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Growth dynamics of *Rhizoctonia solani* isolates causing web blight in black gram (*Vigna mungo* L.)

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Abstract
Rhizoctonia solani, the causative agent of web blight in black gram (*Vigna mungo*), poses a significant threat to pulse productivity during wet seasons. This study aimed to characterize the pathogen's growth dynamics under laboratory conditions. Symptomatology revealed typical necrotic lesions and sclerotia formation on infected tissues. The pathogen was morphologically identified by right-angle branching. Colony growth comparisons of three *R. solani* isolates on potato dextrose agar (PDA) showed statistically significant differences across 5-time intervals. Isolate Rs3 exhibited the highest mean radial growth (5.832 cm), indicating substantial genetic variability. Further evaluation across five culture media revealed Czapek Dox Agar as the most conducive medium (mean growth 6.127 cm), followed closely by Sabouraud's Dextrose Agar. The findings underscore the variability in pathogen behavior.

Keywords: *Rhizoctonia solani*, web blight, black gram, symptoms, culture media

Introduction
Black gram is cultivated since prehistoric times and believed to have originated in the Indian subcontinent according to archaeological records. This growth habits span from dwarf and erect to semi-prostrate and branched forms, with pods typically 3-5 cm long and bearing variable apical hairs. It is a major *kharif*-season crop with high protein content (24%), significant mineral contributions, and exceptional phosphoric acid concentration. Urdbean is used in traditional medicine and serves as nutritious fodder for livestock. Additionally, it improves soil fertility by fixing atmospheric nitrogen and helps conserve soil moisture. Globally, it is cultivated in countries including Bangladesh, Myanmar, China, Indonesia, Nepal, Pakistan, Sri Lanka, and Thailand. India, however, dominates world production with over 70% of the production, with key growing states being Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Karnataka, and Andhra Pradesh. Black gram is a vital pulse crop in Odisha (Mahapatra, 2020) [5], contributing significantly to the state's pulse production and nutrition security. Grown across diverse agro-climatic regions like the Mahanadi Delta and Rusikulya plains, it accounts for 28.04% of the pulse area and 25% of total production. The crop is susceptible to major diseases including yellow mosaic, *Cercospora* leaf spot, powdery mildew, anthracnose, dry root rot, web blight and leaf crinkle virus. These diseases affect plants at various growth stages, resulting in symptoms such as leaf yellowing, necrotic lesions, wilting, premature defoliation, and pod malformation. One of the most destructive fungal diseases of black gram is web blight, caused by *Rhizoctonia solani* Kuhn, a soil-borne polyphagous fungal pathogen producing resilient sclerotia. *R. solani* is among the most significant and destructive fungal pathogens affecting global agriculture, noted for its broad host range and high ecological adaptability, making it challenging to manage. The fungus attacks members of diverse botanical families. The fungus colonizes seeds and below-ground plant tissues most aggressively, but can also infect above-ground stems and pods in certain conditions. The ability of *R. solani* to form sclerotia contributes to long-term persistence in soils across multiple cropping cycles, while its highly competitive behaviour in the rhizosphere allows it to displace beneficial microbes. Chemical control using fungicides, while often effective in the short term, presents several significant disadvantages. A primary concern is the potential for the pathogen to develop resistance due to repeated and prolonged fungicide applications, ultimately reducing long-term efficacy.

Moreover, with diverse geographical locations and host crops, the pathogen exhibits substantial variation in behaviour. Hence, the goal of the current study is to assess the cultural variability among the *R. solani* isolates.

Materials and Methods

Diseased sample collection

Black gram (*Vigna mungo*) plants showing symptoms of disease were carefully collected from the SOA INS farm, Khordha, for laboratory analysis. Both healthy and diseased plants were selected randomly from different locations across the field to ensure representative sampling. Collected samples included roots, stems, and leaves, especially those exhibiting typical lesions or discoloration. Each sample was placed in sterile paper bags, labelled with details (location, date, and symptoms), and kept at 4°C until further isolation of pathogens.

Cleaning and sterilisation of glassware and equipment

Glassware, including beakers, test tubes, conical flasks, Petri dishes and other reusable items, was initially soaked in a detergent solution after washing with potassium dichromate solution. Then, the items were rinsed with tap water and subsequently with distilled water to remove any detergent residues. These items were sterilised in hot air oven at 160°C for 2 hours. Moist heat sterilisation of culture media and distilled water was performed using an autoclave at 121°C and 15 psi pressure for 20 minutes. During inoculation and culture transfer procedures, small instruments such as scalpels, needles, and forceps were sterilised using 70% ethanol followed by incineration.

Isolation, purification and preservation of the pathogen

Infected plant samples exhibiting both diseased and healthy portions, particularly on leaves, were brought to the laboratory in sterile paper bags for pathogen isolation. Small bits (1-2 mm) were excised from the junction of healthy and diseased leaf tissues. These segments were surface sterilized by immersion in 0.1% mercuric chloride solution and rinsed thoroughly thrice with a series of sterile distilled water. The tissues were aseptically placed onto solidified water agar plates and incubated at 27±2°C for 2-5 days. Any visible mycelial growth was carefully transferred to solidified PDA plates. The fungal colonies were observed for characteristic morphological features using standard literature and purified by the hyphal tip method. The cultures were maintained at 27±2°C for 3-5 days. The cultures obtained by this method were preserved on PDA slants at 4°C. Different isolates of the fungus were tested for their morphological characters and growth dynamics over PDA. For the same, 3 isolates of the fungus were tested at 5 time-intervals (24, 48, 72, 96 and 120 hours) with 5 replicates for each treatment combination. Statistical analysis was done with the help of OPSTAT.

Culture media for fungal growth

For the isolation, cultivation, and morphological studies of fungal pathogens, various culture media were prepared using standard procedures. All media components were weighed accurately and dissolved in distilled water, where required. The media were then sterilised in an autoclave at 121°C and 15 psi for 20 minutes. After sterilisation, the media were poured aseptically into sterile Petri dishes under a laminar airflow cabinet.

1. Potato Dextrose Agar (PDA) was prepared by boiling 200 g of peeled and chopped potatoes in 1 litre of distilled water for 30 minutes, filtering the extract, and adding 20 g dextrose and 20 g agar to the potato extract.
2. Richard's Agar consisted of 10 g sucrose, 5 g potassium nitrate (KNO_3), 5 g magnesium sulfate ($MgSO_4.7H_2O$), 2.5 g potassium dihydrogen phosphate (KH_2PO_4), 0.02 g ferric chloride ($FeCl_3$), and 20 g agar per litre of distilled water.
3. Czapek Dox Agar was prepared using 30 g sucrose, 3 g sodium nitrate ($NaNO_3$), 1 g dipotassium phosphate (K_2HPO_4), 0.5 g magnesium sulfate ($MgSO_4.7H_2O$), 0.5 g potassium chloride (KCl), 0.01 g ferrous sulfate ($FeSO_4$), and 20 g agar in 1 litre of distilled water.
4. Wheat Extract Agar was made by soaking 100 g of wheat grains in distilled water overnight, boiling the mixture for 30 minutes, filtering the extract, and adding 20 g agar to the filtrate.
5. Yeast Extract Agar is composed of 10 g yeast extract, 20 g glucose, and 20 g agar per litre of water.
6. Sabouraud's Dextrose Agar was prepared by dissolving 40 g dextrose and 10 g peptone in 1 liter of distilled water, with the addition of 20 g agar to solidify the medium.
7. Water Agar was prepared by dissolving only 20 g agar in 1 litre of distilled water. Except PDA and water agar, other five culture media were used to study the growth dynamics of the fungal pathogen isolates. This experiment was conducted with 5 media, 4 time-intervals (24, 48, 72 and 96 hours) and 3 pathogenic isolates. Each combination was executed with four replications each. The data was analyzed using OPSTAT online portal.

Results and Discussion

Water-soaked lesions appear on leaves, stems, and pods, enlarging rapidly and coalesce into irregular necrotic patches. Characteristic white, cottony, web-like mycelial growth with sclerotia on the infected portions is a distinguishing sign for identification of the disease (Fig-1a,b). The pathogen was full grown in the Petri plate in about 5-7 days at 27 ± 2°C by hyphal tip method. The cultures appeared cottony and fast growing. Later with age, there were production to off white to brown sclerotia, which later turn chocolaty to dark coloured. Right angle branching of the mycelium was noticed, which is a typical characteristic associated with *R. solani* (Fig-2a,b). These findings agree with the findings of Desvani et al. (2018) and Akber et al. (2023) [2, 4].

The colony growth characteristics of three *R. solani* isolates causing web blight of black gram were systematically evaluated on potato dextrose agar (PDA) medium over five-time intervals (24, 48, 72, 96, and 120 hours). The experimental design employed a factorial arrangement in a completely randomized design with five replications, allowing for comprehensive statistical analysis of isolate behavior and temporal growth patterns. There was substantial variation in colony growth among the three *R. solani* isolates and progressive increase in colony growth over time. The growth patterns of isolates varied differently across the observation periods.

Isolate Rs3 exhibited the highest overall mean colony growth (5.832 cm), followed by isolate Rs1 (5.648 cm), while isolate Rs2 demonstrated the lowest growth rate

(5.032 cm). This variation in growth characteristics among isolates is consistent with the established understanding that *R. solani* is a species complex with considerable genetic diversity manifested through different biological characteristics (Ajayi-Oyetunde and Esker, 2018) [1]. The colony growth measurements across time intervals revealed a consistent progressive increase for all isolates. At 24 hours post-inoculation, the mean colony growth was 2.580 cm, which increased systematically through subsequent observation periods: 3.813 cm (48 hours), 5.500 cm (72 hours), 6.987 cm (96 hours), and 8.640 cm (120 hours). Individual isolate performance varied across time intervals, contributing to the significant interaction effect observed. Isolate Rs1 showed colony growth ranging from 2.640 cm (24 hours) to 8.820 cm (120 hours), demonstrating consistent growth acceleration. Isolate Rs2, despite having the lowest overall mean, maintained steady growth from 2.160 cm to 8.140 cm across the same period. Isolate Rs3 exhibited the most robust growth pattern, with measurements ranging from 2.940 cm to 8.960 cm, consistently outperforming the other isolates at most time intervals. The significant interaction between isolates and time intervals indicates differential growth responses among isolates, suggesting potential variations in their competitive ability and pathogenic potential (Table-1).

Substantial variation in growth responses across different media compositions was observed. Among the culture media evaluated, Czapek Dox Agar demonstrated superior performance with the highest mean radial growth of 6.127 cm, followed by Sabouraud's Dextrose Agar at 6.035 cm and Yeast Extract Agar at 5.985 cm. Wheat Extract Agar showed intermediate performance with a mean growth of 5.906 cm, while Richard's Agar exhibited the lowest mean radial growth of 5.548 cm. A clear progressive increase in radial growth was observed with increasing incubation time. The mean radial growth increased from 4.892 cm at 24 hours to 5.410 cm at 48 hours, further expanding to 6.265 cm at 72 hours, and reaching maximum growth of 7.115 cm at 96 hours. Maximum growth rate occurred during the initial 72-96 hours of incubation. The mean radial growth varied from 5.880 cm (Rs1) to 5.974 cm (Rs3), with Rs2 showing intermediate growth of 5.908 cm. Isolate Rs3 demonstrated the highest growth rate, suggesting potentially higher aggressiveness, while isolate Rs1 showed the most restricted growth pattern. The significant interaction between culture media and isolates ($p < 0.001$, $F = 8.393$) demonstrates that isolate performance varies depending on the medium used. This finding suggests that different isolates may have varying nutritional preferences or metabolic capabilities, which could reflect their adaptation to different environmental conditions or host tissues. The significant three-way interaction among medium, time, and isolate ($p < 0.001$, $F = 119.077$) demonstrates the complexity of growth responses, indicating that the combined effects of these factors cannot be predicted from their individual or two-way interactions alone (Table-2-5).

Fig 1a: Infected leaf with water soaked lesion and webbing (Initial stage) **b:** Infected stem with necrosis, cankerous lesion and sclerotia

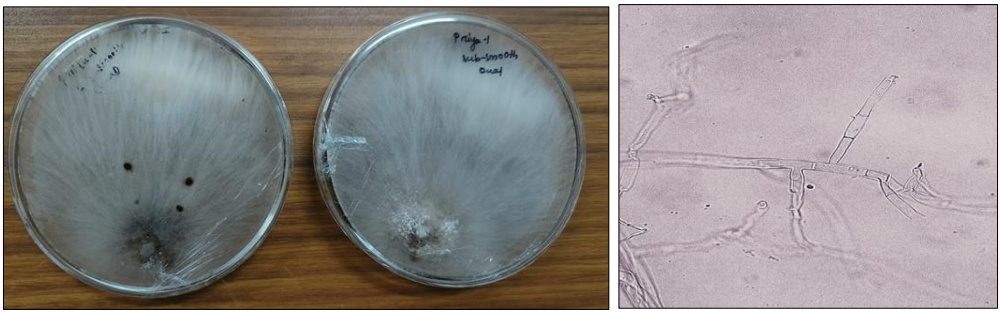

Fig 2a: Two cultures of *R. solani*: Dark brown sclerotia (left) and White sclerotia (right) **2b:** Right angle branching and septation in mycelium of *R. solani* (40X)

Table 1: Effect of isolates and time interval on colony diameter of *R. solani* in PDA

	Colony diameter (cm)					
	24h	48h	72h	96h	120h	Mean
Rs1	2.640	4.040	5.700	7.040	8.820	5.648
Rs2	2.160	3.320	4.940	6.600	8.140	5.032
Rs3	2.940	4.080	5.860	7.320	8.960	5.832
Mean	2.580	3.813	5.500	6.987	8.640	

Factors	SE(m)±	C.D.
Isolate	0.019	0.055
Time	0.025	0.071
Isolate X Time	0.043	0.123

Table 2: Effect of culture media, time interval and isolates on the colony diameter of *R. solani*

	Richard's Agar				**Czapek Dox Agar**				**Wheat Extract Agar**				**Yeast Extract Agar**				**Sabouraud's Dextrose Agar**			
	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
Rs1	2.100	8.475	6.875	4.675	3.225	8.625	7.500	5.225	3.075	8.525	7.025	4.900	2.975	8.400	7.000	5.100	2.100	8.975	8.025	4.800
Rs2	4.275	2.325	8.475	6.975	4.925	3.300	8.575	7.575	4.625	3.000	8.675	7.275	5.025	2.975	8.100	7.800	4.625	2.475	8.975	8.175
Rs3	6.875	4.425	2.525	8.575	7.325	5.025	3.400	8.825	7.100	4.775	3.075	8.825	7.200	5.075	3.175	9.000	7.925	4.775	2.575	9.000

	SE(m)±	**C.D.**
Factor(A)	0.010	0.028
Factor(B)	0.009	0.025
Interaction A X B	0.020	0.055
Factor(C)	0.008	0.021
Interaction A X C	0.017	0.048
Interaction B X C	0.015	0.043
Interaction A X B X C	0.034	0.095

Table 3: Effect of culture media and time interval on the colony diameter of *R. solani*

	Colony Diameter (cm)				**Mean**
	24h	**48h**	**72h**	**96h**	
Richard's Agar	4.417	5.075	5.958	6.742	5.548
Czapek Dox Agar	5.158	5.650	6.492	7.208	6.127
Wheat Extract Agar	4.933	5.433	6.258	7.000	5.906
Yeast Extract Agar	5.067	5.483	6.092	7.300	5.985
Sabouraud's Dextrose Agar	4.883	5.408	6.525	7.325	6.035
Mean	4.892	5.410	6.265	7.115	

SE(m) ± C.D.

Factor(A=media)	0.010	0.028
Factor(B=time interval)	0.009	0.025
Interaction A X B	0.020	0.055

Table 4: Effect of culture media and isolates on the colony diameter of *R. solani*

	Colony Diameter (cm)			
	Rs1	Rs2	Rs3	Mean
Richard's Agar	5.531	5.513	5.600	5.548
Czapek Dox Agar	6.144	6.094	6.144	6.127
Wheat Extract Agar	5.881	5.894	5.944	5.906
Yeast Extract Agar	5.869	5.975	6.113	5.985
Sabouraud's Dextrose Agar	5.975	6.063	6.069	6.035
Mean	5.880	5.908	5.974	

Factor(A=media)	0.010	0.028
Factor(C=isolates)	0.008	0.021
Interaction A X C	0.017	0.048

Table 5: Effect of time interval and isolates on the colony diameter of *R. solani*

	Rs1	Rs2	Rs3	Mean
24h	2.695	4.695	7.285	4.892
48h	8.600	2.815	4.815	5.410
72h	7.285	8.560	2.950	6.265
96h	4.940	7.560	8.845	7.115
Mean	5.880	5.908	5.974	

	SE(m)±	C.D.
Factor(Time)	0.009	0.025

Factor(Isolate)	0.008	0.021
Time X Isolate	0.015	0.043

Conclusion

The research employed a multifaceted approach encompassing symptomatology, pathogen isolation, and comparative growth studies to advance understanding of this economically significant plant disease. The comparative analysis of three *R. solani* isolates on potato dextrose agar revealed significant variability in growth characteristics, with isolate Rs3 demonstrating the highest overall mean colony growth, followed by Rs1 and Rs2. The temporal analysis showed consistent progressive growth across all isolates, with colony diameters increasing from 2.580 cm at 24 hours to 8.640 cm at 120 hours post-inoculation. The evaluation of growth dynamics across different culture media demonstrated that Czapek Dox Agar provided optimal conditions for *R. solani* growth, followed by Sabouraud's Dextrose Agar and Yeast Extract Agar.

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