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CHARCATERIZATION AND GROWTH KINETICS OF *Phomopsis psidii* CAUSING STYLER END ROT OF GUAVA (*Psidium guava*)

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ABSTRACT

Styler end rot is one of the most significant diseases which affects the styler end of guava fruit causing direct yield loss. The present study was aimed to characterize the causal pathogen of styler end rot (*Phomopsis psidii*) on physiological basis, and to assess its growth kinetics (ΔrAUKC) under laboratory conditions. The impact of various nutrient media, temperature regimes, pH, carbon, nitrogen and trace elements on pathogenic growth was evaluated. Among the six nutrient media tested, potato dextrose agar (PDA) gave highest diametric growth (90 mm) and growth rate (0.59 mm h^{-1}) with a ring-pattern white mycelial growth. The maximum diametric growth (90 mm) with higher growth rate (0.56-0.59 mm h^{-1}) was observed at a temperature of 25ºC and pH 6.5 after 144 h incubation. Further, the peak growth of fungus was noted between 48-72 h incubation, irrespective of nutrient media, temperature or pH. In terms of biomass production, the highest mean biomass was observed with starch as carbon source (1141.87 mg biomass), potassium nitrate as nitrogen source (282.30 mg biomass) and magnesium sulphate as trace element (858.33 mg). Understanding the optimal growth conditions of *Phomopsis psidii* lays the foundation for developing targeted cultural and chemical control methods against guava styler end rot.

Keywords: Fungal biomass, growth kinetics, guava, styler end rot, *Phomopsis psidii*, physiological studies

INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical fruit widely grown in India, especially in tropical and sub-tropical areas (Marin *et al*., 2021). Guava belongs to the family Myrtaceae and is considered a "super-fruit" due to its high vitamin C, fibre, antioxidant and mineral contents (Sumra *et al*., 2018). However, guava is susceptible to several pathogens, and around 177 pathogens have been reported to incite diseases in the plant with 167 being of fungal origin (Misra and Panday, 2013). One of its most significant diseases is styler end rot, caused by *Phomopsis psidii*, which affects the styler end of fruit and leads to direct yield loss. It was first reported in India by Rai in 1956 from Lucknow, and recently outbreaks of this disease have occurred in Himachal Pradesh (India).

Morphologically, *P. psidii* produces dark, carbonaceous pycnidia on rotting fruits and in culture, with pycnidia ranging from 140 to 400 µm (mean 225 µm) in dia (Rai, 1956). The fungus forms two types of conidia i.e., hyaline, aseptate, cylindrical to sub-cylindrical α-conidia measuring around 5-9 x 2.5-4 µm; and longer, slender, curved β-conidia measuring 16- 32 x 1.5-8.0 µm (Rai, 1956; Borhade, 2023). The mycelium is hyaline, septate, branched, and varies in thickness from 1.3 µm on host surfaces to 5 µm in culture (Borhade, 2023). On potato dextrose agar (PDA), the

pathogen produces white floccose mycelium with brown, globose to irregular pycnidia densely distributed, producing numerous α-conidia measuring 8.8 x 3.2 µm (Borhade, 2023). These morphological features are critical for distinguishing *P. psidii* from other similar pathogens. While morphology remains key to preliminary identification, molecular methods are increasingly used to confirm the identity due to overlapping characteristics in related species (Van *et al*., 2005).

Fungal physiology encompasses the nutrition, metabolism, growth, reproduction and death of fungal cells (Walker and White, 2017). The growth and development of a fungus is largely affected by various external factors, with growth medium, temperature and pH being the most crucial. The quality of medium in which a fungus thrives plays a vital role in its growth performance. Further, the morphological and physiological traits of fungi are largely determined by the characteristics of growth medium (Parvathy *et al*., 2023). Rohini *et al*. (2016) reported PDA as the best medium for the growth and sporulation of 23 isolates of *Phomopsis vexans*. Many workers have reported PDA as best medium for various *Phomopsis* sp. (Patil *et al*., 2016; Jakatimath *et al*., 2017). The temperature and pH are important environmental factors affecting the growth and metabolic activity of pathogenic fungi (Selvig and Alspaugh, 2011; Thiyam and Sharma, 2014). The ideal temperature range for the growth of most fungi lies between 25 and 30ºC, however growth is significantly hindered at temperatures > 40˚C, which could inflict mortality (Sharma and Rajak, 2003). Many *Phomopsis* sp. thrive well at 25ºC (Tian *et al*., 2018; Wrona *et al*., 2020). When the pathogens infect host plants, ambient temperature and pH play vital role in colonization and expansion of pathogens. Maqsood *et al*. (2014) reported that *P. mangiferae* exhibited maximum growth on media having pH 6.5. Though much research has been done on several *Phomopsis* species, yet very little is known about *P. psidii.*

The nature of carbon source influences the growth and metabolic activity of fungi, as fungi rely on these sources for energy and structural development (Wiriya *et al*., 2014). Carbohydrates is the primary source of carbon and is essential for biosynthesis, energy generation and microbial fermentation (Kupradit *et al*., 2020). The type and concentration of carbon source influences the nutrient assimilation efficiency and metabolic activity of fungi, resulting in differential mycelial growth (Lazarevic *et al*., 2016). Nitrogen sources play a vital role in controlling mycelial growth, protein synthesis and enzyme function in fungi. Fungi can use both organic and inorganic nitrogen forms (Costa *et al*., 2002). It not only facilitates the accumulation of mycelial biomass but also boosts sporulation in fungi. Fang and Zhong (2002) have reported that the use of single nitrogen source leads to less mycelial production, while combined nitrogen sources enhances mycelial growth. The interplay between C and N significantly influences the overall metabolic processes in fungi. Roy *et al*. (2014) assessed the impact of various C and N sources on radial growth and biomass of *P. vexans* and observed that the media-enriched with monosaccharides like glucose and disaccharides like sucrose and maltose promote better growth than polysaccharides, including water-soluble cellulose. Among the nitrogen sources, the nitrate and amide forms of N are most effective in supporting the growth of *P. vexans* isolates. The mycelial growth is also greatly affected by trace elements found in nutrient medium which are required in minute amounts, but are vital for biochemical and physiological processes involved in mycelial development (Kaur and Atri, 2016). Due to the scanty literature available on *P. psidii*, this study was undertaken to evaluate and characterize its physiological requirements. The present work was aimed to fill the existing knowledge gaps by assessing the optimal environmental and nutritional conditions for the growth and development of fungus. By assessing the factors like temperature, pH, C, N sources and trace elements and other essential growth parameters, this study was aimed to generate valuable insights into the biology of *P. psidii*.

MATERIALS AND METHODS

The present research was conducted in Research Laboratory, Department of Plant Pathology, College of Horticulture & Forestry Neri, Hamirpur, Himachal Pradesh, India in the year 2023-2024.

Collection, isolation and identification of pathogen

Diseased guava fruit cv. "Allahabad Safeda" samples, affected by styler end rot were collected from the Bhota Research Farm, COHF, Neri and transported to the laboratory. The samples were thoroughly washed 3-5 times with tap water. Small bits of diseased tissues along with some healthy tissue were excised from young lesions on infected fruit using a sterilized blade. These tissue samples were surface sterilized using a 1% NaOCl solution and rinsed 3 times with sterilized distilled water. After drying with sterilized blotting paper, the tissues were aseptically placed on sterilized petri-plates/slants containing PDA and incubated at 25±1ºC. Once fungal growth appeared, a small piece of agar from the edge of actively growing mycelium was transferred to fresh sterilized PDA slants. The purified cultures were stored in a refrigerator at 4-5ºC for future use.

After confirming the pathogenicity, the fungus was tentatively identified on comparison with the available literature pertaining to the cultural and microscopic characteristics such as mycelium type, conidia type and the presence of pycnidia (Rai, 1956). For molecular identification, the pure culture was sent to Eurofins Genomics India Limited, Bengaluru (India) for sequencing of the ITS region using primer pair ITS1/ ITS4 (White *et al.,* 1990). The obtained sequence was analysed using NCBI nucleotide BLAST and compared with the existing gene sequences (Benson *et al*., 2013; *https://blast.ncbi.nlm.nih.gov/Blast.cgi*]. A phylogenetic tree was constructed using MEGA11 software for further confirmation (Tamura *et al*., 2021).

Effect of nutrient media on mycelial growth of pathogen

The growth of test pathogen was assessed using 6 solid media *viz*., PDA, Czapek's Dox agar (CDA), Richard's agar (RA), malt extract agar (MEA), oat meal agar (OMA) and host extract agar (HEA). For this, culture bits (5 mm dia) of fungus were inoculated in the center of petri-plates, each containing a test medium (Bhogal *et al*., 2022). The plates were subsequently placed in an incubator set at 25±1ºC. Each treatment was replicated three times in a completely randomized design (CRD) and data noted at 24 h intervals for 6 days. The data collected included the average diametric growth (mm) of fungus and cultural characterization for mycelial colour, type of growth and growth pattern. Growth rate (mm h^{-1}) of fungus on each medium was calculated as per the following formula (Bhogal *et al*., 2022):

$$\text{Growth rate, } r_g \text{ (mm h}^{-1}) = \frac{d_g t_2 - d_g t_1}{t_2 - t_1}$$

Where: dgt_1 and dgt_2 are diametric mycelial growth (mm) at time t_1 and t_2, respectively

A calculation model based on the area under the growth/kinetic curve (AUKC) was developed to track the changes in growth rates and identify significant shifts at specific time points (Bhogal *et al*., 2022). The relative AUKC (rAUKC) for each interval was calculated using the formula:

$$\text{rAUKC} = \frac{dgt_1 + dgt_2}{2} \times (t_2 - t_1)$$

The changes in rAUKC (ΔrAUKC) were computed for each time-point to estimate the growth rate of fungus. The ΔrAUKC values for each nutrient medium were plotted over time to observe growth trends. The optimal nutrient medium was selected for further experiments based on these analyses.

Effect of temperature regimes on growth of pathogen

In order to assess the impact of various temperature regimes on mycelial growth of pathogen, the best nutrient medium was selected. Petri dishes containing the best medium were inoculated with a 5 mm culture bit of test fungus and exposed to several temperature regimes *viz*., 18, 20, 22, 25, 28, 30, 32 and 35ºC in BOD incubators for 144 h (Drais *et al*., 2023). Each treatment was replicated 3 times and measurements of average diametric growth were taken at 24 h intervals. The growth rate of the fungus at each temperature was then calculated. Furthermore, ΔrAUKC values at varying temperature conditions were plotted over time as mentioned earlier. These analyses provided valuable insights that led to the selection of the optimal temperature for subsequent experiments.

Effect of different pH levels on the growth of the pathogen

The mycelial growth of test fungus was studied to assess the effect of pH (Gwon *et al*., 2022). The best nutrient medium was adjusted to several pH levels *viz*., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0

by using 1N HCl and 1N NaOH. Each prepared medium was poured into petri-plates and inoculated with a 5 mm culture bit. The petri-plates were incubated at optimum temperature up to 144 h. Each treatment was replicated 3 times in CRD and the data on average diametric growth and growth rate recorded at 24 h interval for 144 h. Further, ΔrAUKC values at several pH levels were plotted against time. Finally, rAUKC values obtained in three experiments were pooled for each duration and ΔrAUKC calculated. These values were plotted against time to get a clear idea of peak growth of pathogen during incubation.

Effect of carbon, nitrogen sources and trace elements on biomass of pathogen

The influence of various nutrient factors on mycelial biomass of fungus was systematically studied using Richard's broth as the basal medium. To evaluate the effect of carbon sources, sucrose in the basal medium was replaced with different carbohydrates, like arabinose, dextrose, fructose, galactose, lactose, mannitol, starch, sucrose and xylose, each adjusted to provide an equal carbon content based on molecular weight. For N sources, sodium nitrate was substituted with ammonium sulphate, asparagine, aspartic acid, potassium nitrate and sodium nitrate, ensuring equal amounts of nitrogen. For determination of the impact of trace elements, ferric chloride in medium was replaced with ammonium molybdate, cupric sulphate, ferrous sulphate, zinc sulphate and magnesium sulphate, each at an equivalent molar concentration. For each treatment, 100 mL modified broth was taken in 150 mL Erlenmeyer flask, inoculated with a 5 mm culture bit of test fungus and incubated at optimal temperature for 14 days. The experimental setup included three replicates/treatment and the average dry weight of mycelium was noted after 7 and 14 days (Sharma *et al*., 2025). A control (Richard's broth without C, N source and trace element supplementation) was maintained for comparison.

Statistical analysis

All the experiments involving *in vitro* growth studies of *P. psidii* were conducted using a completely randomized design [CRD] (Panse and Sukhatme, 2000). For cultural studies (media, temperature regimes and pH levels) and for nutritional studies [carbon, nitrogen, and trace element sources], three replications were maintained for each treatment. The data collected from *in vitro* experiments were subjected to the analysis of variance (ANOVA). The significance of treatment effects was tested at 5% level of probability ($p \geq 0.05$). The entire statistical analysis, including ANOVA and calculation of was performed using standard statistical software OPSTAT (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

Collection, isolation and identification of pathogen

The disease manifested on unripe winter guava fruits during December and January, with initial symptoms of light brown discoloration at the styler end. The lesion progressively enlarged and darkened as the disease advanced, ultimately encompassing the entire fruit. Black pycnidial bodies appeared on the brown lesions (Fig. 1a-c). The infection led to dark brown discoloration and softening of internal tissues. As the disease advanced, white mycelium was evident on fruit in which pycnidia containing α- and β-conidia were seen when observed under a microscope. Pycnidia were globose in shape, measured 48.98-52.53 µm (Fig. 1d). The α-conidia were hyaline, aseptate, fusiform and 10.34 to 12.88 µm in size (Fig. 1e), while β-conidia were sparse, curved (60.9 to 62.3 µm) (Fig. 1f). Similar symptoms were previously reported by (Rai, 1956; Borhade, 2023).

The pathogen in pure culture on PDA depicted white mycelium with a ring pattern and suppressed, thread-like structures, turning from light green to dark green as the incubation progressed (Fig. 1g-j). After 15 d, abundant minute pycnidial bodies were observed densely distributed in the mycelium from centre towards the periphery of culture (Fig. 1k). No conidia were formed even after one month's incubation in culture. Microscopically, the mycelium was hyaline, septate and branched. Based on the comparison of symptoms and cultural characteristics with the

Fig. 1: *Phomopsis psidii;* Symptoms of styler end rot of guava under field conditions (top row figures). Pycnidium (2nd row left), α-conidia (2nd row centre), β-conidia (2nd row right), Pure culture of *Phomopsis psidii* (3rd row figures), Pycnidial bodies in culture (last row figure)

available literature (Rai, 1956; Borhade, 2023), the fungus was tentatively identified as *Phomopsis* sp. The pathogenicity was confirmed by inoculating healthy guava fruits with the pathogen, which produced the same symptoms as observed in the field. The ITS region was amplified using primersITS1/ITS4, yielding a 600 base pair product (Fig. 2A). NCBI BLAST analysis showed 97.25% identity with *Phomopsis* sp., with 100% query coverage. A phylogenetic tree, based on the ITS sequence was constructed (Fig. 2B) thus confirmed the pathogen to be *Phomopsis psidii* based on the previous reports (Rai, 1956; Nag Raj and Ponnappa, 1974).

Effect of different nutrient media

The fungus grew well on all the nutrient media tested (Table 1) with maximum diametric growth (90 mm) on PDA, which was statistically at par with RA (89.3 mm), followed by HEA (61.7 mm). The minimum diametric growth (38 mm) was observed on MEA, followed by OMA (59.4 mm) after 144 h incubation. The growth curves exhibited a linear to sigmoid growth pattern throughout the 144 h period. The fungus on different media after 144 h of incubation exhibited highest growth rate on PDA (0.59 mm h^{-1}), followed by HEA (0.39 mm h^{-1}). The minimum growth rate (0.23 mm h^{-1}) was observed on MEA followed by CDA and OMA (0.38 mm h^{-1}).

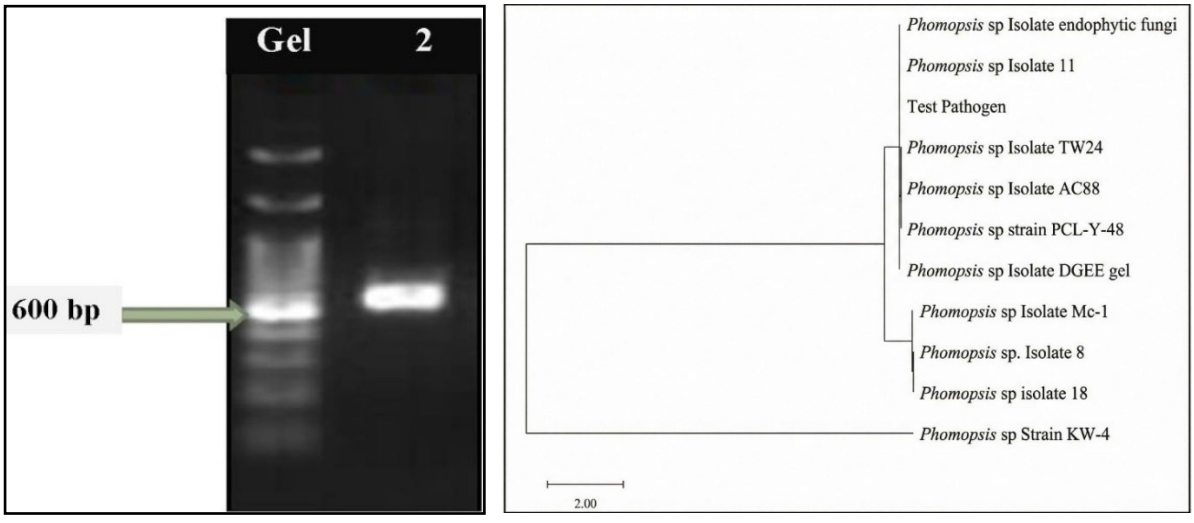

Fig. 2: Molecular identification of pathogen; Amplified ITS gene of DNA isolated (left side), and the phylogenetic analysis of ITS sequence of isolated fungus (right side)

Table 1: Effect of different nutrient media on mycelial growth and growth rate of *Phomopsis psidii*

Nutrient medium	Diametric growth (mm)	Growth rate (mm h^{-1})	Colour of mycelium	Growth pattern	Type of growth
Potato dextrose agar	90.00	0.59	White	Ring	Suppressed thin threads
Host extract agar	61.67	0.39	Creamish white	Ring	Cottony but suppressed
Richard's agar	89.34	0.58	White	Ring	Suppressed & very sparse mycelium
Czapek's dox agar	60.33	0.38	Creamish white	Ring	Suppressed
Malt extract agar	38.00	0.23	Creamish white	Ring	Cottony and dense
Oat meal agar	59.34	0.38	Creamish white	Ring	Cottony and fluffy
$CD_{0.05}$	2.87	0.02	-	-	-
$SE_{(d)}$	1.30	0.009	-	-	-

*Growth after 144 of incubation (h)

The relative area under the kinetic curve (ΔrAUKC) values were plotted against time intervals to find the peak growth period for each nutrient medium. The highest peak growth was observed between 24-48 h incubation in PDA, RA, CDA and MEA. While the highest peak growth in HEA and OMA occurred between 48-72 h incubation. Overall, the highest ΔrAUKC value was recorded for PDA during 24-48 h period, indicating the rapid growth of fungus on this medium from the very beginning.

The predominant growth pattern observed was ring pattern on all the media. However, growth types varied from suppressed growth on PDA, OMA and RA, but cottony supressed growth on HEA, cottony and dense growth on CDA and cottony and fluffy growth on MEA. On PDA thin thread-like mycelium was observed, while on HEA sparse mycelium was seen. The mycelium colour varied slightly across the media, ranging from white on PDA and HEA to creamish white on RA, OMA, CZA and MEA. Nutrients in growth medium directly or indirectly influence the growth of any microorganism, due to their varied nutritional requirements for mycelial growth. Our results are in line with Rohini *et al*. (2016) who reported PDA as best medium for the growth and sporulation of *P. vexans*. Borhade (2023) reported PDA as best medium for the growth of *P. psidii*. Our findings are supported by Mahadevakumar and Janardhana (2016) who observed that the pathogen exhibited a range of colours, ranging from white to pale pink on PDA.

Effect of different temperature regimes

The study on growth dynamics of *P. psidii* at various temperatures (18 to 35ºC) revealed that maximum diametric growth (90 mm) was observed at 25ºC after 144 h incubation, followed by that observed at 24ºC (83 mm) and 26ºC (81 mm) [Table 2]. However, the minimum diametric growth (40.34 mm) was found at 35ºC which was at par with the growth observed at 32ºC (41 mm). The growth curves obtained at different test temperatures reflected sigmoid curves to straight line. The maximum growth rate of test pathogen after 144 h incubation was observed at 25ºC (0.59 mm h^{-1}) which was followed by growth rate recorded at 24ºC (0.54 mm h^{-1}) and 26ºC (0.52 mm h^{-1}). However, minimum growth rate (0.24 mm h^{-1}) of fungus was noted at 35ºC which was at par with the growth rate at 32ºC (0.25 mm h^{-1}). which further did not differ from that recorded at 30ºC (0.26 mm h^{-1}). The ΔrAUKC plotted against time indicates that at temperatures 20-35ºC, except 28ºC, the relative growth rate attained its peak between 48-72 h incubation. However, no distinct peak was recorded at 18 and 28ºC up to 144 h. The highest ΔrAUKC was recorded between 120-

Table 2: Effect of different temperature regimes on mycelial growth and growth rate of *Phomopsis psidii*

Temperature (ºC)	Diametric growth (mm)	Growth rate (mm h^{-1})
18	52.34	0.32
20	72.66	0.47
22	77.66	0.50
24	83.00	0.54
25	90.00	0.59
26	81.00	0.52
28	65.34	0.42
30	43.00	0.26
32	41.00	0.25
35	40.34	0.24
$CD_{P\geq0.05}$	2.14	0.01
$SE_{(d)}$	1.02	0.007

*Growth after 144 of incubation

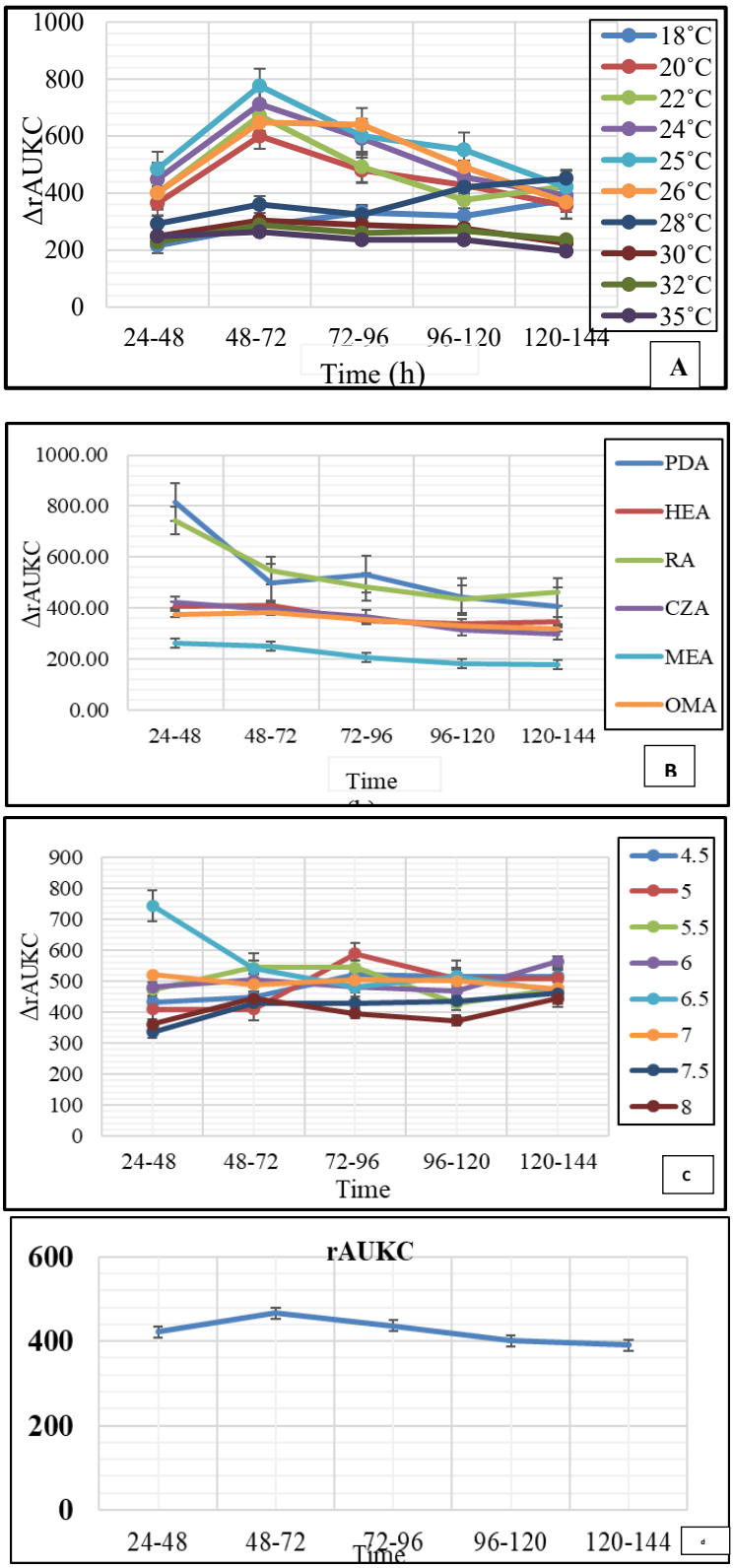

Fig. 3: Effect of temperature, nutrient media and pH on the relative area under kinetic curve of *Phomopsis psidii;* a) Temperature, b) Nutrient media and c) pH d) Changes in relative area under kinetic curve values with time.

Table 3: Effect of pH on mycelial growth and growth rate of *P. psidii*

pH	Diametric growth (mm)	Growth rate (mm h^{-1})
4.5	79.00	0.51
5.0	79.00	0.51
5.5	80.00	0.52
6.0	82.00	0.52
6.5	90.00	0.56
7.0	81.00	0.52
7.5	68.34	0.46
8.0	66.34	0.45
$CD_{p\geq0.05}$	1.18	0.05
$SE_{(d)}$	0.55	0.02

* Growth after 144 of incubation

144 h at these temperatures, indicating continued growth (Fig. 3A). Our findings are supported by Sugha *et al*. (2012) who recorded optimum growth of *P. vexans* at 25ºC. Maqsood *et al*. (2014) reported that the highest growth of *P. mangiferae* was observed when it was incubated at 25ºC. Results are further supported by Borhade (2023) who recorded maximum growth of *P. psidii* at 25ºC.

Effect of pH level

The maximum diametric growth of *P. psidii* (90 mm) was recorded at pH 6.5, followed by pH 6.0 (82 mm), which was at par with the growth at pH 7.0 (81 mm) [Table 3]. In contrast, minimum growth was observed at pH 8.0 (66.34 mm), followed by pH 7.5 (68.34 mm). The growth curves demonstrated that the fungus exhibited exponential growth across the tested pH levels, suggesting that these conditions support fungal development, although the growth was optimal at pH 6.5 (Fig. 3C). Additionally, the growth rate was significantly maximum (0.56 mm h^{-1}) at pH 6.5 which was at par with the growth rate recorded at pH 5.5, 6.0, 7.0, 4.5 and 5.0. The minimum growth rate (0.45 mm h^{-1}) was observed at pH 8.0 but was at par

with treatment pH 7.5. The ΔrAUKC values plotted against time revealed that the growth peak for the pathogen occurred at pH 6.5 and 7.0 between 24 and 48 h incubation. For pH levels 4.5, 5.0 and 5.5, the maximum ΔrAUKC values were observed between 72 and 96 h. At pH 6.0 and 7.5, peak growth was reached between 120 and 144 h, revealing continued growth, while at pH 8.0 maximum growth occurred between 48 and 72 h incubation (Fig. 3C). In present study, the pathogen could grow well at all pH levels ranging from 4.5-7.0 with best growth at pH 6.5. Maqsood *et al*. (2014) and Borhade (2023) reported that *P. mangiferae* and *P. psidii* on PDA exhibited their maximum growth at pH 6.5 which is in conformity our observations.

The fungus grew best between 48-72 h incubation as a clear peak of ΔrAUKC was obtained in this duration, irrespective of any kind of nutrient medium, temperature or pH used (Fig. 3 A-C). It appeared that during 0-48 h incubation, the fungus adapted itself to the new growth conditions and attained its maximum growth in next 24 h. A sharp decline in ΔrAUKC values after 72 h might be due to the gradual exhaustion of available nutrients in the growing medium ultimately leading to decline in growth rate. Overall, the fungus exhibited its maximum growth between 48-72 h incubation which is in line with Srivastava *et al*. (2011) who studied the changes in biomass of *Fusarium oxysporum* over a period and found that the fungal biomass increased at a slow rate till 48 h and thereafter the growth increased at an exponential rate. After approximately 8 d, the rapid growth plateaued, moving into a more stationary phase. The lag phase, which may extend for as long as 24 h, usually occurs when fresh medium is inoculated with cells from a more mature culture. During this phase, the cells are deficient in crucial enzymes, and growth will only progress at an optimal rate until the necessary concentrations of these substances for synthesis are restored (Granjo *et al*., 2007). Decline in growth rate of fungus after 72 h can be explained with the fact that the concentration of essential nutrients might have reduced and the mycelial biomass increased which resulted in reduced growth rate.

Effect of carbon sources on mycelial biomass of P. psidii

The highest biomass (1141.87 mg) of *P. psidii* was achieved when starch was used as a carbon source, followed by arabinose (473.91 mg) [Table 4]. The lowest biomass (104.68 mg) was observed without a carbon source, followed by galactose as carbon source producing slightly more biomass (213.90 mg). Regardless of the carbon source, the minimum biomass (198.78 mg) was recorded after 7 d incubation, which increased significantly to 494.05 mg after 14 d incubation. After 14 d, starch resulted in the highest biomass (1604.86 mg), followed by arabinose (675.26 mg). The control (no carbon source) yielded lowest biomass (70.46 mg), with galactose (105.6 mg) and sucrose (105.86 mg) showing similar biomass levels after 7 d. Other carbon sources produced intermediate biomass levels after both 7 and 14 d. Fungi prefer starch as a carbon source to provide nutrition for their growth and development (Wei *et al*., 2022) and starch is the main carbohydrate found in potato, so explaining why best growth was achieved on PDA. Filamentous fungi secrete a large number of starch hydrolytic enzymes and the monosaccharides thus obtained can be utilized in energy production and growth and development of fungi (Wang *et al.,* 2020). Our results are in line with Gurgel *et al*. (2002) who reported starch, glucose and sucrose to be good C sources for the mycelial biomass production of *P. anacardii*. The results are also in agreement with Girish and Bhat (2011) who

Table 4: Effect of carbon sources on mycelial biomass of *Phomopsis psidii*

Carbon source	Dry weight (mg)		Overall mean
	7 d	14 d	
Arabinose	272.56	675.26	473.91
Dextrose	132.76	415.30	274.03
Fructose	138.66	381.93	260.30
Galactose	105.60	322.20	213.90
Lactose	197.40	353.70	275.55
Mannitol	164.30	396.67	280.53
Starch	678.76	1604.86	1141.87
Xylose	121.40	307.53	214.46
Sucrose	104.86	344.03	224.95
Control	70.46	138.90	104.68
Overall mean	198.78	494.05	
Factors	$CD_{0.0}$	$SE(_d)$	
Carbon source	1.85	0.91	
Duration	0.83	0.40	
Carbon x Duration	2.62	1.29	

Table 5: Effect of nitrogen sources on mycelial biomass of *Phomopsis psidii*

Carbon source	Dry weight (mg)		Overall mean
	7 d	14 d	
Ammonium sulphate	73.10	284.26	178.68
Asparagine	82.66	308.43	195.50
Aspartic acid	36.60	214.06	125.33
Potassium nitrate	141.40	423.20	282.30
Sodium nitrate	106.43	413.16	259.80
Control	25.13	82.40	53.76
Overall mean	77.56	287.58	
Factors	$CD_{0.05}$	$SE_{(d)}$	
Nitrogen source	3.57	1.71	
Duration	2.06	0.99	
Nitrogen x Duration	5.04	2.43	

Table 6: Effect of trace elements on mycelial biomass of *Phomopsis psidii*

Carbon source	Dry weight (mg)		Overall mean
	7 d	14 d	
Cupric sulphate	130.10	1108.20	619.15
Ferrous sulphate	144.06	1288.93	716.50
Zinc sulphate	153.13	1539.76	846.45
Magnesium sulphate	158.56	1558.10	858.33
Ammonium sulphate	133.53	1132.13	632.83
Ferric chloride	154.66	1550.46	852.56
Control	98.13	435.03	266.58
Overall mean	136.25	1177.02	
Factors	$CD_{0.05}$	$SE_{(d)}$	
Trace elements	4.82	2.34	
Duration (D)	2.58	1.25	
Trace elements x D	6.82	3.31	

revealed maximum weight of different isolates of *P. azadirachtae* in starch amended liquid basal medium.

Effect of nitrogen sources on mycelial biomass of P. psidii

The highest biomass of *P. psidii* was recorded with potassium nitrate (282.30 mg), followed by sodium nitrate (259.80 mg), asparagine (195.50 mg) and ammonium sulphate (178.68 mg) {Table 5}. The lowest biomass (53.76 mg) was observed in medium without a nitrogen source, followed by aspartic acid producing slightly higher biomass (125.33 mg). Regardless of the nitrogen source, the minimum biomass (77.56 mg) was recorded after 7 d, which increased significantly to 287.58 mg after 14 d. After 14 d, potassium nitrate resulted in the highest biomass (423.20 mg), followed by sodium nitrate (413.16 mg). The control (no nitrogen source) had the lowest biomass (25.13 mg), followed by aspartic acid (36.60 mg) also showing low biomass after 7 d. Other nitrogen sources produced intermediate biomass levels at both 7 and 14 d incubation. Overall, potassium nitrate and sodium nitrate were the most effective nitrogen sources for the growth of *P. psidii*. Present findings are in conformity with Gurgel *et al*. (2002) who reported asparagine and potassium nitrate to be best nitrogen source for growth of *P. anacardii* and *P. mangiferae*. Results are further supported by Girish and Bhat (2011) who revealed that some of the isolates of *P. azadirachtae.* exhibited maximum growth with potassium nitrate as a nitrogen source. Pawar (2021) also reported barium nitrate, cobalt nitrate and potassium nitrate to be the best nitrogen source which also supports our findings to a certain extent.

Effect of trace elements on mycelial biomass of P. psidii

The highest biomass of *P. psidii* (858.33 mg) was recorded when magnesium sulphate was used as a trace element, followed by ferric chloride (852.56 mg) [Table 6]. The lowest biomass (266.58 mg) was produced when no trace element was used, followed by copper sulphate producing slightly higher biomass (619.15 mg). Regardless of the trace element, a significant increase in fungal biomass was observed after 14 d of incubation (1177.02 mg) compared to 7 d (136.25 mg). After 14 d, magnesium sulphate resulted in the highest biomass (1558.10 mg), followed by ferric chloride (1550.46 mg). The control (no trace element) showed the lowest biomass (98.13 mg) after 7 d, with copper sulphate producing (130.10 mg). These findings indicate that magnesium sulphate and ferric chloride were the most effective trace elements for the growth of *P. psidii*. Magnesium is a key element for fungi playing vital role in oxidation process. Magnesium has a role in the activity of certain enzymes and in respiration being a component of protein substances with a special importance for microorganisms being included in phosphorylation and reducing enzymes and for protein synthesis too (Rozsa *et al*., 2021). However, iron is essential to fungi for their survival as it plays an important role in DNA respiration, transcription, metabolism and energy growth

essentially during infection process (Valkovic and Domic, 2022). So, the media having magnesium and iron in their composition are preferred by the fungi and same happened in our studies too.

Conclusion: The pathogen causing styler end rot in guava was identified as *Phomopsis psidii.* The fungus exhibited maximum growth and growth rate on PDA as nutrient medium, temperature of 25ºC and pH 6.5. The fungus attained maximum growth in 48-72 h incubation, irrespective of any of nutrient media, temperature or pH used. Starch was found as best carbon source, and potassium nitrate as best nitrogen sources; while magnesium sulphate proved best trace element for the growth of *P. psidii*.

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