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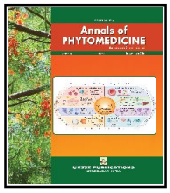

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Cultural, molecular characterization and *in vitro* sensitivity of major rice fungal pathogens to fungicides and bioagents

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Abstract

Rice production faces severe challenges from major fungal diseases such as blast, brown spot, and sheath blight, caused by *Magnaporthe grisea*, *Bipolaris oryzae*, and *Rhizoctonia solani*, respectively. This study aimed to identify these pathogens using symptomatological, cultural, morphological, and molecular techniques, and to assess the *in vitro* efficacy of selected bioagents and fungicides against them. Diseased rice samples were collected from the Crop Research Centre at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, and pathogens were isolated and maintained on potato dextrose agar. Molecular characterization through ITS rDNA amplification produced approximately 600 bp amplicons with 98.54-99.25% similarity with reference sequences in GenBank. These sequences were submitted with accession numbers MW287325.1 (*M. grisea*), MW309505.1 (*B. oryzae*), and MW009705.1 (*R. solani*). In dual-culture assays, *Pseudomonas fluorescens* showed greater inhibition of mycelial growth of *M. grisea* (58.15%), *B. oryzae* (58.52%), and *R. solani* (51.48%) than *Trichoderma harzianum* (54.44%, 52.96%, and 24.81%, respectively). Among fungicides, propiconazole, tricyclazole, and hexaconazole showed the highest efficacy, inhibiting *M. grisea* by 76.30-87.41%, *B. oryzae* by 57.04%, and *R. solani* by 68.15%, with a clear dose-dependent response. These results confirm the identification of major rice pathogens through morphological and molecular methods and highlight effective bioagents and fungicides that can be incorporated into sustainable rice disease management strategies.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world, supplying nearly 20% of the global dietary energy and playing a crucial role in food security, particularly in Asia (Pooja and Katoch, 2014). India is the second largest producer of rice and a leading exporter of premium Basmati rice to international markets (Agricultural Statistics at a Glance, 2019). Despite its economic and nutritional importance, rice productivity is significantly constrained by several diseases, among which blast caused by *M. grisea*, brown spot caused by *B. oryzae*, and sheath blight caused by *R. solani* are among the most destructive. Under favourable environmental conditions, these diseases can cause yield losses ranging from 10% to more than 50%, thereby posing a serious threat to global rice production and food security (Kandhari, 2005; Singh, 2009; Kumar *et al.,* 2018). The management of these diseases is complicated by the high level of variability within pathogen populations. *M. grisea* is known for its rapid evolutionary adaptability and ability to overcome host resistance. *B. oryzae* exhibits significant pathogenic diversity, and *R. solani* possesses a wide host range with considerable variability in virulence (Manandhar *et al.,* 1998; Goswami *et al.,* 2010; Valarmathi and Ladhalakshmi, 2018). Consequently, accurate identification and characterization of these pathogens using morphological as well as molecular tools are essential for effective disease diagnosis and management. Chemical fungicides such as tricyclazole, carbendazim, propiconazole, and hexaconazole are commonly used for the management of rice diseases due to their high efficacy (Bhat *et al.,* 2012; Neelakanth *et al.,* 2017). However, continuous and indiscriminate application of fungicides has raised serious concerns regarding fungicide resistance and pesticide residues in rice grains. In recent years, detection of tricyclazole residues beyond permissible limits has resulted in the rejection of several Basmati rice consignments in international markets. The European Union has further tightened regulations by reducing the maximum residue limit to 0.01 ppm in 2018, significantly affecting rice exports (Anonymous, 2014; Singh, 2017; APEDA, 2020). These developments emphasize the need for sustainable and residue-compliant disease management strategies. In this context, biological control agents such as *T. harzianum* and *P. fluorescens* have gained considerable attention due to their antagonistic activity against plant pathogens and their role in promoting environmentally safe crop protection. Integration of such bioagents with judicious fungicide use forms the basis of integrated disease management (IDM), which aims to reduce chemical dependency while maintaining effective disease control. However, limited studies have simultaneously focused on the molecular confirmation of major rice pathogens along with the comparative evaluation of bioagents and fungicides against

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them under *in vitro* conditions. Therefore, the present study was undertaken to isolate and characterize major rice fungal pathogens using symptomatological, cultural, morphological, and molecular approaches, and evaluate the *in vitro* efficacy of selected bioagents and fungicides against these pathogens to identify potential components for integrated disease management in rice.

2. Materials and Methods

2.1 Chemicals and reagents

10×Taq DNA buffer B (Genie, Bangalore), 6× loading dye (Genie, Bangalore), agarose (SRL Pvt. Ltd., Mumbai, India), chloroform (*E. Merck*, Mumbai, India), CTAB (CDH Pvt. Ltd., New Delhi, India), dNTP mix (Genie, Bangalore), EDTA (SRL Pvt. Ltd., Mumbai, India), ethidium bromide (SRL Pvt. Ltd., Mumbai, India), forward and reverse primers (Sigma, USA), isoamyl alcohol (*E. Merck*, Mumbai, India), isopropanol (SRL Pvt. Ltd., Mumbai, India), $MgCl_2$ (Genie, Bangalore), NaCl (E. Merck, Mumbai, India), RNase (Genie, Bangalore), Taq DNA polymerase (Genie, Bangalore), Tris-HCl (SRL Pvt. Ltd., Mumbai, India) and β-mercaptoethanol (SRL Pvt. Ltd., Mumbai, India).

2.2 Equipment

The following instruments were used: analytical weighing balance (Citizen), water bath (Mackflow), refrigerated centrifuge (Biogen), vortex shaker (GeNei), PCR thermal cycler (Bio-Rad), gel documentation system (Uvitech, Cambridge), hot air oven (Biogen), laminar air flow (Scientech), BOD incubator (Biogen) and deep freezer (-20°C).

2.3 Collection and isolation of pathogens

Rice plants showing typical symptoms of blast, brown spot, and sheath blight were collected from experimental fields of Variety Pusa Basmati-1121 at the Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India (29.08°N, 77.69°E). A total of 30 diseased samples (10 per disease) were collected during the cropping season and transported to the laboratory in sterile paper envelopes for pathogen isolation. Isolation of fungal pathogens was carried out in the laboratory of the Centre of Excellence for SPS, Department of Plant Pathology, SVPUA&T, Meerut. Infected plant tissues (3-5 mm pieces) were washed thoroughly under running tap water, followed by surface sterilization with 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and blot dried using sterile filter paper. The sterilized tissues were aseptically transferred onto potato dextrose agar (PDA) plates and incubated at 25 ± 1°C for 5-7 days. Emerging fungal colonies were purified using hyphal tip and single-spore isolation techniques and maintained on PDA slants for further studies (Holder and Keyhani, 2005). In total, three representative isolates corresponding to each pathogen (*M. grisea*, *B. oryzae,* and *R. solani*) were selected for detailed characterization.

2.4 Symptomatology

Disease symptoms were recorded under natural field conditions at different crop growth stages through regular visual observations. Symptom expression was documented primarily to confirm disease identity and to capture variability in symptom severity and progression across growth stages. Disease diagnosis and symptom characterization were carried out following standard descriptions and evaluation scales as outlined by Agrios (2005) and the IRRI Standard Evaluation System for Rice (IRRI, 2002), with photographic documentation used to support visual comparison.

2.5 Cultural characteristics

Purified isolates of *M. grisea*, *B. oryzae,* and *R. solani* were grown on PDA and incubated at 25 ± 1°C. Observations on colony colour, texture, margin, growth pattern, and pigmentation were recorded at regular intervals up to 7 days after incubation. Cultural variability among isolates was documented to support pathogen identification and to complement molecular characterization. Standard mycological procedures were followed as described by Barnett and Hunter (1998) and Agrios (2005).

2.6 Molecular identification and phylogenetic analysis

Genomic DNA was extracted from 7-day-old fungal mycelium using the CTAB extraction method. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using universal primers ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCCTCCG CTTATTGATATGC-3) (White *et al.,* 1990). PCR amplification was carried out in a 25 µl reaction mixture containing 2.5 µl 10×PCR buffer, 2.0 µl $MgCl_2$ (25 mM), 2.0 µl dNTP mix (2.5 mM each), 1.0 µl each of forward and reverse primers, 0.2 µl Taq DNA polymerase, 2.0 µl genomic DNA template, and nuclease-free water to make the final volume. The PCR conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 min. The amplified products were resolved on 1.2% agarose gel electrophoresis and visualized under UV illumination. PCR products were purified and sequenced using the Sanger sequencing, and the obtained sequences were compared with reference sequences available in the NCBI database using BLAST analysis (Altschul *et al.,* 1990). Phylogenetic analysis was conducted using MEGA version 11 (Tamura *et al.,* 2021). Sequences were aligned using ClustalW, and phylogenetic trees were constructed using the Neighbor-Joining method with 1000 bootstrap replications to determine genetic relationships among isolates.

2.7 *In vitro* evaluation of fungicides and bioagents against pathogens

The *in vitro* antagonistic activity of *T. harzianum* and *P. fluorescens* against the fungal pathogens was evaluated using the dual culture technique. A 5-mm mycelial disc taken from the actively growing margin of a 5-7-day-old culture of each pathogen was placed on potato dextrose agar (PDA) plates. *T. harzianum* was inoculated on the opposite side of the plate at a distance of 3 cm from the pathogen disc, while *P. fluorescens* was streaked at a distance of 3 cm from the pathogen inoculum (Yadav and Yadav, 2018). Plates were incubated at 26 ± 1°C with three replications for each treatment, and radial mycelial growth was recorded after 7 days. Percent inhibition of pathogen growth was calculated using the formula:

$$I = \frac{C - T}{C} \times 100$$

where C represents the radial growth of the pathogen in the control, and T represents radial growth in the treatment.

In addition, six fungicides, carbendazim 50% WP, tricyclazole 75% WP, propiconazole 25% EC, hexaconazole 5% SC, mancozeb 75%

WP, and isoprothiolane 40% EC were evaluated using the poisoned food technique. The required concentrations of fungicides (25, 50, and 100 ppm) were incorporated into sterilized PDA medium before pouring into Petri plates, while PDA without fungicide served as the control. A 5 mm mycelial disc of the test pathogen was placed at the center of each plate and incubated at 26 ± 1°C with three replications per treatment. Radial mycelial growth was measured after 7 days of incubation, and percent inhibition of fungal growth was calculated using the same formula described above.

2.8 Statistical Analysis

The experiments were laid out in a completely randomized design (CRD) with three replications. Analysis of variance (ANOVA) was performed on the data, and critical difference (CD) was used to compare treatment means at the 5% level of significance ($p<0.05$) following standard procedures (Bhaskar *et al.,* 2023).

3. Results

3.1 Symptomatology and pathogen variability

Under natural field conditions, typical symptoms of blast, brown spot, and sheath blight were consistently observed at different crop growth stages; however, marked variability in symptom expression and severity was evident across locations and plant stages. Such variation is epidemiologically important, as it reflects differences in infection timing, host response, and pathogen aggressiveness.

3.1.1 Rice blast

Rice blast symptoms varied from foliar lesions at early stages to severe neck and panicle infection at heading, often resulting in partial to complete panicle sterility. Variation in lesion size,distribution, and grain filling suggested differential infection pressure and pathogenic fitness among isolates (Plate 1). Similar stage-specific symptom progression and yield impact have been reported earlier by Hajano *et al*. (2011) and Kulkarni and Peshwe (2019), highlighting the adaptive nature of *M. grisea* across crop phenology.

3.1.2 Brown spot

Brown spot symptoms ranged from mild leaf spotting to extensive necrosis and blighting, particularly under the seedling and reproductive stages. Early infections caused seedling stunting and mortality, whereas later infections affected glumes and grains, resulting in poor grain filling and discoloration (Plate 2). This variability corroborates earlier reports describing *B. oryzae* as highly influenced by host nutrition and environmental stress, thereby contributing to inconsistent disease severity (Nghiep *et al.,* 2004; Sunder *et al.,* 2014).

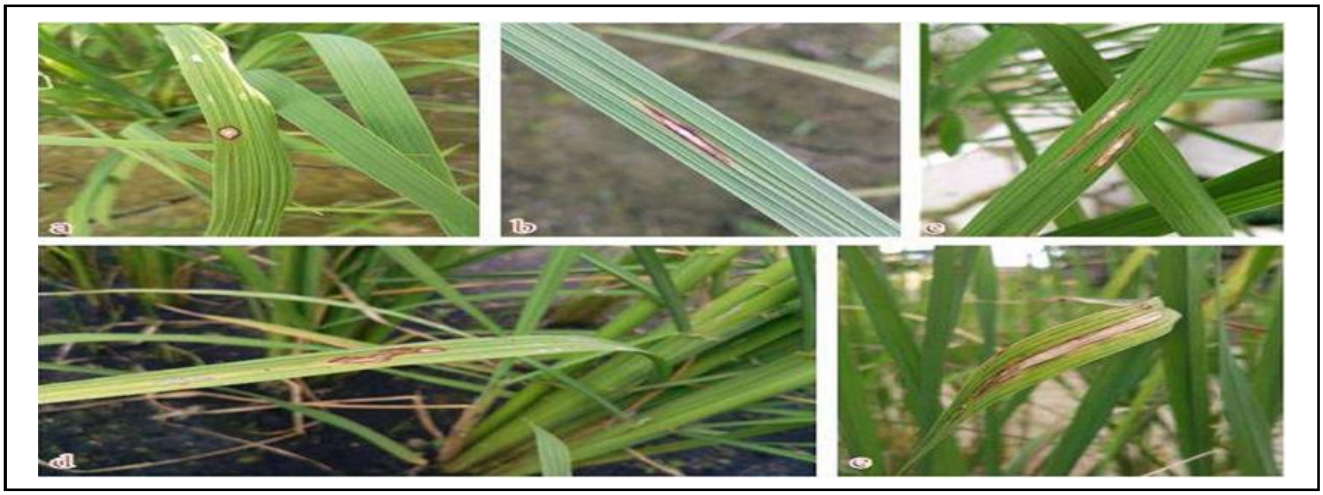

Plate 1 a, b, c, d:Blast symptoms on leaves; e: Severe infection of blast on leaves.

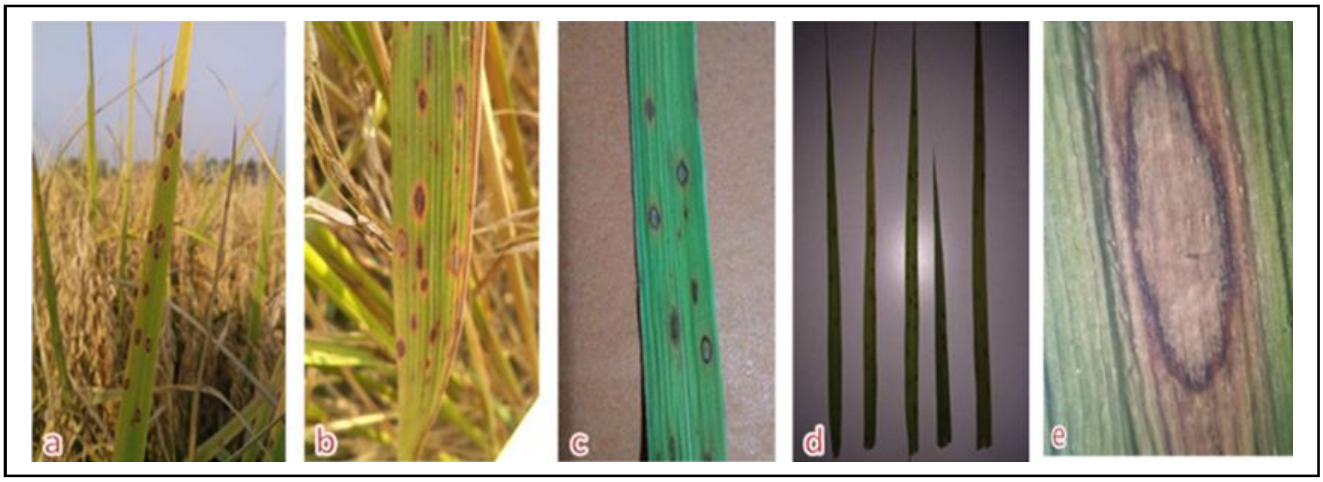

Plate 2 a, b, c, d, e: Different symptoms of Brown spot on leaves.

3.1.3 Sheath blight

Sheath blight symptoms initially appeared on lower leaf sheaths and progressed upward under favourable microclimatic conditions. Differences in lesion expansion, tiller collapse, and lodging intensity suggested variation in aggressiveness and vertical spread among *R. solani* isolates (Plate 3). Comparable disease progression patterns were described by Ou (1972) and Yu*et al*. (1980), emphasizing the role of pathogen vigour and canopy humidity in disease escalation.

Plate 3 a, b, c, d: Symptoms of Sheath blight on leaf sheath.

3.1.4 Cultural characteristics and management relevance

Distinct cultural variability among the pathogens further supported their identification and biological diversity. *M. grisea* isolates exhibited moderate to rapid growth on PDA with colonies ranging from light grey to dark grey (Plate 4). *B. oryzae* isolates showed olivaceous to dark brown colonies with differences in mycelial density (Plate 5), agree with reports by Valarmathi and Ladhalakshmi (2018), indicating adaptive variability that may influence survival and infection efficiency. *R. solani* isolates displayed rapid mycelial growth with variable sclerotial production (Plate 6). Differences in sclerotial size and distribution are particularly relevant for disease management, as sclerotia are primary survival structures that contribute to persistence and recurrent field infections. Such cultural variability underscores the need for integrated approaches rather than reliance on single control measures.The observed variability in symptom expression and cultural characteristics confirms the heterogeneous nature of major rice fungal pathogens. This variability has direct implications for disease forecasting, fungicide sensitivity, and long-term management strategies, justifying the integration of molecular characterization, bioagents, and judicious fungicide use in sustainable rice disease management.

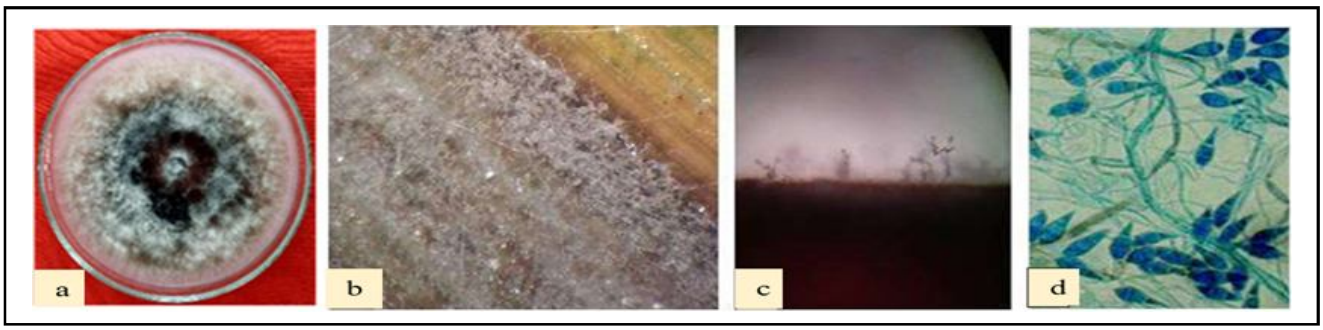

Plate 4 a: Pure culture of *M. grisea* PDA; b: Growth of mycelium of *M. grisea* on rice leaf *in vitro*; c, d: Microscopic view of conidia of *M. grisea*.

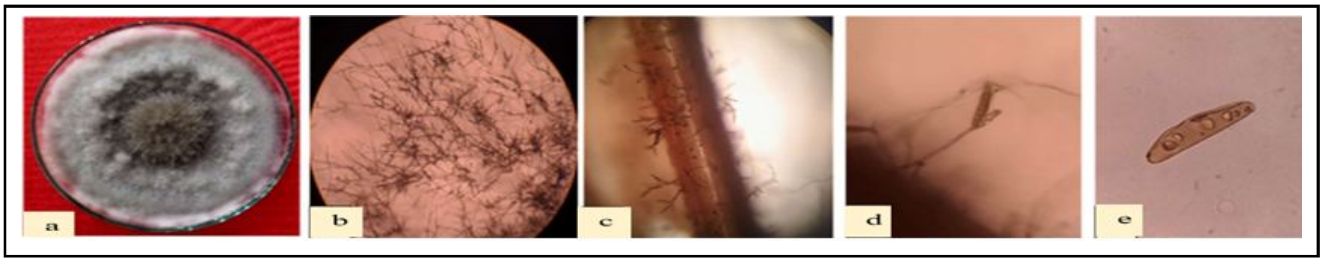

Plate 5 a: Pure culture of *B. oryzae*on PDA; b:Mycelium of *B. oryzae;*c: Production of conidia and conidiophores on infected leaf; d:Conidiophore bearing conidia; e: Microscopic view of conidia of *B. oryzae*.

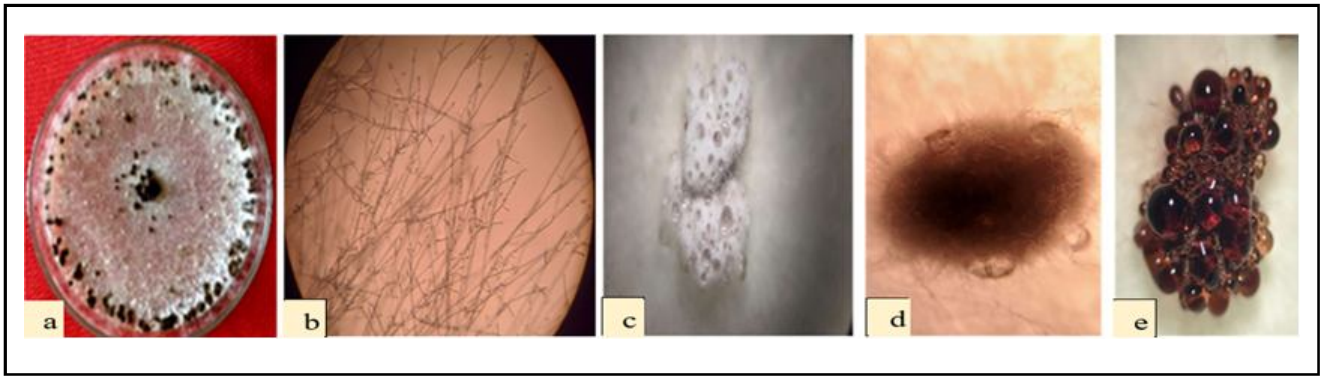

Plate 6 a: Pure culture of *R. solani*; b: Mycelium of *R. solani;* c, d, and e:Different stages of sclerotia formation by *R. solani*: Microscopic view.

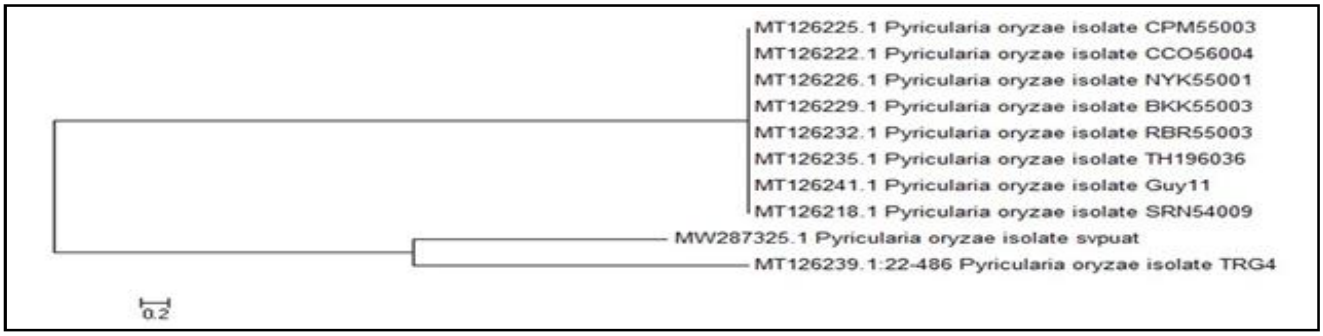

Figure 1: Neighbor-joining phylogenetic tree based on ITS rDNA sequences showing the genetic relationship between *P. oryzae* isolate SVPUAT and reference *P. oryzae* isolates retrieved from GenBank (NCBI). The scale bar represents nucleotide substitutions per site.

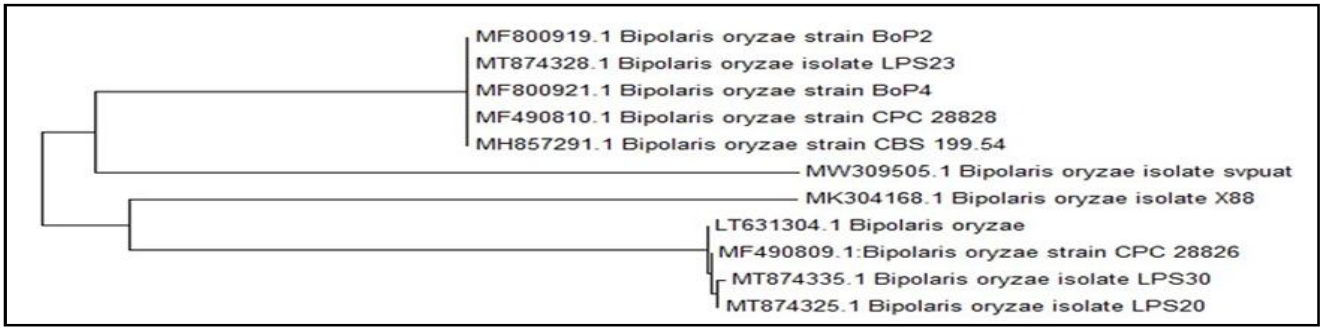

Figure 2: Neighbor-joining phylogenetic tree based on ITS rDNA sequences showing the genetic relationship between *B. oryzae* isolate SVPUAT and reference *B. oryzae* isolates retrieved from GenBank (NCBI). The scale bar represents nucleotide substitutions per site.

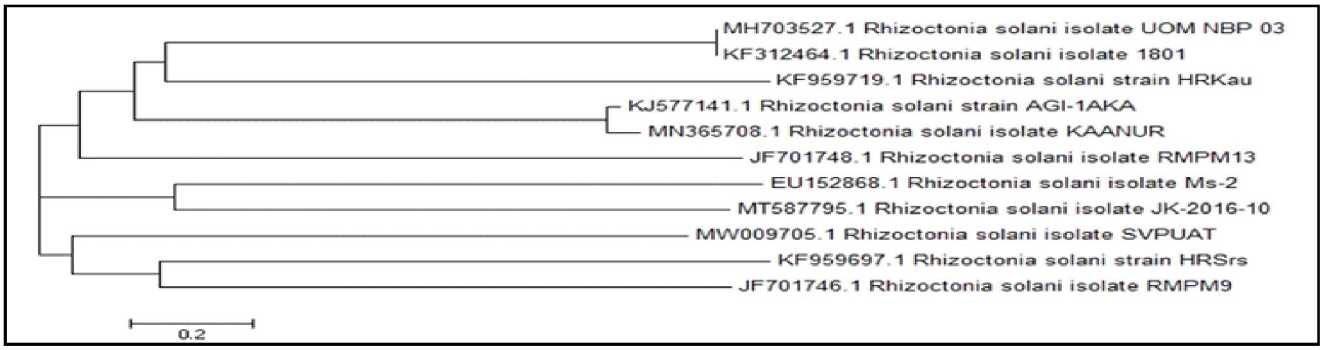

Figure 3: Neighbor-joining phylogenetic tree based on ITS rDNA sequences showing the genetic relationship between *R. solani* isolate SVPUAT and reference *R. solani* isolates retrieved from GenBank (NCBI). The scale bar represents nucleotide substitutions per site.

3.2 Phylogenetic characterization of pathogens

ITS rDNA-based molecular characterization unequivocally confirmed the identity of *P. oryzae* (syn. *M. grisea*), *B. oryzae,* and *R.solani*. The ITS sequences of the present isolates exhibited high nucleotide similarity (98.54-99.25%) with authenticated reference sequences available in GenBank (NCBI) and were deposited under accession numbers MW287325.1 (*P. oryzae*), MW309505.1 (*B. oryzae*), and MW009705.1 (*R. solani*), respectively. These results validate the robustness of integrating morphological, cultural, and molecular approaches for precise identification of rice fungal pathogens. Neighbor-joining-based phylogenetic analysis revealed that the *P. oryzae* isolate SVPUAT clustered within the major *P. oryzae* clade together with globally reported isolates, although it formed a distinct sub-branch, indicating slight ITS-based sequence divergence. This divergence reflects limited intraspecific variability within *P. oryzae* populations, which has been widely reported in earlier studies using ITS-based molecular markers (Muni and Nadarajah, 2014). Such genetic variation among geographically distinct isolates is considered a common feature of this pathogen.The *B. oryzae* isolate SVPUAT clustered closely with reference isolates originating from diverse geographical regions, reflecting a highly conserved ITS region and indicating comparatively low genetic variability within the species. However, molecular marker-based studies have demonstrated the presence of genetic variability within *B. oryzae* populations (Kumar *et al.,* 2011), suggesting that while ITS sequences are conserved, population-level diversity may exist when assessed using more discriminatory markers. In contrast, the *R. solani* isolate SVPUAT formed a separate branch within the *R. solani* clade, indicating relatively higher intraspecific genetic diversity. This observation is in agreement with earlier reports describing the complex population structure and high genetic heterogeneity of *R. solani* isolates based on ITS and other molecular markers (Nadarajah *et al.,* 2014). The ITS-based phylogenetic reconstruction not only confirmed the taxonomic identity of the three major rice pathogens but also revealed varying degrees of intraspecific genetic variability among them. Similar ITS-based identification and sequence homology patterns have been reported earlier for *P. oryzae* (Muni and Nadarajah, 2014) and *R. solani* (Nadarajah *et al.,* 2014), while molecular evidence for genetic diversity within *B. oryzae* populations has been documented by Kumar *et al.* (2011). These findings reaffirm the reliability of ITS sequencing as a powerful tool for species-level identification and phylogenetic placement of rice fungal pathogens, thereby supporting its application in epidemiological and disease management studies (Figures 1-3).

3.3 *In vitro* efficacy of bioagents against pathogens

Among the tested bioagents *P. fluorescens* showed the highest antagonistic activity against all three pathogens (Table 1; Plate 7). It inhibited mycelial growth of *M. grisea*, *B. oryzae,* and *R. solani* by 58.15%, 58.52%, and 51.48%, respectively. In comparison, *T. harzianum* recorded 54.44% inhibition of *M. grisea* and 52.96% inhibition of *B. oryzae*, while comparatively lower inhibition (24.81%) was observed against *R. solani*. The differences among treatments were statistically significant at $p<0.05$, as indicated by the critical difference values presented in Table 1. The higher inhibitory activity of *P. fluorescens* may be attributed to its ability to produce diffusible antibiotics, siderophores, and lytic enzymes that suppress fungal growth. In contrast, the relatively lower inhibition of *R. solani* by *T. harzianum* may be associated with the rapid mycelial growth and sclerotial nature of the pathogen, which often reduces the effectiveness of mycoparasitic fungi. These findings are consistent with earlier reports. Nirmalkar *et al.* (2017) reported significant inhibition of *M. grisea* by *P. fluorescens*, while Meghana *et al.* (2019) observed higher suppression of *R. solani* by *P. fluorescens* compared with *Trichoderma* spp. The present results, therefore, indicate that *P. fluorescens* is a comparatively more effective antagonist against the major rice pathogens evaluated in this study and may serve as a potential component of integrated disease management strategies.

Table 1: Efficacy of different bioagents against *M. grisea*, *B. oryzae*, and *R. solani* under *in vitro* conditions

S. No.	Bioagents	*M. grisea*		*B. oryzae*		*R. solani*	
		Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition
1	*Trichoderma harzianum*	41.00	54.44	42.33	52.96	67.67	24.81
2	*Pseudomonas fluorescens*	37.67	58.15	37.33	58.52	43.67	51.48
	Control	90.00	–	90.00	–	90.00	–
	CD at 5%	**1.90**	**–**	**2.12**		**3.55**	

3.4 *In vitro* efficacy of fungicides against pathogens

All the tested fungicides significantly ($p\leq0.05$) inhibited the mycelial growth of *Magnaporthe grisea*, *Bipolaris oryzae,* and *Rhizoctonia solani* compared with the untreated control (Table 2; Plate 8). In general, inhibition increased with increasing fungicide concentration, indicating a clear dose-dependent response.

3.4.1 *M. grisea*

All fungicides suppressed the mycelial growth of *M. grisea*, although their efficacy varied across concentrations. Propiconazole recorded the highest inhibition at 25 ppm (64.44%) and 50 ppm (76.30%), whereas at 100 ppm, tricyclazole exhibited maximum inhibition (87.41%), followed by propiconazole and hexaconazole. The superior efficacy of tricyclazole against the blast pathogen is widely reported and is primarily attributed to its ability to inhibit melanin biosynthesis, a process essential for appressorium formation and host penetration in the rice blast fungus. Similar results were reported by Bhat *et al.* (2012), who identified tricyclazole as one of the most effective fungicides for rice blast management. Neelakanth *et al.* (2017) also reported strong inhibitory effects of tricyclazole and triazole fungicides against *M. grisea* under laboratory conditions.

3.4.2 *B. oryzae*

The sensitivity of *B. oryzae* to the tested fungicides varied with concentration. Carbendazim showed the highest inhibition at 25 ppm

(46.67%) and 50 ppm (51.10%), while hexaconazole recorded maximum inhibition at 100 ppm (57.04%). The effectiveness of carbendazim may be related to its action as a mitotic inhibitor, interfering with fungal cell division, whereas azole fungicides such as hexaconazole act by inhibiting ergosterol biosynthesis, thereby disrupting fungal membrane integrity. Similar findings were reported by Meghana *et al*. (2019), who observed significant suppression of *B. oryzae* by azole fungicides and carbendazim.

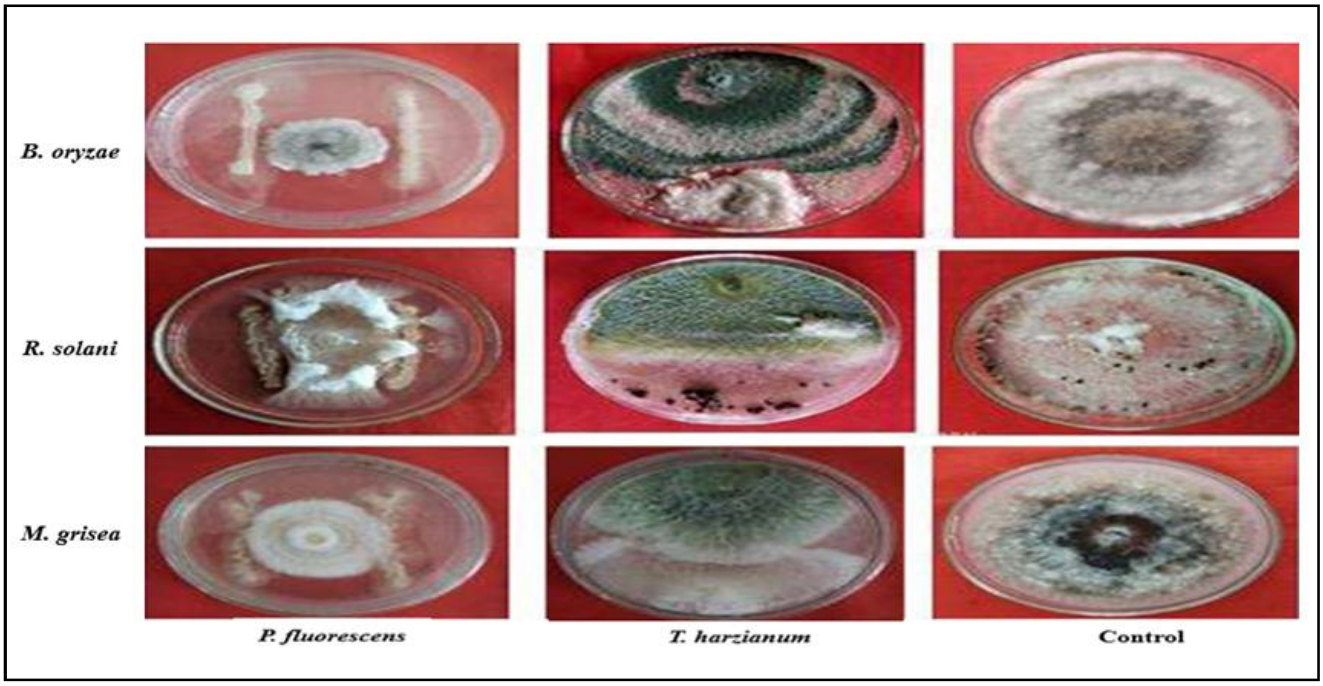

Plate 7: Efficacy of different bioagents against *M. grisea, B. oryzae, and R. solani in vitro* conditions.

3.4.3 *R. solani*

In the case of *R. solani*, fungicidal efficacy varied with concentration (Table 2; Plate 8). Hexaconazole recorded the highest inhibition at 25 ppm (51.48%), whereas propiconazole was the most effective at 50 ppm and 100 ppm, showing 56.67% and 68.15% inhibition, respectively. In contrast, tricyclazole exhibited comparatively lower activity against *R. solani*. This observation is expected because tricyclazole primarily inhibits melanin biosynthesis, a mechanism that is particularly important for infection structure development in the rice blast pathogen but less relevant for basidiomycetous fungi such as *R. solani*.The higher efficacy of triazole fungicides against *R. solani* may be attributed to their ability to inhibit ergosterol biosynthesis, thereby disrupting fungal cell membrane integrity and growth. Similar findings have been reported by Adhikari *et al*. (2018), who observed significant suppression of *R. solani* by hexaconazole and carbendazim-based fungicides under *in vitro* conditions.

Table 2: Efficacy of different fungicides against *M. grisea*, *B. oryzae*, and *R. solani* under *in vitro* conditions

Fungicide name	Concentration (ppm)	*M. grisea* Radial growth (mm)	*M. grisea* Per cent inhibition	*B. oryzae* Radial growth (mm)	*B. oryzae* Per cent inhibition	*R. solani* Radial growth (mm)	*R. solani* Per cent inhibition
Carbendazim	25	72.70	19.26	48.00	46.67	62.67	30.37
	50	70.00	22.22	44.00	51.11	49.00	45.56
	100	66.67	25.93	42.00	53.33	32.00	64.44
Tricyclazole	25	69.70	22.59	65.70	27.04	86.00	4.44
	50	33.33	62.96	51.33	42.96	80.30	40.74
	100	11.30	87.41	49.67	44.81	35.00	61.11
Propiconazole	25	32.00	64.44	77.67	13.70	46.00	48.89
	50	21.30	76.30	75.00	16.67	39.00	56.67
	100	17.00	81.48	68.67	23.70	28.67	68.15

Hexaconazole	25	35.30	60.74	64.67	28.15	43.67	51.48
	50	25.30	71.85	46.33	48.52	40.33	55.19
	100	17.00	80.74	38.67	57.04	29.67	67.04
Mancozeb	25	54.33	39.63	59.67	33.70	67.33	25.19
	50	48.67	45.93	53.67	40.37	52.33	41.85
	100	44.64	50.37	49.67	44.81	35.67	60.37
Isoprothiolane	25	68.00	24.44	73.00	18.89	47.67	47.04
	50	65.00	27.78	63.67	29.26	41.67	53.70
	100	46.00	48.89	60.00	33.33	35.67	60.37
Control	–	90.00	–	90.00	–	90.00	–
CD at 5%	–	2.73	–	3.76	–	3.70	–

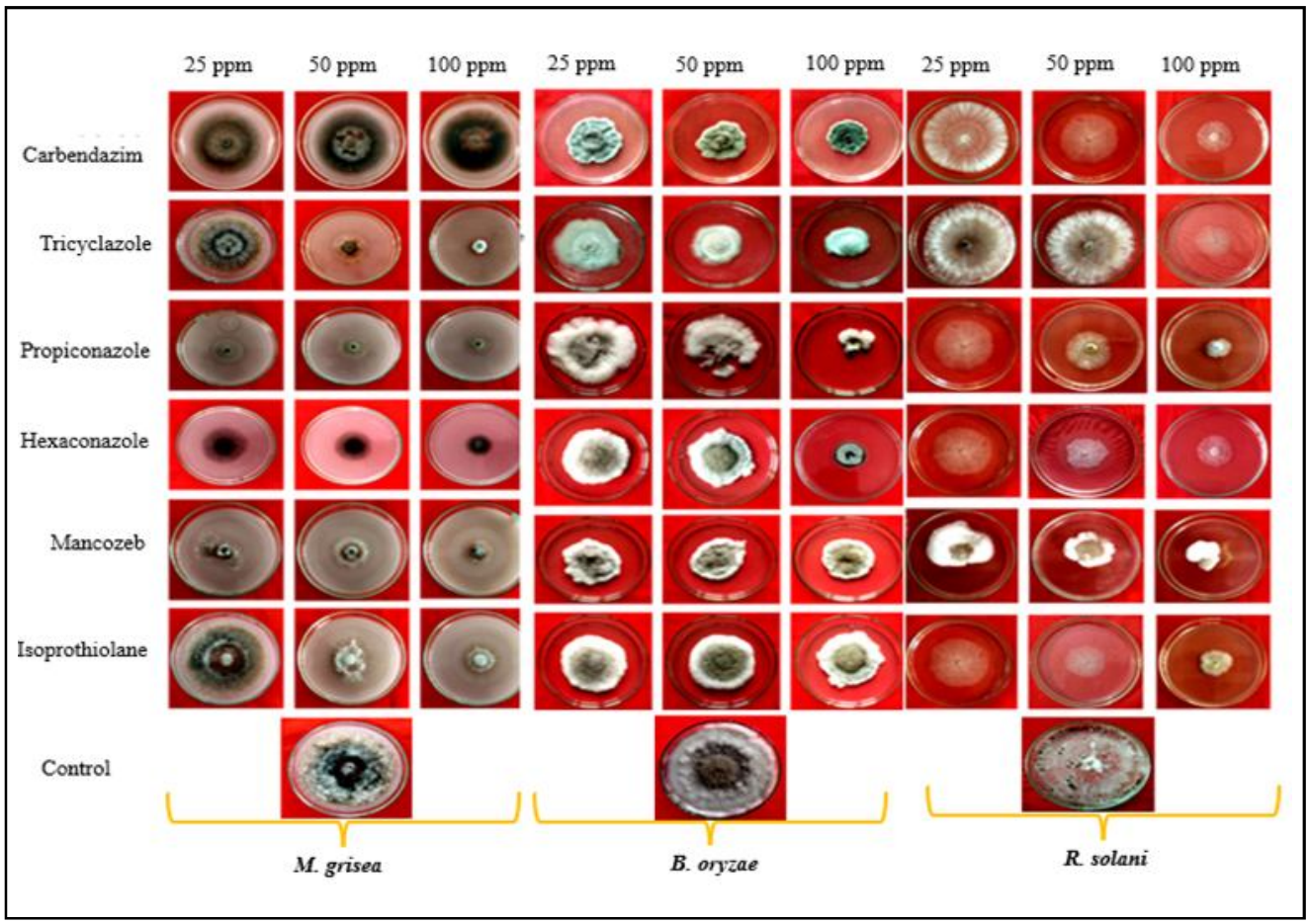

Plate 8: Efficacy of different fungicides against *M. grisea, B. oryzae* and *R. solani in vitro* conditions.

4. Discussion

The present findings are in close agreement with earlier scientific reports, confirming that variability in symptom expression and disease severity among major rice pathogens is largely influenced by host growth stage, environmental conditions, and pathogen aggressiveness, as also documented by Hajano *et al*. (2011) and Kulkarni and Peshwe (2019) for rice blast. Similar variability observed in brown spot supports the observations of Nghiep *et al*. (2004) and Sunder *et al.* (2014), who emphasized the role of host nutrition and environmental stress in disease development. The progressive nature of sheath blight and differences in lesion expansion corroborate the findings of Ou (1972) and Yu *et al.* (1980), highlighting the importance of canopy humidity and pathogen vigour. Cultural and morphological diversity among isolates aligns with reports by Valarmathi and Ladhalakshmi (2018), indicating adaptive variability affecting survival and infection efficiency. Molecular characterization based on ITS rDNA is

consistent with studies by Muni and Nadarajah (2014) and Nadarajah *et al*. (2014), confirming its reliability for species identification while also indicating varying levels of intraspecific diversity, particularly higher in *R. solani* compared to *M. oryzae* and relatively conserved sequences in *B. oryzae*, as also supported by Kumar *et al*. (2011). The superior antagonistic activity of *P. fluorescens* observed in the present study agrees with findings of Nirmalkar *et al*. (2017) and Meghana *et al.* (2019), attributing its effectiveness to antibiotic production and other inhibitory mechanisms, whereas the comparatively lower efficacy of *T. harzianum* against *R. solani* may be due to its rapid growth and sclerotial nature. Furthermore, the differential efficacy of fungicides corresponds with earlier reports by Bhat *et al*. (2012), Neelakanth *et al.* (2017), and Adhikari *et al.* (2018), where tricyclazole was highly effective against *M. oryzae* due to inhibition of melanin biosynthesis, while triazoles such as propiconazole and hexaconazole effectively suppressed *B. oryzae* and *R. solani* by targeting ergosterol biosynthesis. Collectively, these findings reinforce previous scientific evidence that pathogen variability significantly influences disease development and management efficacy, thereby emphasizing the need for integrated and adaptive disease management strategies in rice.

5. Conclusion

The study confirmed *M. grisea*, *B. oryzae,* and *R. solani* as the major fungal pathogens of rice through morphological and ITS-based molecular characterization. Among the tested bioagents, *P. fluorescens* exhibited the highest antagonistic activity against all pathogens under *in vitro* conditions. Among fungicides, propiconazole, tricyclazole, and hexaconazole showed superior efficacy in suppressing mycelial growth of the tested pathogens. These findings highlight the potential of combining effective bioagents with selective fungicides as components of integrated disease management strategies for sustainable rice production. However, further field validation is necessary before practical recommendations can be made.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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