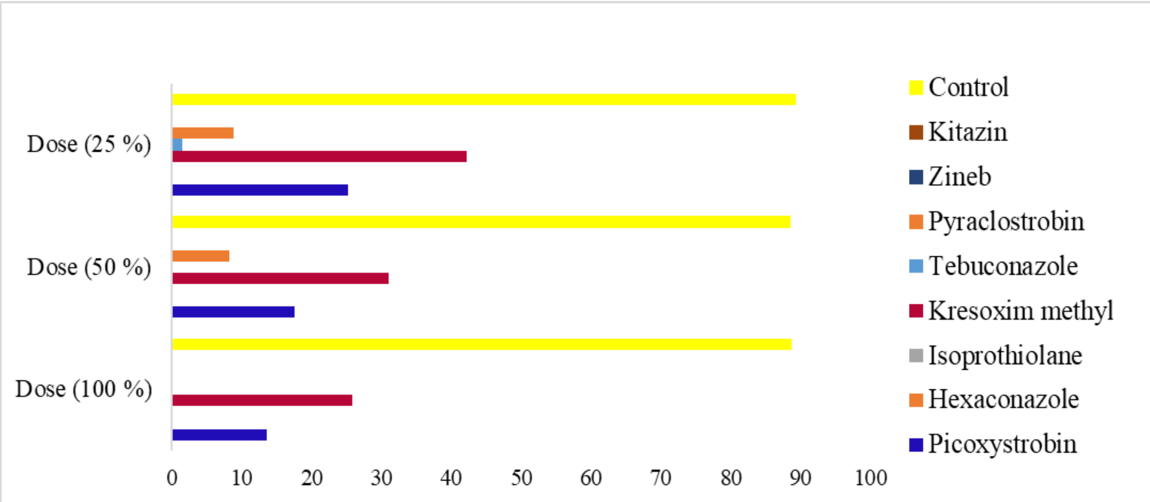

Fig. 1. *In vitro* efficacy of fungicides against *pyricularia oryzae*

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* [18,19,20]. Additionally, Mohammad et al*.* [21] 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 [22].

5. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Law Jodi Woan-Fei, Ser Hooi-Leng, Khan Tahir M, Chuah Lay-Hong, Pusparajah Priyia, Chan Kok-Gan, Goh Bey-Hing, Lee Learn-Han. The potential of Streptomyces

as biocontrol agents against the rice blast fungus, Magnaporthe oryzae (*Pyricularia oryzae*). Frontiers in Microbiology; 2017. DOI.org/10.3389/fmicb.2017.00003

2. Wilson RA, Talbot NJ. Under pressure: Investigating the biology of plant infection by Magnaporthe oryzae. Nat. Rev. Microbiol. 2009;7:185–195. DOI: 10.1038/nrmicro2032.
3. Netam RS, Bahadur AN, Tiwari U, Tiwari RKS. Efficacy of plant extracts for the control of (*Pyricularia oryzae*) Blast of rice under field condition of Bastar, Chhattisgarh. Research of Journal of Agricultural Science. 2011;2(2):269-271.
4. Akhilesh Kumar Kulmitra, Neha Sahu, Durgesh Kumar Sahu and VB. Sanath Kumar. *In vitro* evaluation of animal products against *Pyricularia oryzae* (Cav.) causing rice blast disease. Journal of Entomology and Zoology Studies. 2017;5(4): 98-101
5. Nazifa Z, Aminuzzaman F, Laila L, Rehena M. *In Vitro* Efficacy of Botanicals against Rice Blast Pathogen Magnaporthe oryzae oryzae. American Journal of Plant Sciences. 2021;12:662-678. DOI: 10.4236/ajps.2021.124045.
6. Wang G, Valent B. Durable resistance to rice blast. Science. 2017;355:906–907. DOI: 10.1126/science.aam9517.
7. Gladieux P, Condon B, Ravel S, Soanes, Maciel JLN, Nhani A, Chen L, Terauchi R, Lebrun M, Tharreau D, Mitchell T, Pedley KF, Valent B, Talbot NJ, Farman M, Fourni er E. Gene Flow between Divergent Cereal- and Grass-Specific Lineages of the Rice Blast Fungus Magnaporthe oryzae. mBio. 2018;9:10.1128/mbio.01219-17. DOI.org/10.1128/mbio.01219-17
8. Kumar Kiran N, Nagamani P, Viswanath K, Prasanthi L. Lab and Field Level Potential of Endophytic Bacteria in Managing the Dry Root Rot Disease in Black Gram Incited by Rhizoctonia bataticola (Taubenh) Butler. Legume Research. 2024;47(4):637-644. DOI: 10.18805/LR-4522.
9. Kongcharoen N, Kaewsalong N, Dethoup T. Efficacy of fungicides in controlling rice blast and dirty panicle diseases in Thailand. Scientific Reports. 2020;10: 16233. DOI.org/10.1038/s41598-020-73222-w
10. Mohiddin FA, Padder SA, Bhat AH, Ahanger MA, Shikari AB, Wani SH, et al. Phylogeny and optimization of Trichoderma harzianum for Chitinase production: evaluation of their antifungal behaviour against the prominent soil borne Phyto-pathogens of temperate India. Microorganisms. 2021;9:1962. DOI: 10.3390/microorganisms9091962
11. Fei L, Hao L. *In Vitro* and *Ex Vivo* Antifungal Activities of Metconazole against the Rice Blast Fungus Pyricularia oryzae. Molecules. 2024;29(6):1353. DOI: 10.3390/molecules29061353
12. Choi YW, Hyde KD, Ho WH. Single spore isolation of fungi. Fungal diversity. 1999;1-6
13. Nene YL, Thapliyal PN. Fungicide in Plant Diseases Control. (Third Edition; Oxford and IBH publishing Co. Pvt. Ltd., New Delhi. 1973;325.
14. Vincent JM. Distribution of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159:850.
15. Peterson LG. Tricyclazole for Control of Pyricularia oryzae on Rice: the Relationship of the Mode of Action and Disease Occurrence and Development. In: Grayson, B.T., Green, M.B., Copping, L.G. (eds) Pest Management in Rice. Springer, Dordrecht; 1990. Availalble:https://doi.org/10.1007/978-94-009-0775-1_8
16. Nidhee Yadav, RS Netam, Prahlad Singh Netam and Nitesh Mehar. 2022. Evaluation of fungicides against Pyricularia oryzae causing blast disease of rice. The Pharma Innovation Journal. 2022;11(7): 647-652
17. Horo JT, Gudisa T. Efficacy of different fungicides for the control of rice blast (Pyricularia oryzae) disease under field conditions at Pawe, Northwest Ethiopia. International Journal of Phytopathology. 2021;10(3):215–224. Available:https://doi.org/10.33687/phytopath.010.03.3649
18. Balol, Gurupada, Talekar SC, Satish RG. Efficacy of various fungicides on the management of rice blast disease caused by *Pyricularia oryzae* (Cav.) under field condition in Dharwad district of Karnataka. Internat. J. Plant Sci. 2022;17(2):214-217. DOI: 10.15740/HAS/IJPS/17.2/214-217
19. Neelakanth*, Sidde Gowda DK. 1, Chethana B. S. 2 and Parasappa H. H. 3. *In vitro* and *In vivo* Evaluation of Fungicides Against Pyricularia oryzae Causing Blast of Rice. Int. J. Pure App. Biosci. 2017;5(3):259-263.

20. Chen Yu, Yang X, Yuan SK, Li YF, Zhang AF, Yao J, Gao TC. Effect of azoxystrobin and kresoxim-methyl on rice blast and rice grain yield in China. Annals of Applied Biology. 2015;166. DOI:10.1111/aab.12202.
21. Mohammad Najeeb Mughal, Sabiya Bashir, N.A Sofi and Shabir Ahmed Wani. Bio-efficacy of some Fungicides against rice blast (*Pyricularia grisea*) under temperate agro-climatic conditions of Kashmir. Int. J. Curr. Microbiol. App. Sci. 2018;7(7):3511-3516
22. Jamal-u-ddin Hajano, A. Mubeen Lodhi, Mumtaz A. Pathan, M. Ali Khanzada and G. Serwar Shah. *In-vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen Magnaporthe oryzae Couch. Pakistan Journal of Botany. 2012;44(5): 1775-1778.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/120442