

**Survey of rice sheath blight disease in Kuttanad and
in vitro management using biocontrol agents and
fungicides**

UNDER PEER REVIEW

ABSTRACT

Aims: Rice is an important crop cultivated across the world whose production is influenced by numerous factors like pest, disease, weather parameters. In India, diseases accounts for 35 per cent in yield reduction, with sheath blight being significant. This disease is notably prevalent in Kuttanad region in Kerala. This study involves a survey conducted in Kuttanad to assess the extent of the disease and *in vitro* management using biocontrol agents and fungicides.

Study design: Study was outlined in Completely Randomised Design (CRD) to evaluate the *in vitro* efficacies of bioagents.

Place and Duration of Study: Department of Plant Pathology, College of Agriculture, Vellayani and M.S. Swaminathan Rice Research Station, Moncompu, between 2022 and 2023

Methodology: Survey was conducted in forty locations of Kuttanad. The pathogenicity studies were conducted by artificial inoculation on rice variety, Uma. Cultural characteristics of forty isolates were studied. The virulent culture obtained through pathogenicity testing was further advanced for the *in vitro* studies with biocontrol agents and fungicides. The inhibition of fungal growth was also recorded.

Results: Forty isolates of sheath blight pathogen (*Rhizoctonia solani*) were obtained. The virulent isolate among these was identified on the basis of number of days taken for symptom development by artificial inoculation and this was further advanced for the *in vitro* studies. *Bacillus amyloliquefaciens* was found to be more effective with an inhibition percentage of 68.64. Fungicides were evaluated and at 500 ppm and 1000 ppm, fungicide Kresoxim methyl 40% + Hexaconazole 8% WG showed complete inhibition of the pathogen which was on par with Azoxystrobin 18.2% + Difenoconazole 11.4% SC at 1000 ppm.

Conclusion: Observations in this study suggests that these biocontrol agents and fungicides are very effective in the management of the pathogen and hence can be used for further field-level studies in management of the disease.

Keywords: Rhizoctonia solani; Rice; Sheath blight; In vitro; Biocontrol agents; Fungicides

1. INTRODUCTION

Rice is a staple food crop consumed by a majority of the world population and is being cultivated in over 114 countries worldwide. Approximately 90 per cent of the global rice production is concentrated in Asia with China and India being the top producers [1]. The increasing human population is leading to the increased demand of rice whereas, rice crop is highly susceptible to various biotic and abiotic stress, causing the deterioration of both quality and quantity [2]. The overall yield loss due to diseases in rice cultivation is 10.25 per cent while in India, it is specifically estimated at 35 per cent. Out of these, yield reduction due to blast accounts for 25 per cent, followed by sheath blight and bacterial leaf blight by 20 and 10 per cent respectively [3].

Sheath blight is one of the most important diseases affecting rice worldwide and is caused by the necrotrophic fungus *Rhizoctonia solani* Kuhn [4]. It is ranked as second most important disease affecting rice crop after rice blast [5]. It is an important location specific disease prevalent in Kuttanad region of Kerala leading to 30-37% yield loss [6]. The disease is currently controlled using various biocontrol agents and fungicides. Regular assessment of bioagents and fungicides are necessary to develop effective management of the disease. This study aims to evaluate the incidence of the disease in Kuttanad tract (AEU – 04) of Kerala and to assess the efficacy of biocontrol agents and fungicides on the inhibition of sheath blight pathogen, *Rhizoctonia solani* under *in vitro* conditions.

2. MATERIALS AND METHODS

2.1 Survey and collection of sheath blight samples

A survey was conducted in major rice growing regions of Kuttanad tract (AEU – 04) viz., Alappuzha, Kottayam and Pathanamthitta during the year 2022 – 2023 to collect sheath blight infected samples for the study. Survey was conducted in 40 locations (Alappuzha – 22 locations, Kottayam - 12 locations and Pathanamthitta – 6 locations). From each field, a plot of area of one square meter was selected randomly.

Symptoms on the plant specimens were recorded. Plants expressed symptoms like presence of grey to brown water-soaked lesions on sheaths [7].

Percent disease incidence was recorded on the basis of number of healthy and diseased leaf sheaths in each locations using the given formula [8].

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tillers}}{\text{Total number of tillers}} \times 100$$

2.2 Isolation of sheath blight pathogen

Samples collected were washed under running water to remove dirt and other contaminants. The diseased samples were cut into small bits (5mm) containing both healthy and infected regions using a sterile scalpel. The bits were then surface sterilized using 0.1 per cent mercuric chloride for 30 seconds and then rinsed three times with sterile water. The sterilized bits were then transferred to sterile plates containing Potato Dextrose Agar (PDA) media in aseptic condition. The plates were then sealed and incubated at 28 ± 2 °C. The mycelial growth and sclerotia formation were recorded for seven days. The pure culture was maintained in PDA slants and then incubated at 28 ± 2 °C.

2.3 Pathogenicity studies

Artificial inoculation method was conducted to test the pathogenicity of the fungal isolates. The plants were artificially inoculated at tillering stage of the crop. Seven days old cultures of the fungal isolates were used for artificial inoculation. Pinpricking was done using sterile needle to make injury on the sheaths between tillers, just above the water level. Mycelial bits of 5mm size were placed on the pinpricked areas. Over these bits, a thin layer of moistened cotton was placed [9]. Inoculated plants were labelled and observed for symptom development. Periodic observations were taken including the time taken for symptom development for each isolate.

2.4 Cultural characterization of fungal isolates

The fungal isolates were sub - cultured to sterile petri plates containing PDA media. 5mm mycelial disc of seven-day old culture was kept in the center of the petri plates and these were incubated at room temperature (28 ± 2 °C). Observations on the mycelial growth were recorded. The cultural characters viz., colony colour, number of days taken for full growth in petri plates, number of days for the formation of sclerotia, colour and pattern of formation of sclerotia were recorded.

2.5 *In vitro* evaluation of bacterial biocontrol agents and fungicides against sheathblight pathogen

2.5.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture

In vitro evaluation of biocontrol agents *Bacillus amyloliquefaciens* (B15) and *Pseudomonas fluorescens* (PN026) was conducted using Dual culture technique [10]. Sterilized PDA media was poured in 9 cm sterile petri plates. A mycelial disc (5 mm) of *Rhizoctonia solani* was placed on the centre of the petri plates. Using a sterile inoculation loop, a loopful of bacterial biocontrol agent was streaked on two sides of the pathogen at 1.5 cm away from periphery of the petri plate. Seven replications were kept for each treatment. A control plate consisting of only pathogen mycelial bit was used for the assessment. The plates were incubated at room temperature. The observations of the radial growth of the pathogen were recorded from second day until the pathogen attained full growth in the control plate. The percent inhibition of pathogen over the control was calculated as per the formula [11].

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

where,

PI = Per cent Inhibition (%)

C = Growth of the pathogen in control plates (cm)
T = Growth of the pathogen in dual culture (cm)

2.5.2 In vitro evaluation of fungicides against sheath blight pathogen by poisoned food technique

In vitro evaluation of the fungicides listed below was carried out using poisoned food technique [12]. The evaluation of these fungicides against the virulent isolate of the pathogen was done at three different concentrations i.e., recommended dose, half the recommended dose and quarter the recommended dose.

The treatments are as follows.

T1 - Azoxystrobin 18.2% + Difenconazole 11.4% SC - 250 ppm

T2 - Azoxystrobin 18.2% + Difenconazole 11.4% SC - 500 ppm

T3 - Azoxystrobin 18.2% + Difenconazole 11.4% SC - 1000 ppm

T4 - Kresoxim methyl 40% + Hexaconazole 8% WG - 250 ppm

T5 - Kresoxim methyl 40% + Hexaconazole 8% WG - 500 ppm

T6 - Kresoxim methyl 40% + Hexaconazole 8% WG - 1000 ppm

T7 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 100 ppm

T8 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 200 ppm

T9 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 400 ppm

T10 - Control

Molten sterilized PDA was used as nutrient medium for pathogen and required quantity of each fungicide was added in the 250 ml conical flask separately so, as to get a requisite concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 20 ml poisoned medium was poured to each of the 90 mm sterilized petri plates and allowed for solidification. Mycelial disc of 5 mm diameter was cut from seven-day old culture of fungal pathogen and transferred aseptically to the center of each petri plates containing the poisoned solid medium. Three replications were maintained for each of the concentrations of fungicides, while the plates without fungicides served as control. The plates were incubated at the temperature $27 \pm 2^\circ\text{C}$. The observations were recorded for radial growth in cm on daily basis. Per cent growth inhibition (PI) of each treatment was calculated as described below [11].

$$PI (\%) = \frac{C - T}{C} \times 100$$

where,

PI = Per cent inhibition

C = Radial growth of the pathogen in control plate (cm)

T = Radial growth of the pathogen in treatment plate (cm)

3. RESULTS AND DISCUSSION

3.1 Survey and collection of sheath blight samples.

Samples collected from different locations were observed and symptoms appeared on leaf sheaths of the plants as grey water-soaked lesions near the water level which further elongated and progressed to larger lesions. In severe infections the whole plant was affected leading to discoloration in panicles and grains, followed by appearance of mycelial strands and dark brown mustard-like sclerotia (Fig.1.). The percentage disease incidence (PDI) was calculated and it varied from 15 – 72%. The highest disease incidence was found in Neelamperoor region (72.73%) followed by Kunnumma (69.23%), Kidangara (64.28%) and Pulinkunnu (63.34%) in Alappuzha district. According to previous studies, data can be classified in to four groups such as very high (>50%), high (31 – 50%), moderate (20 – 30%) and low (<20%)[8] Similarly, by the assessment of PDI, it was observed that among the survey locations, eight showed very high PDI (>50%), eighteen were high (30 – 50%), eleven were moderate (20 – 30%) and three locations were observed with low PDI (<20%) (Fig. 2, Table 1).



Fig. 1. Symptoms of sheath blight disease in rice.
a - Water-soaked lesions on sheaths; b - White sclerotia on the sheath; c - White mycelia on the surface of the plant

Table 1. Survey locations and Percent Disease Incidence (PDI) in each location

Isolate	District	Location	PDI (%)
RS 1		Karumadi .	58.33
RS 2		Thakazhi	45.40
RS 3		Edathua	31.25
RS 4		Mampuzhakari	33.33
RS 5		Champakulam	40.00
RS 6	ALAPPUZHA	Kainakary	41.66
RS 7		Nedumudi	50.00
RS 8		Kidangara.	64.28
RS 9		Pulinkunnu.	63.64
RS 10		Kunnumma.	69.23
RS 11		Ramankary	50.00
RS 12		Kavalam.	58.33
RS 13		Narakathara	27.27
RS 14		Vazhappally.	58.33

RS 15		Moncompu	36.36
RS 16		Koilmukku	35.71
RS 17		Kainady	30.00
RS 18		Chungam	18.19
RS 19		Kannadi	36.37
RS 20		Puthukary	42.86
RS 21		Neelamperoor.	72.73
RS 22		Kunnamkari	30.00
RS 23		Onamthuruth	22.23
RS 24		Neendoor	46.15
RS 25		Parampuzha	27.27
RS 26		Mannar	41.67
RS 27		Eara	26.67
RS 28	KOTTAYAM	Kallara	15.39
RS 29		Koduthuruth	54.55
RS 30		Kumarakom	31.25
RS 31		Kaipuzha	33.34
RS 32		Vechoor	30.77
RS 33		Thalayolaparambu	40.00
RS 34		Vaikom	22.22
RS 35		Kadapra	30.77
RS 36		Niranam	50.00
RS 37	PATHANAMTHITTA	Thiruvalla	27.28
RS 38		Nedumpuram	31.25
RS 39		Peringara	18.19
RS 40		Perumthuruthy	25.00

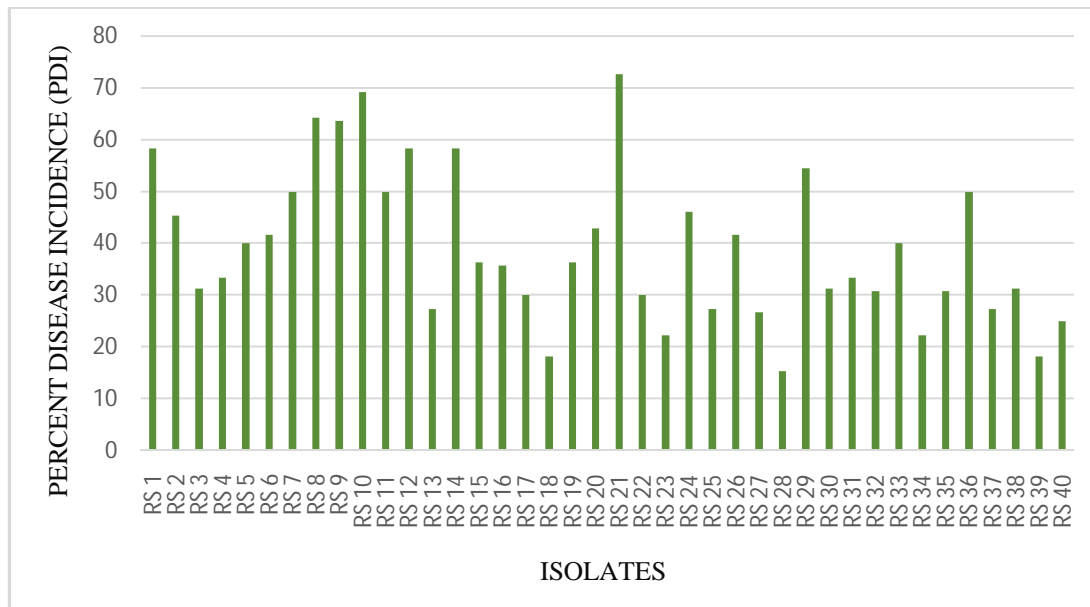


Fig. 2. Disease incidence of sheath blight in rice in the surveyed locations

3.2 Isolation of sheath blight pathogen

The mycelia was formed in different colours ranging from white, cream to dark brown. The growth pattern was flat along with the production of aerial mycelia. The sclerotia was produced within 2-3 days. It was white in the beginning and then became light brown to dark brown after maturation (Fig. 3.). Many of the research findings have recorded that the mycelial colour of *Rhizoctonia solani* varied from white to dark brown depending on the isolate [14].



Fig. 3. Pure culture of *Rhizoctonia solani*

3.3 Pathogenicity studies

The isolates obtained were tested for pathogenicity by artificial inoculation in rice variety, Uma at tillering stage of the crop which revealed symptoms similar to that observed in the field. The characteristic symptom was the presence of grey to brown water-soaked lesions on the rice sheath near the water level. This finding was in accordance with the presence of water-soaked lesions on the infected leaf sheath which further progressed upwards in severe infections[13]. The pathogens were reisolated and it was found to be similar to that of the inoculated pathogens. Based on the number of days taken to induce disease symptoms on healthy plants, the most virulent was recorded as R₂₁ isolated from

Neelamperoorregion of Alappuzha district which produced disease symptom within 5 days along with the presence of matured sclerotia.

3.4 Cultural characterization of fungal isolates

The cultural characters such as colony colour, number of days taken for the complete growth of mycelia in the petriplate, number of days taken for the formation of sclerotia, colour and pattern of formation of sclerotia were also recorded (Table 2). The isolates were observed to have white, brown, whitish brown, light brown and dark brown coloured colonies. The colony colour ranged from light brown, yellowish brown, very pale brown, brown and dark brown [15]. The sclerotia produced were observed and they were of varying colours from white to yellowish cream to dark brown. Majority of the isolates obtained were observed to produce sclerotia of light brown colour and among them some became dark brown colour on maturation. Colour of sclerotia ranges from white to dark brown and the pattern of sclerotia formation can be diverse which includes concentrated at centre, periphery, scattered [16]. The number of days required for the production of sclerotia ranged from 3 to 9. The virulent isolate R₂₁ formed sclerotia within 3 days which also took the least number of days for complete growth in the petriplate compared to the other isolates.

Table 2. Cultural characters of fungal isolates

Isolate	Colony colour	No. of days taken for the complete growth of mycelia in petriplate	No. of days taken for the formation of sclerotia	Colour of sclerotia	Sclerotia formation pattern
R ₁	Whitish brown	5	8	Light brown	Scattered
R ₂	White	6	7	Light brown	Central
R ₃	Whitish brown	6	8	Light brown	Peripheral
R ₄	White	8	5	Dark brown	Peripheral
R ₅	White	11	9	White	Scattered
R ₆	White	8	6	Dark brown	Scattered
R ₇	White	9	8	White	Peripheral
R ₈	Whitish brown	6	7	Light brown	Central
R ₉	White	5	6	Light brown	Scattered
R ₁₀	White	6	7	Light brown	Concentric circles
R ₁₁	Light brown	6	7	Light brown	Peripheral
R ₁₂	White	5	8	Light brown	Scattered
R ₁₃	White	6	8	Light brown	Scattered
R ₁₄	Light brown	7	4	Dark brown	Scattered
R ₁₅	White	11	9	White	Peripheral
R ₁₆	Light brown	4	5	Dark brown	Scattered

R ₁₇	Yellowish brown	5	7	Light brown	Scattered
R ₁₈	Centre–dark brown Periphery–Light brown	5	4	Dark brown	Scattered
R ₁₉	Light brown	6	4	Dark brown	Scattered
R ₂₀	Light brown	6	5	Light brown	Scattered
R ₂₁	Dark brown	3	3	Dark brown	Scattered
R ₂₂	Dark brown	4	5	Light brown	Scattered
R ₂₃	Light brown	7	8	Light brown	Peripheral
R ₂₄	Light brown	9	7	Light brown	Scattered
R ₂₅	Light brown	6	7	Light brown	Peripheral
R ₂₆	White	5	6	Light brown	Central
R ₂₇	White	9	8	White	Central
R ₂₈	White	6	5	Light brown	Central
R ₂₉	Light brown	6	6	Light brown	Scattered
R ₃₀	Whitish brown	11	8	Whitish brown	Peripheral
R ₃₁	White	6	5	Dark brown	Scattered
R ₃₂	White	5	6	Light yellow	Peripheral
R ₃₃	Light brown	4	5	Dark brown	Scattered
R ₃₄	Light brown	8	7	Whitish brown	Peripheral
R ₃₅	White	5	7	Light yellow	Central
R ₃₆	Light brown	8	4	Dark brown	Concentric circles
R ₃₇	Centre–dark brown Periphery–Light brown	6	5	Dark brown	Scattered
R ₃₈	Dark brown	4	4	Dark brown	Scattered
R ₃₉	Light brown	5	7	Light brown	Scattered
R ₄₀	White	8	7	Light brown	Concentric circles

3.5 *In vitro* evaluation of bacterial biocontrol agents and fungicides against sheathblight pathogen

3.5.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture

In vitro evaluation of biocontrol agents conducted as per the dual culture technique against the virulent fungal isolate R₂₁ recorded the highest inhibition of 68.64 per cent when *Bacillus amyloliquefaciens* (B15) was studied. However, *Pseudomonas fluorescens* (PN026) inhibited the growth of the pathogen by 49.78% (Fig. 4, Table 3). Similar results were observed in previous studies. *Bacillus* sp. was found to inhibit mycelial growth at a higher per cent than *Pseudomonas fluorescens*[17]. *Bacillus amyloliquefaciens* demonstrated highest inhibition of *R. solani* *in vitro*, by inhibiting the mycelial growth by 36 per cent [18].

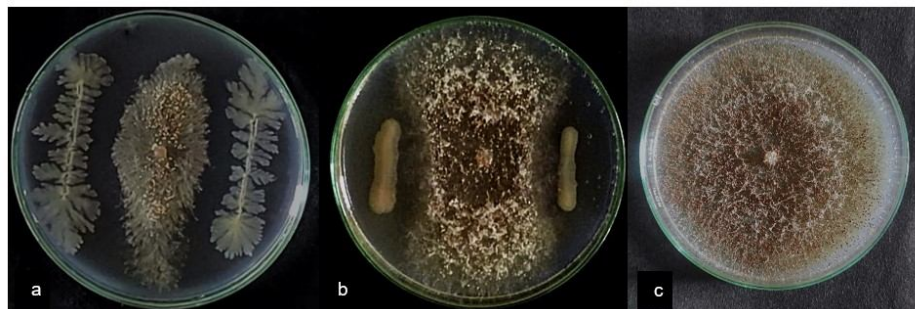


Fig. 4. *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture
a - *Rhizoctonia solani* against *Bacillus amyloliquefaciens*; b - *Rhizoctonia solani* against *Pseudomonas fluorescens*; c - Control

Table 3. *In vitro* evaluation of bacterial biocontrol agents against virulent isolate.

Treatments	Colony diameter (cm)*	Percent inhibition**
<i>Bacillus amyloliquefaciens</i> (B15)	(2.823 ± 0.09) ^c	68.64(55.945) ^a
<i>Pseudomonas fluorescens</i> (PN026)	(4.520 ± 0.25) ^b	49.78(44.876) ^b
Control	(9.00 ± 0.00) ^a	-
C. V	2.82	2.404
SE(m)	0.06	0.458
CD (0.05)	0.17	1.411

#Values are means of seven replications

*Mean of three replication ± Standard deviation

**Values in the parenthesis are arc sine transformed

Values followed by similar superscripts are not significantly different at 0.05% level [19]

3.5.2 *In vitro* evaluation of fungicides against sheath blight pathogen by poisoned food technique

In vitro evaluation of fungicides against sheath blight pathogen carried out by the poisoned food technique revealed complete inhibition when Kresoxim methyl 40% + Hexaconazole 8% WG at 500 ppm and 1000 ppm and Azoxystrobin 18.2% + Difenconazole 11.4% SC at 1000 ppm were assessed. Treatments using Kresoxim methyl 40% + Hexaconazole 8% WG at 250 ppm inhibited the mycelial growth by 90.16% whereas Azoxystrobin 18.2% + Difenconazole 11.4% SC at 250 ppm and 500 ppm showed 86.07% and 88.19% inhibition respectively. Among the three fungicides assessed, lowest inhibition was exhibited by Trifloxystrobin 25% + Tebuconazole 50% 75 WG. The percentage inhibition was 76.71%, 80.26% and 87.63% @ 100 ppm, 200 ppm and 400 ppm concentrations respectively. (Fig.5, Table 4). The findings agreed with similar works. In *in vitro* condition, the fungicide Azoxystrobin + Difenconazole gave approximately 80 per cent inhibition to *R. solani* at 100 ppm [20]. Azoxystrobin +

Difenoconazole was effective in inhibition of sheath blight pathogen [21]. Hexaconazole was found to inhibit mycelial growth more than 80 per cent at 100 ppm concentration and was recorded to have 100 per cent inhibition of *R. solani* at higher concentrations. [22]. Under in vitro conditions it was observed that fungicide Kresoxim methyl 40% and Hexaconazole 8% was found to be most effective against *R. solani* mycelial inhibition [23].

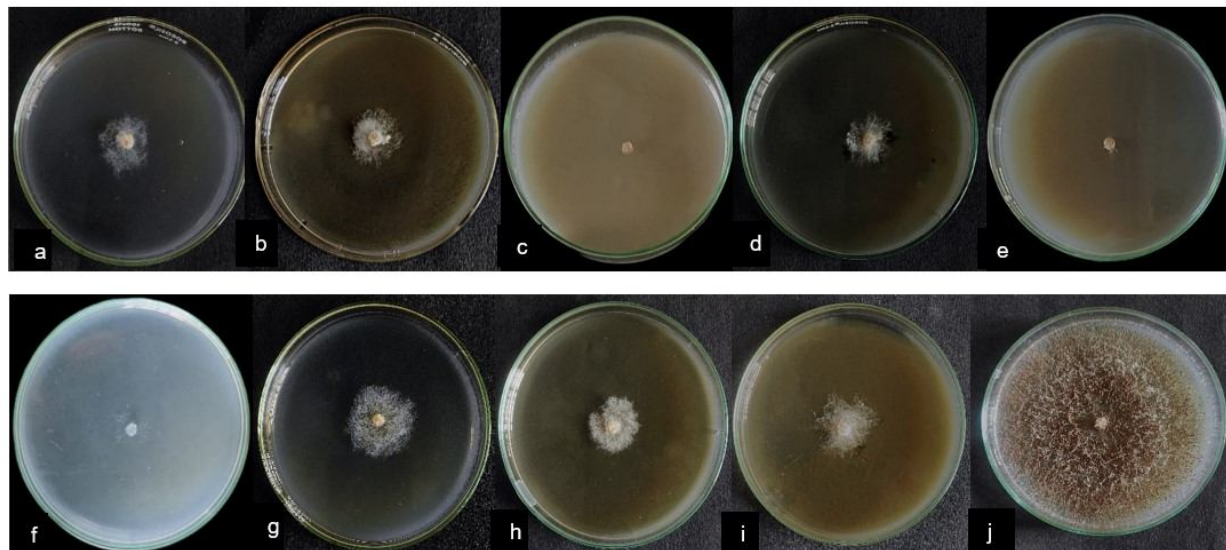


Fig. 5. In vitro evaluation of fungicides against sheath blight pathogen using poisoned food technique

a - Azoxystrobin 18.2% + Difenoconazole 11.4% SC - 250 ppm; b - Azoxystrobin 18.2% + Difenoconazole 11.4% SC - 500 ppm; c - Azoxystrobin 18.2% + Difenoconazole 11.4% SC - 1000 ppm; d - Kresoxim methyl 40% + Hexaconazole 8% WG - 250 ppm; e - Kresoxim methyl 40% + Hexaconazole 8% WG - 500 ppm; f - Kresoxim methyl 40% + Hexaconazole 8% WG - 1000 ppm; g - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 100 ppm; h - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 200 ppm; i - Trifloxystrobin 25% + Tebuconazole 50% 75 WG - 400 ppm; j - Control

Table 4. In vitro evaluation of fungicides against sheath blight pathogen using poisoned food technique

Treatments		Dose (ppm)	Colony diameter (cm)*	Percent inhibition
Chemical				
Azoxystrobin 18.2% + Difenoconazole 11.4% SC (T ₁)		250	1.25 (1.324 ± 0.008) ^d	86.07 (68.089) ^e
Azoxystrobin 18.2% + Difenoconazole 11.4% SC (T ₂)		500	1.06 (1.250 ± 0.005) ^f	88.19 (69.89) ^c
Azoxystrobin 18.2% + Difenoconazole 11.4% SC (T ₃)		1000	0.00 (0.707 ± 0.00) ^h	100 (90.00) ^a
Kresoxim methyl 40% + Hexaconazole 8% WG (T ₄)		250	0.89 (1.178 ± 0.002) ^g	90.16 (71.71) ^b

Kresoxim methyl 40% + Hexaconazole 8% WG (T ₅)	500	0.00 (0.707 ± 0.00) ^h	100 (90.00) ^a	
Kresoxim methyl 40% + Hexaconazole 8% WG (T ₆)	1000	0.00 (0.707 ± 0.00) ^h	100 (90.00) ^a	
Trifloxystrobin 25% + Tebuconazole 50% WG (T ₇)	75	100	2.09 (1.611 ± 0.004) ^b	76.71(61.14) ^g
Trifloxystrobin 25% + Tebuconazole 50% WG (T ₈)	75	200	1.78 (1.509 ± 0.002) ^c	80.26 (63.62) ^f
Trifloxystrobin 25% + Tebuconazole 50% WG (T ₉)	75	400	1.12 (1.271 ± 0.002) ^e	87.63 (69.41) ^d
Control (T ₁₀)		9.00 (3.082 ± 0.00) ^a	-	
C. V		0.25	0.122	
SE(m)		0.002	0.053	
CD (0.05)		0.06	0.157	

#Values are means of three replication

*Square transformed values ± Standard deviation

**Values in the parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level [19] (Gopinath *et al.*, 2021)

4. CONCLUSION

Sheath blight of rice caused by *Rhizoctonia solani* is one of the major diseases causing severe yield loss in rice production. The capability of the pathogen to survive in the adverse conditions through the production of sclerotia makes it difficult to manage. The frequent assessment of biocontrol agents and new fungicides are essential to reduce the spread of the pathogen. In the current study, the efficacy of biocontrol agents and fungicides are evaluated under *in vitro* conditions. It was observed that among the bacterial biocontrol agents *Bacillus amyloliquefaciens* was found to be effective against the pathogen which inhibited the mycelial growth by 68.64%. Among the fungicides evaluated, it was observed that at concentrations of 500 ppm and 1000 ppm Kresoxim methyl 40% + Hexaconazole 8% WG exhibited 100% inhibition in the growth of the pathogen compared to control which is on par with the fungicide Azoxystrobin 18.2% + Difenconazole 11.4% SC at 1000 ppm. The findings of this study suggests that these biocontrol agents and fungicides can be used for further field-level studies in management of sheath blight disease.

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.

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