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Molecular Characterization and Fungicidal Efficacy Against *Rhizoctonia* Isolates from Rice and Potato

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ABSTRACT

This study aimed to characterize *Rhizoctonia* isolates from rice sheath blight (R-1) and potato (P-1) infections and evaluate the efficacy of diverse fungicides against them. Molecular analysis identified the rice isolate (R-1) as belonging to the AG-1 group, displaying a distinct 265 bp band, while the potato isolate (P-1) exhibited amplification at 500 bp using Rs1F2(F)/Rs2R1(R) markers. Fungicide efficacy testing against R-1 revealed significant inhibition by Bavistin 50 EC, Titan Classic 5 EC, Orius 25 EC, and Emesto Prime 240 ES across various concentrations. Tilt 25 EC and Monceren 250 SC exhibited inhibition at 10 ppm and 100 ppm, while Fujita 40 EC showed the least inhibition (88.4% at 100 ppm). For P-1, Emesto Prime 240 ES and Monceren 250 SC consistently inhibited growth across 5–100 ppm. These findings highlight differential responses of *Rhizoctonia* isolates to fungicides, identifying potential candidates for effective disease management in rice and potato cultivation. Understanding fungal behavior and fungicide interactions is crucial for sustainable disease control. Further research should explore the mechanisms driving these responses to develop targeted management strategies, ultimately improving crop yield and quality.

INTRODUCTION

Rice (Oryza sativa L.) is a major cereal crop and a staple food for a large segment of the global population. In India, rice was cultivated over 47.83 million hectares with a record production of 137.82 million metric tonnes during the 2023-24 agricultural year (Anonymous, 2024). To meet the increasing global food demand, enhancing rice productivity remains a priority. However, various biotic and abiotic stresses significantly hinder rice cultivation, leading to substantial yield losses. Among fungal diseases, sheath blight, caused by *Rhizoctonia solani*, is one of the most damaging, with yield losses ranging from 4% to 40%, depending on crop stage, disease severity, and environmental conditions (Zheng et al., 2013; Bhunkal et al., 2015b). The disease severity is exacerbated by higher tiller density, temperatures between 25–30°C, and relative humidity of 80–100% (Bhunkal et al., 2015a).

Similarly, *R. solani* also causes black scurf disease in potato, which can lead to yield losses of up to 30% (Carling et al., 1989). This economically significant disease affects potato quality, yield, and plant productivity (Ahmad et al., 1995; Khan et al., 1995). The pathogen survives as mycelium in decomposing material or sclerotia on potato tubers, and its infection of stolons results in malformed tubers (Weinhold et al., 1978; Escande & Echandi, 1991; Jeger et al., 1996). Such infections impair nutrient and water translocation, further diminishing productivity.

Due to the absence of resistant cultivars, fungicide application remains the primary disease management strategy. However, concerns regarding maximum residue limits (MRLs), environmental contamination, and food safety have led to strict regulatory scrutiny. India, as a leading exporter of agricultural commodities, faces challenges from the USA and European Union, where excessive fungicide residues have resulted in consignment rejections (Kumar et al., 2018). The indiscriminate use of fungicides contributes to ecological imbalances and potential health risks.

Several studies have evaluated fungicidal efficacy against *R. solani* (Moni et al., 2010; Singh et al., 2016) and assessed fungicide residues in soil, rice grains, potato tubers, and straw (Kundu et al., 2011; Arora et al., 2014; Chen et al., 2015). However, there is limited information on developing new fungicidal formulations, their bio-efficacy, and residue management. Addressing these gaps is essential for sustainable

disease control, reducing agricultural losses, and ensuring food security.

The present study aims to evaluate the in vitro efficacy of different fungicides against sheath blight and black scurf disease, providing insights for enhanced disease management strategies in rice and potato cultivation.

2. MATERIAL AND METHODS:

2.1 Collection and isolation:

Sheath blight and black scurf disease samples were collected from Punjab region of India during 2019-2020 and were designated as R-1 and P-1, respectively. Sheath blight infected samples showing typical symptoms were collected, were cut into small bits and sterilized in 1% sodium hypochlorite for 1 minute. Sterilized bits were placed on the Petri plates containing potato dextrose agar (PDA) medium. Potato tubers infected with black scurf were cleaned under tap water. Later, sclerotia pieces were scraped off using a sterilized knife. The isolation was made further, as mentioned above. Both the isolates were incubated at 25±2°C to obtain pure cultures and they were used for the analysis.

2.2 Molecular characterization:

Two *Rhizoctonia* isolates collected from rice and potato infected samples each, were grown on potato dextrose broth (PDB) for genomic DNA isolation. The CTAB technique was used to isolate DNA from fungal mat and the procedure followed as stated by Murray and Thompson (1980).

Table 1: Primer sequences and detailed information

Primer name		Primer sequence (5′- 3′)	Annealing temp. (°C)	Reference
R. solani AG common Primer (Forward)		5′-CTCAAACAGGCATGCTC-3′	50	Matsumoto (2002)
AG-1	AG1-1A	5′-CAGCAATAGTTGGTGGA-3′	48	
	AG1-1B	5′-AAGGTCCTTTGGGGTTGGGG-3′	60	
	AG1-1C	5′-CTTTTTTTGGGGGGCCTTGC-3′	60	
AG-2	AG2-1	5′-AGGCAATAGGTTATTGGACC-3′	56	
	AG2-2	5′-CATGGATGGGAGAACTTTTA-3′	54	
AG-3	Rs1F2	5′- TTGGTTGTAGCTGGTCTATTT-3′	51.6	Lees *et al* (2002)
	Rs2R1	5′- TATCACGCTGAGTGGAACCA-3′	55.9	

PCR profile for AG-1 marker: For **AG 1**: PCR amplifications were performed at initial denaturation at 94^{0}C for 5minute, followed by 30 cycles of Denaturation at 94^0 C for 1 minute, 2-minute primer Annealing at 54^{0}C for, 3 minutes primer extension at 72^{0}C, followed by final extension at 72^{0}C for 10 minutes.

PCR profile for AG-3 PT marker: For **AG 3-PT**: PCR amplifications were carried at initial denaturation at 95^{0}C for 2 minutes, followed by denaturation at 95^0 C for 45 seconds for 35 cycles, annealing at 65^{0}C for 60 seconds, Extension at 72^{0}C for 1 minute 30 seconds, followed by final extension at 72^{0}C for 5 minutes.

2.3 Poison food technique:

Serial dilutions of various concentrations of each fungicide were prepared from a stock solution of 100 millilitres. Three replications were made for each concentration for testing. Along-side, suitable control was maintained without fungicide of both the isolate. The inoculated and control Petri plates were incubated at 25±2°C and the data was recorded after the growth of control plates were covered completely. Per cent inhibition in mycelial growth was calculated based on the formula given by Bliss *et al* (1934).

$$P.I = \frac{C-T}{C}$$

Where, P.I =Percent inhibition; C= Growth in control plate; T = Growth in treated plate

Table 2: Description of chemical fungicides along with mode of action

S.no.	FUNGICIDE	CONCENTRATIONS (ppm)	GROUP	TARGET SITE AND CODE
1	Bavistin 50 EC (Carbendazim)	1,5,10,50,100	B: Cytoskeleton and motor protein	ß-tubulin assembly in mitosis
2	Titan classic 5 EC (Hexaconazole)	5,25,50,100	G: sterol biosynthesis in membranes	G1 C14- demethylase in sterol biosynthesis (erg11/cyp51)
3	Tilt 25 EC (Propiconazole)	1,5,10,50	G: sterol biosynthesis in membranes	G1 C14- demethylase in sterol biosynthesis (erg11/cyp51)
4	Fujita 40EC(Isoprothiolane)	5,25,50,100	F: lipid synthesis or transport / membrane integrity or function	F2 phospholipid biosynthesis, methyltransferase
5	Pulsor 24 SC (Thifluzamide)	5,25,50,100	C. respiration	C2 complex II: succinate-dehydro-genase
6	Galileo Way 18.76 SC (Picoxystrobin + Propiconazole)	5,25,50,100	G: sterol biosynthesis in membranes	G1 C14- demethylase in sterol biosynthesis (erg11/cyp51)
7	Amistar 250EC (Azoxystrobin)	1,5,10,50,100	C. respiration	C3 complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)
8	Orius 25 EC(Tebuconazole)	5,25,50,100	G: sterol biosynthesis in membranes	G1 C14- demethylase in sterol biosynthesis (erg11/cyp51)
9	Emesto prime 240 ES (Penflufen)	5,25,50,100	C. respiration	C2 complex II:succinate-dehydro-genase
10	Monceren 250 SC(Pencycuron)	5,25,50,100	B: Cytoskeleton and motor protein	B4 cell division (unknown site)

FRAC CODE LIST (2025)

3. RESULTS AND DISCUSSION:

3.1 Molecular characterization of *Rhizoctonia* isolates

Rhizoctonia isolate (R-1) collected from infected rice sheath blight, showed positive result for AG-1 group with band size of 265bp and other markers belonging to different AGs did not show any amplification. This result confirmed that rice isolate belonged to AG-1 group. Whereas, potato isolate (P-1) amplified at a band size of 500 bp with markers Rs1F2(F)/ Rs2R1(R).

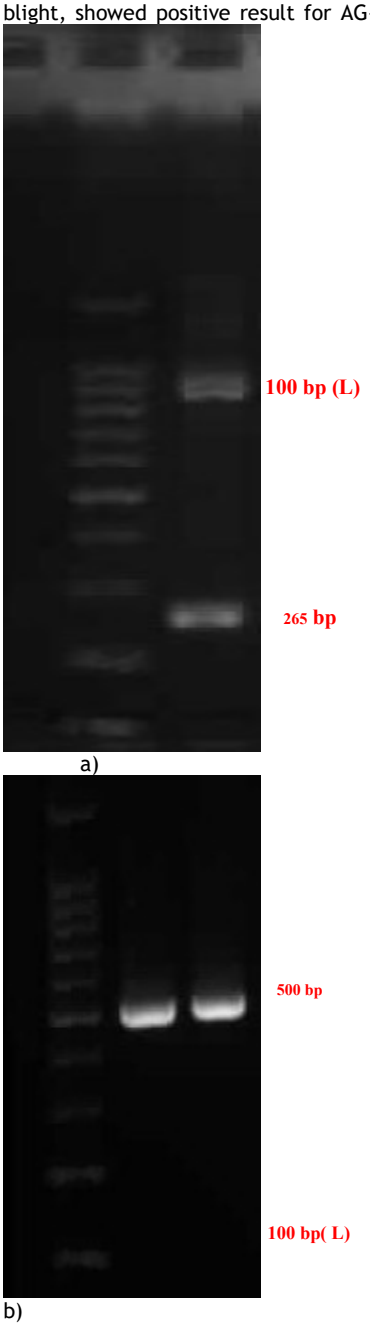
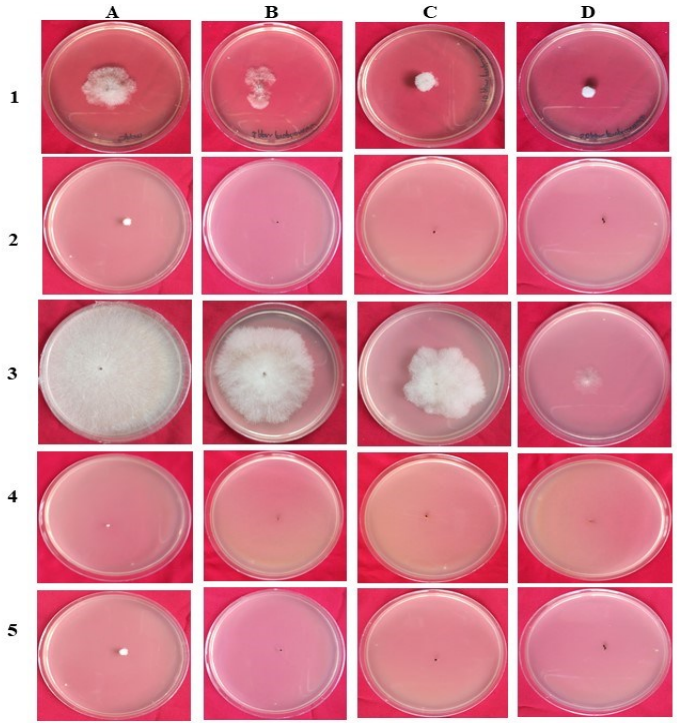


Fig.1: *Rhizoctonia* isolate (R-1) collected from infected rice sheath blight, showed positive result for AG-1 group with band size of 265bp and other markers belonging to different AGs did not show any amplification. This result confirmed that rice isolate belonged to AG1 group. Fig.2: Potato isolate was characterized to AG3- PT with an amplified product of 500bp.

Fig 1: PCR profile of *Rhizoctonia*solanigenerated by AG subgroups specific marker AG1-1A (265bp) and AG3-PT (500 bp) a) rice isolate b) potato isolate; Lane 1: 100 bp

3.2 Screening of fungicides against *Rhizoctonia* isolates:

The efficacy of ten fungicides tested against *Rhizoctonia* isolate (R-1) were tested as represented in table 3. The percent inhibition of fungus was calculated at different concentration. Out of all the fungicides tested, Bavistin 50 EC, Titan classic 5 E C, Orius 25 EC, Emesto prime 240 ES showed maximum inhibition at all the concentrations used in this experiment. Followed by, Tilt 25 E C and Monceren 250 S C started inhibiting fungus at 10 ppm and 100 ppm. The least inhibition of fungus showed by Fujita 40EC even at 100 ppm (88.4 %). Similarly, two fungicides were tested against *Rhizoctonia* isolate (P-1) as represented in table 2. Both Emesto prime 240 ES and Monceren 250 SC inhibited fungus at maximum at all the concentration ranging from 5 to 100 ppm.

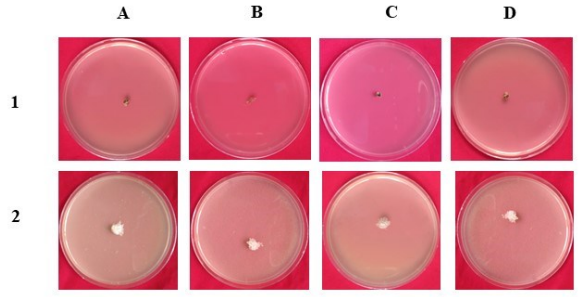


Fungicides:
1. Propiconazole(Ppm =1,5,10,50)
2. Hexaconazole (Ppm=5,25,50,100)
3. Isoprothiolane=(Ppm=5,25,50,100)
4. Tebuconazole (Ppm=5,25,50,100)
5. Thifluzamide(Ppm=5,25,50,100)

Isolate: Rice (R-1)

Control

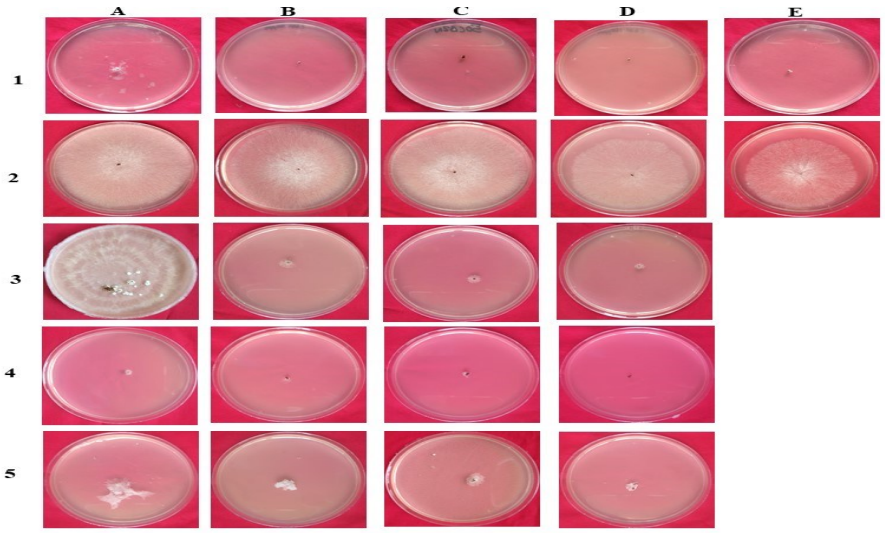
Plate 1: Effect of different fungicides against rice isolate (R-1) under *in vitro* condition



Fungicides:
1. Penflufen= 5,25,50,100
2. Pencycuron= 5,25,50,100

Isolate: Potato (P-1)

Control



Fungicides:
6.Carbendazim= 1,5,10,50,100
7. Azoxystrobin =1,5,10,50,100
8. Picoxystrobin + propiconazole=5,25,50,100
9. Penflufen= 5,25,50,100
10. Pencycuron= 5,25,50,100

Isolate: Rice (R-1)

Control

Plate 2: Effect of different fungicides against potato isolate (P-1) under *in vitro* condition

Table 3: Effect of different fungicides against rice isolate (R-1) under *in vitro* condition

S.no.	Fungicide	Percent Inhibition					
		1 ppm	5 ppm	10 ppm	25 ppm	50 ppm	100 ppm

1	Bavistin 50 EC	100.0	100.0	100.0	-	100.0	100.0
2	Titan classic 5 E C	-	100.0	-	100.0	100.0	100.0
3	Tilt 25 E C	77.3	85.6	100.0	-	100.0	-
4	Fujita 40EC	-	27.1	-	45.2	69.1	88.4
5	Pulsor 24 SC	-	85.5	-	89.8	94.9	100.0
6	Galileo Way 18.76 SC	-	0.0	-	87.4	93.1	97.0
7	Amistar 250EC	0.0	0.0	0.0	-	0.0	0.7
8	Orius 25 EC	-	100.0	-	100.0	100.0	100.0
9	Emesto prime 240 ES	-	100.0	-	100.0	100.0	100.0
10	Monceren 250 SC	-	83.3	-	86.9	94.2	100.0
Control		0.0	0.0	0.0	0.0	0.0	0.0

Table 4: Effect of different fungicides against potato isolate (P-1) under *in vitro* condition

S. no.	Fungicide	Percent inhibition					
		1 ppm	5 ppm	10 ppm	25 ppm	50 ppm	100 ppm
1	Emesto prime 240 ES	-	100.0	-	100.0	100.0	100.0
2	Monceren 250 S C	-	100.0	-	100.0	100.0	100.0
Control		0.0	0.0	0.0	0.0	0.0	0.0

Matsumoto (2002) standardized PCR profile for direct identification of *Rhizoctonia* isolates using AG-specific markers belonging to AG-1 and AG-2 subgroups. Lees *et al* (2002) standardized PCR profile for AG3-PT subgroup identification with markers Rs1F2(F)/ Rs2R1(R). Goswami *et al* (2017) identified one hundred and twelve isolates causing sheath blight in rice as AG-1-1A subgroup, indicating its dominancy over other subgroups. El-Zaidy *et al* (2018) found twenty-six *Rhizoctonia* isolates infecting potato when amplified with AG-3 PT specific primers produced a band size of 500bp.

Sheath blight of rice is not controlled by resistance breeding till date. So, the better way to manage of sheath blight is by chemical control. Hunjan *et al* (2010) reported that Trifloxystrobin+ tebuconazole inhibited the sheath blight disease in both field and lab conditions. They confirmed that newly available fungicides like tebuconazole, pencycuron and thifluzamide were found effective in managing the disease. Similarly, Lore *et al* (2012) reported that under both lab and field conditions, propiconazole, hexaconazole and carbendazim showed maximum inhibition of fungus. Prakash *et al* (2013) found that carbendazim and hexaconazole showed maximum growth inhibition of 86.62 and 71.22 per cent, but propiconazole and tebuconazole found to have moderate inhibition. Datta *et al* (2017) studied the comparative effectiveness of the triazoles group on *Rhizoctonia solani* and reported that among four fungicides (hexaconazole, propiconazole, difenoconazole, tebuconazole), hexaconazole found to have maximum inhibitory effect with lowest EC50 value.

Treatment of Moncern fungicide for tubers affected with black scurf greatly reduced the scleotial viability (Campion *et al* 2003). Different anastomosis groups (AG 3, AG 2-1 and AG 5) showed difference in percent inhibition against Monceren fungicide with AG 5 isolates showing moderate resistant with highest EC50 value. But with respect to AG 3, Monceren showed inhibition of 84.75 per cent (Malik *et al* 2014). Agarwal *et al* (2008) reported that different potato isolates showed variation in inhibition of mycelial growth with Monceren fungicide, the maximum inhibition found was 77.1 per cent and minimum was 70.4 per cent.

CONCLUSION

The study confirms the rice isolate (R-1) as part of the AG-1 group based on positive amplification at 265bp. The potato isolate (P-1) differs, displaying a distinct genetic profile with a 500bp band using specific markers Rs1F2(F)/Rs2R1(R). These distinct genetic signatures highlight unique characteristics of each isolate, crucial for tailored disease management in rice and potato crops. The study highlighted varied responses of fungicides against *Rhizoctonia* isolates R-1 and P-1. Bavistin 50 EC, Titan classic 5 EC, Orius 25 EC, Emesto prime 240 ES, and Monceren 250 SC exhibited high inhibition for both isolates across different concentrations. Fujita 40EC showed the least efficacy against R-1. This emphasizes the need for tailored fungicide selection based on isolate-specific responses for effective disease control.

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6. CONFLICT OF INTERESTS

The authors declare no conflict of interest in relation to this publication. All forms of help and financial assistance have been duly acknowledged.

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