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Unveiling the Virulence Arsenal: Cultural Characterisation and Growth Kinetics of *Rhizoctonia solani* Isolates Associated with Damping-off and Bottom Rot in Broccoli

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A B S T R A C T

Rhizoctonia solani is a soil-borne fungal pathogen with a broad host range that inflicts severe damage on diverse agricultural crops worldwide. This study investigated the physiological adaptation of *R. solani* by assessing how various nutrient media, temperature regimes, and pH levels influence its mycelial growth and sclerotia formation. The pathogen was isolated from infected broccoli roots showing damping-off symptoms and characterized morphologically and microscopically. Radial growth, colony coloration, mycelial density, and type were evaluated across seven culture media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), Richard's Agar, Komada's Medium (KM), and Oatmeal Agar (OMA). Growth kinetics were modeled using the Area Under the Kinetic Curve (AUKC). Additionally, mycelial proliferation was evaluated across temperatures ranging from 15°C to 30°C and pH levels from 5.0 to 7.0. Considerable physiological variation occurred across different culture media. PDA supported the highest radial growth diameter, followed by KM (89.42 mm) and Richard's Agar (81.32 mm). Kinetic curve modeling revealed optimal mycelial growth rates between 120 and 144 hours of incubation on PDA. Environmental testing identified optimal growth at 30°C and pH 6.5, whereas significant growth inhibition occurred at extreme temperature and pH values. *R. solani* demonstrates strong adaptability across variable nutrient and environmental conditions, enabling rapid proliferation under favourable settings. These findings clarify the physiological parameters governing *R. solani* development on broccoli, offering actionable insights for optimizing disease forecasting and management strategies.

Introduction

Broccoli, or *Brassica oleracea* L. var. italica, is an important vegetable grown for human consumption in tropical and subtropical regions worldwide. It belongs to the Brassicaceae family and is recognised for its global significance (Rubatzky *et al.*, 1997). India is the second, with an estimated total production of approximately 10.7

million tons and 9.6 million tons of broccoli, respectively (Geeta & Diptimayee, 2023). Broccoli species, which are essentially diploids, were domesticated in the Mediterranean and Asia Minor. It is a globally significant vegetable crop with increasing market demand due to its high nutritional value and health benefits (Branca & Cartea, 2010; Sharma *et al.,* 2022). It is rich in vitamins, minerals, fibre, and bioactive compounds like glucosinolates, which have potential anti-cancer properties (Syed *et al.,* 2023; Li *et al.,* 2022).

Rhizoctonia solani is a highly prevalent and damaging fungus that poses a significant threat to plant health across a wide range of species. It has a diverse host range, causing varied diseases in crops worldwide (Dubey *et al.,* 2014; Akber *et al.,* 2023). It can affect plants at different growth stages, leading to significant yield losses (Baker, 1970; Trkulja *et al.,* 2022; Kuiry *et al.,* 2014). *R. solani* isolates exhibit considerable morphological variability, including differences in colony characteristics, sclerotia formation, and hyphal growth (Goswami *et al.,* 2011; Singh *et al.,* 2014). This variability is shaped by crucial factors, such as the genetic traits of the host, the aggressiveness of the pathogen, and the prevailing environmental conditions. Understanding these influences is essential for effective management and intervention strategies (Kuiry *et al.,* 2014). Traditional identification methods based on morphology and hyphal anastomosis reactions have limitations, prompting the use of molecular techniques for more accurate pathogen detection and classification. Recent research has focused on investigating endogenous double-stranded RNAs and employing 'omics' approaches to better understand *R. solani* biology and pathogenicity (Das *et al.,* 2021). A basic requirement of all the phytopathogenic fungi is nutrition, but their requirements differ among themselves. The growth and sporulation of *Rhizoctonia solani* are influenced by various factors, including the nutritional composition of culture media, as well as environmental conditions such as temperature, moisture levels and pH. Given the significance of this pathogen and the diseases it causes, the current study aims to examine how different nutrient media, varying temperature and pH levels impact the growth of this fungus.

Materials and Methods

The research was carried out at the Research Laboratory in the Department of Plant Pathology at R.G. Bagdia Arts, S.B. Lakhotia Commerce, and R. Bezonji Science College, located in Jalna, India.

Collection, isolation and identification of the pathogen

The diseased samples of Broccoli showing typical symptoms of damping off and bottom rot were procured from the farmer's field area, Tehsil Jalna, and brought to the laboratory. After washing with tap water, small segments of affected and healthy tissues were excised from the infected roots using a sterilised blade. These segments were disinfected in a 1% sodium hypochlorite solution and rinsed with sterile distilled water. After drying on sterilised blotting paper, the tissues were inoculated onto potato dextrose agar (PDA) in sterilised Petri dishes and incubated at 25 °C. Once fungal growth appeared, agar pieces from the growing mycelium were transferred to new PDA slants for culture purity. The purified cultures were stored at 4–5 °C for future use. Initial pathogen identification was based on cultural and microscopic features such as the type of mycelium, colour of mycelium, and whether sclerotia were formed. Confirmatory analysis was conducted at the Agharkar Research Institute in Pune.

Influence of various nutrient media on the mycelial development of *Rhizoctonia solani*

Seven distinct solid media were assessed for the growth of *Rhizoctonia solani*, including Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar with Antibiotics (SDA), Richard's Agar (RA), Komada's Medium (KM), and Oat Meal Agar (OMA). A 5 mm diameter plug was excised from a pure culture using a cork borer and placed at the centre of a Petri dish filled with the respective medium. These plates were then incubated in a BOD incubator at 25 ± 1 °C. Each experimental condition was replicated three times, and measurements were taken at 24-hour intervals for up to 7 days (168 hours) to record the average growth diameter in millimetres, along with observations of cultural characteristics such as mycelial colour, growth type, and overall growth patterns. To analyse the growth data obtained from the various nutrient media over the different incubation times, growth curves were created, plotting mycelial growth on the Y-axis against time on the X-axis. The growth rate in millimetres per hour was calculated for the test fungus on each type of media.

To capture subtle variations in growth rates throughout

the growth curve and identify significant changes in growth dynamics, a calculation model based on the area under the kinetic curve (AUKC) was employed. This approach allowed for the estimation of the relative AUKC (rAUKC) at each time interval (Bhogal *et al.,* 2022).

ΔrAUKC values, calculated by subtracting the rAUKC at each time point from that of the previous point, indicate changes in the fungus's growth rate. A linear increase in ΔrAUKC suggests consistent mycelial growth, while a decrease signals a reduction in growth rate. Higher ΔrAUKC values correspond to faster growth. These values were plotted against time intervals for different nutrient media to analyse growth trends. Additionally, the time to produce sclerotia and the total number produced over 30 days were recorded to identify the best nutrient medium for further research.

Impact of various temperature conditions on the development of *Rhizoctonia solani*

To investigate the impact of various temperature conditions on the mycelial growth of the pathogen, Petri dishes filled with the optimal nutrient medium were inoculated with a 5mm diameter piece of the test fungus. These dishes were then placed in different BOD incubators set at temperatures of 10, 15, 20, 25, 30, 35, and 40 °C, and monitored for up to 168 hours. Each experimental condition was replicated three times, and measurements of average growth diameter (in mm) were taken at 24-hour intervals.

Additionally, the time taken for sclerotia production and the total number of sclerotia formed were recorded over a 30-day incubation period. The growth rate and rAUKC values of the fungus at each temperature setting were computed. Based on these observations, an optimal temperature for subsequent experiments was determined.

Impact of Various pH Levels on the Growth of *Rhizoctonia solani*

To investigate how various pH levels influence the growth of *Rhizoctonia solani*, a nutrient medium was prepared and adjusted to pH values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 using IN HCl and 1N NaOH. This medium was then placed into Petri dishes and inoculated with a 5 mm diameter culture segment. The Petri dishes were incubated for up to 168 hours. Each pH treatment was replicated three times, and measurements of average growth diameter (in mm) were taken at 24-hour intervals over a period of 7 days (168 hours). Additionally, sclerotia production was observed for up to 30 days, and the growth rate of the fungus, along with the relative AUKC (rAUKC) value at each pH level, was subsequently calculated.

Statistical evaluation

This research conducted three experiments that were subjected to statistical analysis using the software OPSTAT in a completely randomised design (Sheoran, 2006).

Results and Discussion

Samples of broccoli roots exhibiting typical damping-off symptoms were gathered from agricultural fields in the Jalna Tehsil region. The identified symptoms included water-soaked lesions appearing on the stem and lower foliage of the broccoli plants. Over time, these lesions enlarge and merge, leading to the formation of a dark, sunken rot near the base of the plant. In severe cases, the afflicted roots develop pronounced dark, sunken areas that may extend upwards. If left untreated, the affected tissue can become necrotic, ultimately causing the death of the plant (Fig 1). The pathogen was successfully isolated and cultured on potato dextrose agar (PDA) and preserved in PDA slants at temperatures between 4 and 5 degrees Celsius in refrigeration. A comprehensive examination of the pathogen's cultural and microscopic features was conducted.

On Potato Dextrose Agar (PDA), *Rhizoctonia solani* exhibits a well-developed, initially densely cottony colony with a flocculent (woolly) central region. The aerial mycelium is initially white to cream-coloured, transitioning to a faintly vinaceous-buff (brownish-white) hue with maturity. Colony margins appear lobate (lobed) or irregular, maintaining a white to cream colouration (Fig. 2). Microscopic examination reveals monomitic (uniform) hyphae with septate (cross-walled) structure. These hyphae appear ochraceous-tawny (yellowish-brown) and thick-walled (Fig.3). Prolonged incubation may induce the formation of sclerotia, the resting structures of the fungus. These sclerotia appear melanistic (dark) or sepia (brown) in color, exhibiting pleomorphic (variable) shapes and sizes. Macroscopically, these sclerotia appear irregular, ranging from light to dark brown, lacking differentiation into a distinct rind and medulla (inner layer). Sclerotial

development is typically observed within one to two weeks of incubation on PDA (Fig.4). These observations are consistent with the findings reported by Thind and Aggarwal (2008); Agarwal (2010); Mishra *et al.,* (2014); Jaaffar *et al.,* (2016).

Impact of Diverse Nutrient Media

The recorded data shows the average diameter growth (in mm) of *Rhizoctonia solani* after 7 days (168 hours) of incubation. Growth was measured at 24-hour intervals on different nutrient media, as shown in Table 1. The results indicate that the fungus thrived on all nutrient media tested. A notable maximum increase in diametric growth of 90.21 mm was observed in Potato Dextrose Agar (PDA), with Komada's Medium (KM) following closely at 89.42 mm, and Richard's Agar (RA) at 81.32 mm. In contrast, the lowest mean mycelial growth was noted in Sabouraud Dextrose Agar with Antibiotics (SDA), measuring 57.23 mm, followed by Malt Extract Agar (MEA) at 60.73 mm. The growth patterns typically exhibited a sigmoid shape across most nutrient media. Regarding growth rates, the test pathogen showed a significantly higher mean growth rate of 0.59 mm h^{-1} in PDA, followed by 0.53 mm h^{-1} in Komada's Medium (KM). The lowest growth rate was recorded in MEA at 0.34 mm h^{-1}, which was statistically similar to that observed in OMA, where the rate was 0.32 mm h^{-1}. Overall, the highest average growth rate of 0.76 mm h^{-1} was noted between 120-144 hours of incubation, while a lower rate of 0.41 mm h^{-1} was achieved during the same interval. The minimum average growth rate was 0.25 mm h^{-1} between 24-48 hours. According to the table data, the peak average growth rate of 0.93 mm h^{-1} for the test fungus occurred between 144-168 hours in PDA, followed by 0.87 mm h^{-1} during the 120-144 hour window in the same medium. Conversely, the lowest average growth rates of 0.16 mm h^{-1} were observed in both MEA and OMA from 0-24 hours. Intermediate growth rates were recorded across other media at various incubation intervals.

The analysis of ΔrAUKC values over time revealed that the most significant growth peak occurred in Potato Dextrose Agar (PDA) during the incubation period of 120 to 144 hours. In contrast, the other media evaluated displayed their peak growth between 72 and 96 hours of incubation. Notably, in the cases of Richard's Agar (RA), Czapek Dox Agar (CDA), and Oatmeal Agar (OMA), a distinct peak in growth was not observed; however, fungal growth persisted even throughout the 144 to 168-hour incubation window. Furthermore, the influence of various nutrient media on sclerotia production was investigated. As summarised in Table 2, the duration required for sclerotia formation varied among the media, with Komada Medium (KM) showing the longest time of 23.82 days for sclerotia production. This was closely followed by Richard Agar, which required 21.21 days. In contrast, Potato Dextrose Agar facilitated the fastest sclerotia production, requiring an average of just 9.28 days. Malt Extract Agar (MEA) also demonstrated a relatively short production time of 12.93 days. Regarding the quantity of sclerotia produced, the highest mean count of sclerotia, 89.1, was recorded on Czapek's Dox Agar, followed by 79.4 on Potato Dextrose Agar. Conversely, the lowest mean count of sclerotia was observed on Oatmeal Agar, which yielded only 37.2 sclerotia.

The cultural characteristics of *Rhizoctonia solani* were evaluated on various nutrient media, revealing significant physiological variations in terms of radial growth, colony colour, mycelium type, and mycelium abundance (Table 2). Potato Dextrose Agar (PDA) and Czapek Dox Agar (CDA) were most effective in promoting abundant mycelial growth. PDA supported the highest radial growth with a dense, fluffy mycelium, while CDA facilitated a flatter, filamentous growth. The presence of potato starch in PDA, which is a natural host component for *R. solani*, likely stimulated significant growth. This is consistent with findings by Nuri *et al.,* (2021), who reported similar growth patterns on PDA. Malt Extract Agar (MEA) and Sabouraud Dextrose Agar with Antibiotics (SDA) resulted in moderate to scanty mycelial growth. The moderate cottony appearance on MEA and the slower growth on SDA can be attributed to their nutrient composition, which may not fully meet the nutritional requirements of *R. solani*. Richard Agar (RA) and Komada's Medium (KM) supported abundant growth, with RA showing a potentially cottony and white appearance and KM exhibiting very cottony and fluffy mycelium initially. These media provide a balanced nutritional environment that supports substantial fungal growth. Oat Meal Agar (OMA) produced moderate growth with a cottony and white appearance, though the growth pattern was slower compared to PDA and CDA.

The results of this investigation align with findings from prior studies, which observed that the growth of *Rhizoctonia solani* is positively influenced by substrates rich in carbohydrates (Ritchie *et al.,* 2009; Nuri *et al.,* 2021; Chaudhary *et al.,* 2018).

Table.1 Mean Diametric Growth and Growth Rate of *R. solani* on Different Nutrient Media after 168 Hours

Sr. No.	Nutrient Medium	Average Diametric Growth (mm) after 168 h	Growth Rate (mm) between interval (h)							Overall Mean
			0-24	24-48	48-72	72-96	96-120	120-144	144-168	
1	Potato Dextrose Agar (PDA)	90.21	0.38	0.52	0.18	0.25	0.67	0.87	0.93	0.59
2	Malt Extract Agar (MEA)	60.73	0.16	0.36	0.12	0.32	0.58	0.68	0.32	0.34
3	Czapek Dox Agar (CDA)	65.98	0.21	0.24	0.37	0.42	0.31	0.78	0.67	0.39
4	Sabouraud Dextrose Agar with Antibiotics (SDA)	57.23	0.29	0.17	0.32	0.37	0.42	0.76	0.89	0.47
5	Richard's Agar (RA)	81.32	0.33	0.21	0.26	0.51	0.62	0.65	0.76	0.47
6	Komada's Medium (KM)	89.42	0.27	0.13	0.39	0.43	0.57	0.96	0.88	0.53
7	Oat Meal Agar (OMA)	61.23	0.16	0.18	0.21	0.29	0.32	0.56	0.53	0.32
	Overall Mean	70.3	0.26	0.25	0.26	0.37	0.49	0.76	0.71	
		CD (p≥0.05) *	**SE (d)**							
	Nutrient Medium	1.08	0.56							
	Duration	2.93	1.32							

Table.2 Morphological and Growth Characteristics of *R. solani* on Different Nutrient Media

Sr. No.	Nutrient Medium	Days of Production of Sclerotia	No. of sclerotia produced by *R. solani* on media (cm^2)	Abundance of mycelium	Growth Pattern	Type of Growth	Mean Texture of sclerotia	Formation of sclerotia	Colony Color
1	Potato Dextrose Agar (PDA)	9.28	79.4	Moderate	Condense	Initially cottony and white, with a dense, fluffy center	Smooth	Peripheral	Brownish white
2	Malt Extract Agar (MEA)	12.93	57.2	Scanty	Moderate	Moderately cottony, with a white to cream-colored area	Rough	Scattered	Yellowish white
3	Czapek Dox Agar (CDA)	17.32	89.1	Abundant	Condense	Less cottony, with a flatter and more filamentous (thread-like) aerial mycelium	Smooth	Peripheral	Yellowish white
4	Sabouraud Dextrose Agar with Antibiotics (SDA)	16.29	39.1	Scanty	Slow	white to cream	Rough	Scattered	White
5	Richard's Agar (RA)	21.21	65.2	Abundant	Condense	potentially cottony and white appearance	Smooth	Central	White
6	Komada's Medium (KM)	23.82	78.3	Abundant	Moderate	Very cottony and fluffy initially, with a dense, white aerial mycelium.	Smooth	Central	Brownish white
7	Oat Meal Agar (OMA	18.21	37.2	Moderate	Slow	cottony and white appearance initially with potential for lobed or irregular margins	Rough	Peripheral	Brownish white
		CD (p≥0.05) *	**SE (d)**						
	Days	1.76	0.36						
	Sclerotia	2.05	0.76						

Table.3 Effect of Temperature on Diametric Growth and Sclerotia Production of *R. solani* After 168 Hours

Sr. No.	Temperature	Average Diametric Growth (mm) after 168 h	Growth Rate (mm) between interval (h							Overall Mean	Days of Production of Sclerotia	No. of sclerotia produced by *R. solani*
			0-24	24-48	48-72	72-96	96-120	120-144	144-168			
1	10	56.95	0.04	0.21	0.29	0.26	0.67	0.53	0.53	**0.36**	12.07	3.9
2	15	72.31	0.04	0.54	0.38	0.39	0.71	0.46	0.31	**0.41**	17.32	5.3
3	20	75.21	0.08	0.43	0.47	0.54	0.49	0.37	0.49	**0.41**	18.09	38.8
4	25	78.43	0.09	0.52	0.49	0.87	0.69	0.72	0.47	**0.51**	18.98	79.7
5	30	83.98	0.07	0.46	0.58	0.68	0.58	0.52	0.58	**0.49**	16.32	63.8
6	35	23.41	0.03	0.38	0.46	0.68	0.32	0.58	0.22	**0.38**	12.09	58.8
7	40	20.14	0.05	0.19	0.39	0.49	0.49	0.45	0.39	**0.36**	11.21	3.27
	Overall Mean	**58.63**	**0.06**	**0.39**	**0.43**	**0.46**	**0.64**	**0.48**	**0.44**	--	**CD (p≥0.05) ***	**SE (d)**
		CD (p≥0.05) *	**SE (d)**							**Days**	1.39	0.22
	Temperature	0.38	0.16							**Sclerotia**	2.24	0.53
	Duration	0.42	0.18									

Table.4 Effect of pH on Diametric Growth and Sclerotia Production of *R. solani* After 168 Hours

Sr. No.	pH	Average Diametric Growth (mm) after 168 h	Growth Rate (mm) between interval (h							Overall Mean	Days of Production of Sclerotia	No. of sclerotia produced by *R. solani* (cm2)
			0-24	24-48	48-72	72-96	96-120	120-144	144-168			
1	5	17.3	0.13	0.19	0.12	0.21	0.09	0.26	0.12	0.16	12.09	5.2
2	5.5	21.6	0.09	0.23	0.29	0.45	0.34	0.38	0.11	0.27	16.02	30.3
3	6	79.2	0.43	0.49	0.42	0.71	0.82	0.57	0.58	0.57	18.76	47.28
4	6.5	88.9	0.31	0.78	0.83	0.79	0.92	0.71	0.68	0.71	13.12	68.7
5	7	72.2	0.56	0.68	0.79	0.91	0.89	0.67	0.72	0.74	29.09	73.3
6	7.5	43.6	0.21	0.19	0.23	0.86	0.79	0.43	0.28	0.42	14.32	47.3
7	8	19.6	0.19	0.13	0.22	0.51	0.46	0.51	0.18	0.31	16.21	25
8	8.5	13.3	0.16	0.18	0.31	0.53	0.42	0.57	0.29	0.35	12.06	8.35
	Overall Mean	44.47	0.26	0.39	0.41	0.62	0.59	0.51	0.36		**CD (p≥0.05) ***	**SE (d)**
		CD (p≥0.05) *	**SE (d)**							**Days**	1.06	0.22
	pH	0.55	0.28							**Sclerotia**	1.74	0.72
	Duration	1.49	0.35									

Fig 1. Root Rot Symptoms of Broccoli Caused by *Rhizoctonia solani*

Fig. 2 Colony Pattern and Morphology of *Rhizoctonia solani*

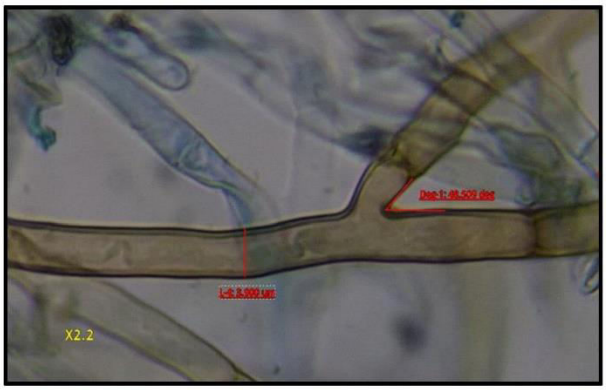

Fig. 3 Hyphae structure

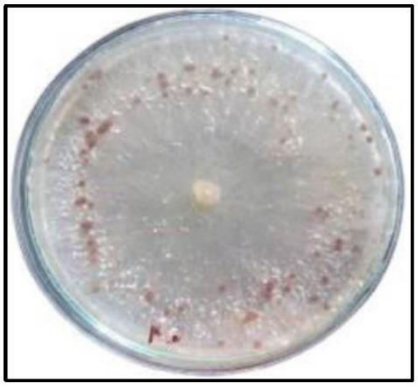

Fig. 4 Sclerotia of *R. solani* in petri dishes

The variability noted in mycelial proliferation suggests that both OMA (Oatmeal agar) and SDA (Sabouraud dextrose agar) may be deficient in certain essential nutrients necessary for robust fungal growth. These media, lacking sufficient nutritional components, do not support vigorous growth, leading to the formation of sparse and irregular mycelial colonies. *R. solani* demonstrates a clear preference for carbon-rich media,

such as potato dextrose agar (PDA) and Komada's Medium (KM), indicating the critical role of carbohydrates in its growth and development. The outcomes of the present study resonate with existing research that underscores the importance of nutrient composition in the growth of fungi. For example, studies by Sneh *et al.,* (1991) and Otten *et al.,* (1998) have shown that nutrient-dense media significantly enhance the growth and pathogenic capacity of soil-borne fungi, including *R. solani*. These findings highlight the necessity of selecting appropriate nutrient media to gain deeper insights into the physiological characteristics and behaviour of fungal pathogens.

Impact of Various Temperature Conditions

The results detailed in Table 3 indicate that the highest average diametric growth of the test fungus (83.98 mm) occurred at 30 °C, closely followed by 78.43 mm at 25 °C. In contrast, the lowest yet statistically comparable average growth was recorded at 40 °C (20.14 mm), with an even lower reading at 35 °C (23.41 mm). Growth curves observed across all temperature conditions exhibited a transition from sigmoid to a linear pattern. The analysis showed that the growth rate peaked at 25 °C, measuring 0.51 mm h^{-1}, and was closely followed by a rate of 0.49 mm h^{-1} at 30 °C. These rates were not significantly different from the other temperatures examined. The minimum growth rate observed was at a low temperature of 40 °C (0.36 mm h^{-1}). Overall, regardless of the temperature variations, the peak average growth rate was noted between 96-120 hours (0.64 mm h^{-1}), which was significantly higher than the subsequent average of 0.48 mm h^{-1} recorded from 120-144 hours. The least significant growth rate of 0.06 mm h^{-1} was observed during the initial 0-24 hour incubation period, followed by a similar rate of 0.39 mm h^{-1} recorded during the 24-48 hour phase. These periods demonstrated statistical comparability with the average growth rates during the other incubation intervals. Intermediate growth rates were noted at various temperatures throughout the different durations of incubation studied. The ΔrAUKC plotted against time indicates that at temperatures between 20 and 25 °C, the relative growth rate attained its peak between 96-120 h of incubation, but the fungus continued to grow at 25 °C up to 120 -144 h of incubation, and no peak was obtained until then. Growth rate attained its peak during 72-96 h of incubation at temperatures of 20, 25, and 30 °C, and after that, the stationary phase was attained at 30 °C. Peak was attained during 72–96 h of incubation at 20 °C. Data pertaining to the duration required for sclerotia production at various temperatures, as well as the total number of sclerotia generated within a 30-day incubation period, indicate significant trends. The average maximum time recorded for sclerotia production was 17.32 days at 15°C, which was statistically comparable to the 18.09 days observed at 20°C. Conversely, the minimum average time for sclerotia production was noted at 40°C (11.21 days), showing statistical similarity to the time reported at 10°C (12.07 days). In terms of the total sclerotia produced across the temperature spectrum, the highest mean count of 79.7 sclerotia was achieved at 25°C, followed closely by 63.8 sclerotia at 30°C. The lowest mean count of sclerotia (3.27) occurred at 40°C, with a statistically marginal increase at 10°C (3.9).

These observations align with previous research conducted by Hemalatha and Singh (2019) and Nuri and Biswas (2021), who identified optimal fungal growth within the 20-30°C range on PDA media. Similarly, Tiwari and Khare (2002) identified 25-30°C as the ideal temperature range for the growth of *Rhizoctonia solani*. Grosch and Kofoet (2003) also supported these findings, noting that various isolates of *R. solani* demonstrated growth capabilities between 15 and 30°C on PDA. Temperature plays a crucial role in influencing fungal growth and development. Literature demonstrates that optimal growth for many fungal species, including *R. solani,* occurs between 20-30°C, while growth is significantly inhibited at temperatures below 10°C and above 35-40°C (Thiyam & Sharma, 2011; Vidal *et al.,* 1997; Ali, 2017; Ayed *et al.,* 2018). Radial growth and mycelial production typically peak at around 25-30°C for numerous fungal species. Additionally, the processes of sclerotia formation and subsequent germination are influenced by temperature, with optimal production occurring in the 25-35°C range (Naik *et al.,* 2016; Ayed *et al.,* 2018).

Impact of pH

The data presented in Table 4 indicates a clear relationship between pH levels and diametric growth rates of the tested fungus. The highest average growth was observed at pH 6.5, measuring 88.9 mm, while a slightly lower growth of 79.2 mm was recorded at pH 6. In contrast, the lowest average growth, at 13.3 mm, occurred at pH 8.5. This lower growth at pH 8.5 was statistically comparable to observations at pH 5 (17.3 mm), pH 8 (19.6 mm), and pH 5.5 (21.6 mm), suggesting a threshold beyond which growth diminishes

significantly. The growth curves demonstrated that the test fungus exhibits adaptability across various pH environments. Notably, pH 6.5 emerged as optimal for growth, with pH 6 closely following. Further analysis reveals that regardless of the incubation time, the test organism experienced its highest mean growth rate at pH 7, recorded at 0.74 mm h^{-1}. This was followed by growth rates of 0.71 mm h^{-1} at pH 6.6 and 0.57 mm h^{-1} at pH 6, all of which were statistically similar. In contrast, lower growth rates were noted at pH 5 and pH 5.5, with rates of 0.16 mm h^{-1} and 0.27 mm h^{-1}, respectively, both significantly lower than those observed at pH 7. Overall, the average maximum growth rate was consistent during the 72-96 hour window, measuring 0.62 mm h^{-1}, followed by a decrease to 0.59 mm h^{-1} during the 96-120 hour period. The minimal growth rate occurred during the initial 0-24 hour incubation phase, recorded at 0.26 mm h^{-1}, with a slight increase to 0.36 mm h^{-1} observed between 144-168 hours.

The analysis of the ΔrAUKC over time reveals that the growth rate of the test pathogen peaked during the 96-120 hour incubation period across all examined pH levels, with the exception of pH 5.0. The longest duration for sclerotia production, averaging 29.09 days, was found at pH 7, significantly exceeding the production times at other pH levels. Conversely, the shortest production time recorded was 12.6 days at pH 8 and 12.9 days at pH 5. In terms of overall sclerotia production, the highest yield was noted at pH 7.0, where 73.3 cm^2 of sclerotia were produced. This was followed by pH 6.5, with 68.7 cm^2, and pH 6.0, which yielded 47.28 cm^2. In contrast, the least amount of sclerotia was produced at pH 5.0, measuring only 5.2 cm^2, with pH 8.5 slightly better at 8.35 cm^2. Throughout our study, the pathogen demonstrated a capacity for growth across the full pH spectrum from 5.0 to 8.5, excelling at pH 7.0. These findings corroborate those of Ben *et al.,* who noted that *R. solani* thrives within a pH range of 5 to 8 when cultured on potato dextrose agar (PDA). Additionally, Hasan *et al.,* affirmed that the ideal pH for *R. solani* growth oscillates between 5 and 7, further validating our results. Aligning with these observations, Nandi *et al.,* (2023) also indicated an optimal pH range of 6.0 to 7.0 for *R. solani* growth. Chaudhary *et al.,* (2018) found that the most favourable conditions for this pathogen were identified at pH levels between 6 and 6.5. The fungus's adaptability is evident, as it can grow within a pH range of 4 to 9, with optimal conditions generally found between pH 5.6 and 7.0 (Ritchie *et al.,* 2009; Goswami *et al.,* 2011). Furthermore, while sclerotial formation occurs within a pH range of 4 to 8, the specific optimal conditions can differ among various isolates (Ritchie *et al.,* 2009). The observed effects of pH on *R. solani* are consistent with patterns seen in other soil-borne pathogens, such as *Sclerotium rolfsii*, which flourishes at pH levels of 5.0-6.0 (Sarker *et al.,* 2013). Collectively, these findings underscore the critical role of environmental parameters in influencing the growth and viability of *R. solani*, highlighting the need for further research into the intricate relationships between pathogens and their surrounding conditions.

The study on *Rhizoctonia solani*, the pathogen responsible for damping off disease in broccoli, revealed significant insights into its isolation, identification, and optimal growth conditions. The pathogen was isolated from diseased broccoli roots and cultured on potato dextrose agar (PDA), exhibiting distinctive morphological features. Various nutrient media were tested for mycelial growth, with PDA and Czapek-Dox agar (CDA) showing the highest growth rates, while oatmeal agar (OMA) and malt extract agar (MEA) showed the least. The study also examined the effects of different temperatures and pH levels on the growth and sclerotia production of *R. solani*. Optimal growth was observed at 25-30°C and pH 6-7, with maximum sclerotia production at 25°C. The findings highlight the importance of nutrient composition, temperature, and pH in the growth and development of *R. solani*, providing valuable information for developing effective disease management strategies.

The research aligns with previous studies, emphasising the need for appropriate media and environmental conditions to understand the physiological behaviour of fungal pathogens.

Adherence to Ethical Standards

Conflict of Interest Disclosure: We, the authors, wish to clearly state that there are no conflicts of interest associated with this research. We remain dedicated to delivering research that is solely guided by the pursuit of knowledge and the betterment of our field.

Author Contributions

Yogesh Urdukhe: Investigation, formal analysis, writing—original draft. Umesh Mogle: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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