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Growth dynamics of *Lasiodiplodia theobromae* in response to environmental factors: Insights from *Parkia roxburghii* Dieback in Manipur, India

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Parkia roxburghii, commonly known as tree bean, is a significant plant for communities in Northeast India, yet its dieback disease caused by *Lasiodiplodia theobromae* is a growing concern. This study aimed to investigate the growth factors of L. *theobromae*, focusing on temperature, pH and sugar concentration. Five isolates were collected from different regions in Manipur and were identified morphologically and molecularly, with internal transcribed spacer (ITS) sequencing confirming their similarity to L. *theobromae*. The growth patterns of these isolates were assessed under varying temperature and pH conditions. Optimal mycelial growth was observed at moderate temperatures (26–32°C) and high sugar concentrations (15g–21g), with radial growth best supported at low pH (4–4.5) and high sugar levels. Fresh and dry weights were maximized at intermediate pH (5–5.5) with moderate to high sugar concentrations. Higher temperatures (34°C) and low sugar levels were detrimental to growth. Statistical analysis revealed significant differences in fungal growth across different treatments, with sugar concentration playing a more crucial role at lower temperatures. The study concluded that a combination of moderate temperature, pH, and high sugar concentration supported optimal fungal growth. Nonetheless, high temperatures and extremely high sugar concentrations suppressed biomass formation. These results helped to elucidate the environmental conditions preferred by L. *theobromae*, which was crucial for tree bean dieback management in infected areas.

Keywords : Climate change, pH, sugar, temperature, tree bean

INTRODUCTION

Tree Bean (*Parkia roxburghii* G. Don Syn. P. *timoriana* (DC.) Merr.) is a leguminous tree, growing up to 25 meters in height, with wide branches (Alam *et al.* 2001).

The species occurs mainly in Southeast Asian nations, such as India, Bangladesh, Myanmar, Java, Thailand, Egypt, and the Malaysian region. In India, its distribution is largely confined to the northeastern states of Mizoram, Nagaland, Manipur, Meghalaya, and Assam (Angami *et al.* 2018). There is an enormous demand-supply gap of tree bean in the northeastern states of India, especially in Manipur, because organized plantations are visible nowhere (Dhandhukia and Thakkar, 2007). Therefore, an enormous amount of tree bean is imported from Myanmar to fulfill the demand (Dhandhukia and Thakkar, 2007). Though it is an underexploited crop, tree bean is highly beneficial and possesses multiple uses (Fernandes *et al.* 2017; Singh *et al.* 2018). Tree Bean is significant in balancing the ecology by supporting and augmenting soil fertility (Firake *et*

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al. 2013). The ideal temperature for luxuriant growth is 15 to 27°C and can endure 35°C. It thrives in areas with an annual rainfall of approximately 3500 mm per year but will manage to survive up to 1750 mm.It grows well in different ranges of altitudes up to 3,000 m above sea level. In north-eastern hilly regions of India, the tree is well adapted to a wide range of soil from clay loam, and deep clay loam to lateritic/acidic soils. In recent years, a widespread dieback of Tree Bean has been observed in the entire northeastern region, notably in Manipur. Over the last 6-7 years, a considerable number of trees have succumbed to decline-related issues. The insects most commonly linked to the decreased growth of tree beans, either partially or entirely, were recognized as Ambrosia beetles and bark beetles, specifically *Blepephaeus succinctor* and *Xystrocera globose* (Sinha *et al.* 2018). But some scientists have also reported a fungus, L. *theobromae* causing dieback in tree bean decline in Northeast India. The symptoms of this affliction manifest as the drying up of half the tree, leaflet loss, wilting, excessive gummosis, easy-to-peel bark, and eventual desiccation of the entire tree. Extensive research has identified *L. theobromae* (synonym *Botryodiplodia theobromae*) as the pathogen associated with the sudden death or decline of Tree Bean (Khanzada *et al.* 2006).

L. theobromae, a facultative parasite, exhibits a broad host range and is known to cause various diseases such as dieback, blights, and root rot in a variety of hosts in tropical and subtropical regions (Kumar and Patel, 2018). While earlier reports have identified the pathogen, limited research has been conducted on the impact of different growth factors (temperature, sugar, and pH) on the fungus. Therefore, the present research investigated the interactive effects of pH, temperature, and sugar concentration on the growth of *L. theobromae*.

MATERIALS AND METHODS

Isolation and identification of the fungal culture

This study was conducted at the Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India. Five distinct pathogens of L. *theobromae* were collected from the Department of plant pathology, College of Agriculture, C.A.U Imphal, which were initially collected from various geographical regions within Manipur – i1 and i2 from Bishnupur, i3 from Tamelong, i4 from Andro village and i5 from C.A.U Iroisemba (Fig. 1) for a previous study by our lab. These isolates were maintained as pure cultures on Potato Dextrose Agar (PDA). Specifically, isolates of *L. theobromae* were sub-cultured on PDA medium within Petri dishes measuring 85 mm in diameter.

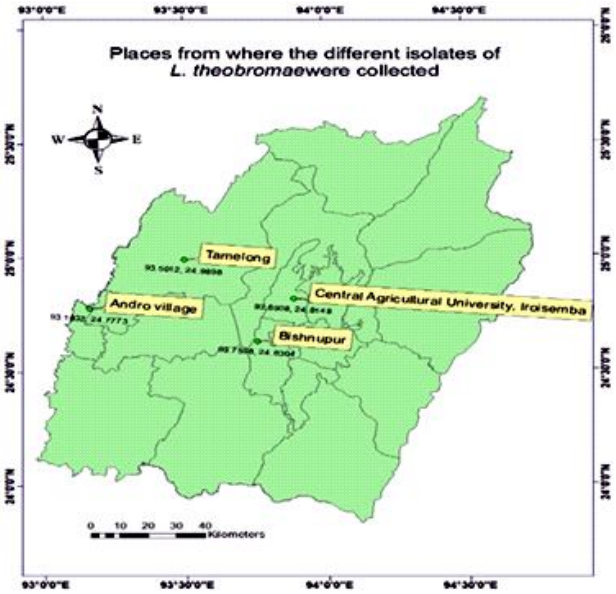

Fig.1: Study area of Manipur

Effect of pH and temperature on growth, dry weight and fresh weight of L. theobromae

For assessing radial growth, PDA was prepared and distributed into five 250 mL Erlenmeyer flasks, each adjusted to a different pH level (4.0, 4.5, 5.0, 5.5 and 6.0). The pH was adjusted using 1 N HCl or 1 N NaOH prior to autoclaving with the digital pH meter (EuTech Instruments, pH700, Thermo Fisher, Scientific, United States). The culture medium was individually prepared for each distinct pH. After sterilization at 121/ °C for 15 minutes, the media were poured into sterile petri plates and allowed to solidify. Once the medium dried, each plate was inoculated with a 5 mm mycelial disc taken from a 7-day-old culture. The inoculated plates were incubated at five different

temperatures (26, 28/ , 30/ , 32/ and 34/ °C) for 5 days in a temperature-controlled incubator. Each treatment (combination of pH and temperature) was replicated three times to ensure reliability.

For examining fresh weight, PDB at pH levels 4.0 to 6.0 was distributed into flasks, autoclaved and inoculated with a 5 mm mycelial disc. It was replicated three times for each treatment, the experiment was performed at different temperatures (26, 28, 30, 32 and 34°C) and the flasks were incubated for 7 days at the given temperatures. To determine the dry weight of the same mycelial mats, fungal cultures were harvested after 7 days and dried in a hot air oven at 60–65/ °C until a constant weight was achieved, after which the dry mycelial weight was recorded.

Effect of interaction between pH and sugar on growth of L. theobromae

For assessing radial growth in this case, PDA was prepared and distributed into five 250 mL Erlenmeyer flasks, each adjusted to a different sugar level (0 g/L (sugar-free), 12 , 15 , 18 and 21 g/L). Then, pH was adjusted to a different pH level (4.0, 4.5, 5.0, 5.5 and 6.0) using 1 N HCl or 1 N NaOH prior to autoclaving. After sterilization the media were poured into sterile petri plates and allowed to solidify. Once the medium dried, each plate was inoculated with a 5 mm mycelial disc taken from a 7-day-old culture. The inoculated plates were incubated for 7 days in incubator at 28°C. Each treatment (combination of pH and sugar) was replicated three times to ensure reliability.

For assessing fresh weight, PDB was prepared and 50 ml media was distributed into 250 mL Erlenmeyer flasks, each adjusted to a different sugar level (0 g/L (sugar-free), 12 , 15 , 18 and 21 g/L). Then, pH was adjusted to a different pH level (4.0, 4.5, 5.0, 5.5 and 6.0) using 1 N HCl or 1 N NaOH prior to autoclaving. It was replicated three times for each treatment and the flasks were incubated for 7 days. After 7 days, then mycelial mats from liquid cultures were harvested by filtration using pre-weighed qualitative filter papers. Fresh weight was recorded after gently blotting excess moisture with sterile filter paper. To determine the dry weight of the same mycelial mats, fungal cultures were harvested after 7 days and dried in a hot air oven at 60–65/ °C until a constant weight was achieved, after which the dry mycelial weight was recorded.

Effect of sugar and temperature on radial growth, dry weight and fresh weight of fungus.

For assessing radial growth, PDA was prepared and distributed into five 250 mL Erlenmeyer flasks, each adjusted to a different sugar level (0 g/L (sugar-free), 12 , 15 , 18 and 21 g/L). After sterilization at 121/ °C for 15 mins, the media were poured into sterile petri plates and allowed to solidify. Once the medium dried, each plate was inoculated with a 5 mm mycelial disc taken from a 7-day-old culture. The inoculated plates were incubated at five different temperatures (26, 28, 30, 32 and 34/ °C) for 7 days in a temperature-controlled incubator. Each treatment (combination of sugar and temperature) was replicated three times. After the incubation period, radial growth was assessed on Potato Dextrose Agar (PDA) plates by measuring the diameter of the fungal colony. Measurements were taken in two perpendicular directions across each colony, and the average was recorded in millimetres to determine the radial growth.

For the fresh weight and dry weight experiment, Potato Dextrose Broth (PDB) media were prepared with five different concentrations of dextrose: 0 g/L (sugar-free), 12 g/L, 15 g/L, 18 g/L, and 21 g/L. After that 50 mL of each medium was dispensed into 250 mL Erlenmeyer flasks and sterilized by autoclaving at 121/ °C for 15 minutes. Upon cooling, each flask was inoculated with a 5 mm diameter mycelial disc obtained from the margin of an actively growing 7-day-old PDA culture. All treatments were performed in triplicate to ensure reproducibility. The flasks were incubated under static conditions at a predetermined temperature (26/ , 28, 30/ , 32 and 34/ °C) for 7 days. Then mycelial mats from liquid cultures were harvested by filtration using pre-weighed qualitative filter papers. Fresh weight was recorded after gently blotting excess moisture with sterile filter paper. The samples were then dried in a hot air oven at 60–65/ °C

until a constant weight was achieved, and then dry weight was calculated.

Statistical analysis and interpretation of data

The data recorded in each experiment were subjected to statistical analysis wherever required. The differences exhibited by treatments were tested for their significance by employing the factorial randomized block design (FRBD). The post hoc method, Duncan multiple range test (DMRT) was used for comparing the set of means. In addition to the factorial randomized block design (FRBD) and the Duncan multiple range test (DMRT), further statistical analyses, including.

RESULT AND DISCUSSION

Morphological characterization of different isolates of L. theobromae

On PDA medium, Isolate1(i1) had creamish white colony colour with white flat appearance and regular margins, isolate 2 (i2) also had creamish white colony colour with sparse and fluffy appearance and regular margins, isolate 3 (i3) had grey white colonies with fluffy appearance and irregular margins, isolate 4(i4) had white colony with sparse and flat appearance and regular margins and isolate 5 (i5) had light brown and white appearance with fluffy appearance and irregular margins (Fig.2). Same was observed by Woodward *et al.* (2005), when examining *Lasiodiplodia theobromae* infecting eggplant, the fungus produced greyish colonies with aerial hyphae and black ostiolate pycnidia clustered in stroma.

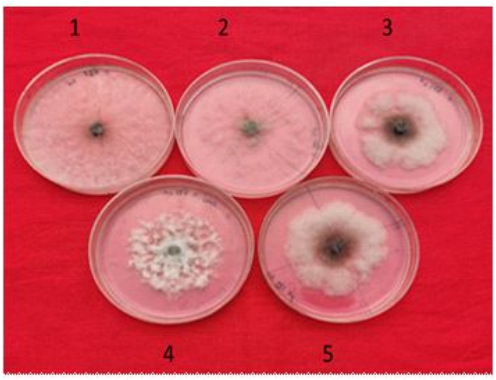

Fig.2 Morphological characterization of fungal isolates

Effect of interaction between pH and temperature on growth of L. theobromae

The growth patterns of various isolates (i1, i2, i3, i4 and i5) of *L. theobromae* were investigated under different pH levels and temperatures (Fig.3). High temperatures (34°C) combined with pH values between 4 and 6 create the best conditions for radial expansions, but the mycelial fresh weight and dry weight was found higher at moderate temperatures(26-30°C) (28°C was selected and treated as the reference (control) condition for fungal growth, as it consistently supported optimal biomass production). But the pH combination with the lower temperature(26°C) was not found conducive for the growth of all the isolates on the PDA medium (Fig.4 a, b and Table.1). Considering *L. theobromae's* typical habitat in subtropical regions, the preference for moderate temperatures aligned with expectations. The fungus showed adaptability, switching between saprophytic and necrotrophic modes in response to environmental stress, including pH conditions, indicating a survival strategy. These findings partially corroborated with studies by Dhandhukia *et al.* (2007) and Khanzada *et al.* (2006), supporting optimal growth conditions for *L. theobromae* at specific pH levels and temperature.

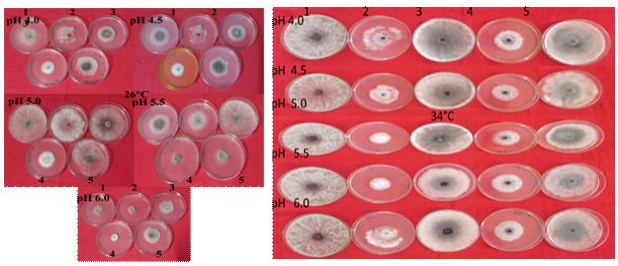

Fig. 3 : Effect of different temperatures and pH on growth of *L.theobromae*

Effect of interaction between pH and sugar on growth of L. theobromae

The mycelial radial growth of *L. theobromae* on PDA medium exhibited variability in response to pH and sugar change **(**Fig.5 **).** The interaction of pH and sugar concentration significantly affected fungal mycelium growth. For optimal growth across all metrics (radius, fresh weight, and dry weight). Radius was best supported at lower pH levels (4-4.5) with high sugar concentrations (18g-

Table 1. Effect of interaction between pH and temperature on the radial growth of mycelium(cm) of L. *theobromae*

S. No	Temperature (^{0}C)	pH	i1	i2	i3	i4	i5	Mean
1.	26	4	4.933^{qrstuvw}	4.567^{stuvwxyz}	4.367^{vwxyzAB}	3.000^{IJKL}	4.300^{vwxyzABC}	4.233
2.		4.5	5.300^{opqrst}	4.533^{stuvwxyzAB}	4.367^{vwxyzAB}	3.033^{HIKL}	4.900^{rstuvw}	4.426
3.		5	8.500^{a}	7.700^{bcdefg}	7.600^{cdefg}	3.867^{zABCDEFGH}	8.000^{abcdef}	7.133
4.		5.5	4.767^{rstuvwx}	3.900^{yzABCDEFG}	4.267^{vwxyzABCD}	3.233^{FGHIJK}	3.267^{EJK}	3.886
5.		6	4.033^{xyzABCDEF}	2.533^{KL}	4.000^{xyzABCDEF}	2.600^{JKL}	3.700^{ABCDEFGHI}	3.373
		Mean	5.506	4.646	4.920	3.146	4.833	4.610
S. No	Temperature (^{0}C)	pH	i1	i2	i3	i4	i5	Mean
1.	28	4	8.500^{a}	8.367^{abc}	8.067^{abcdef}	2.600^{JKL}	7.400^{defgh}	6.986
2.		4.5	8.500^{a}	8.300^{abc}	7.833^{abcdefg}	4.933^{qrstuvw}	8.500^{a}	7.61
3.		5	8.500^{a}	7.800^{abcdefg}	7.433^{defgh}	5.330^{opqrs}	5.233^{pqrstu}	6.859
4.		5.5	8.500^{a}	7.800^{abcdefg}	7.267^{fghi}	5.500^{mnopqr}	7.100^{ghi}	7.233
5.		6	8.333^{abc}	5.330^{opqrs}	7.267^{fghi}	4.000^{xyzABCDEF}	7.300^{efghi}	6.446
		Mean	8.466	7.519	7.573	4.472	7.106	7.026
S. No	Temperature (^{0}C)	pH	i1	i2	i3	i4	i5	Mean
1.	30	4	8.500 a	7.967 abcdef	8.100 abcdef	6.100 klmno	8.067 abcdef	7.746
2.		4.5	8.500 a	7.267 fghi	5.333 opqrs	4.333 vwxyzABC	5.433 nopqr	6.176
3.		5	8.400 ab	7.000 cghij	6.700 hijk	5.767 lmnop	7.667 bcdefg	7.106
4.		5.5	4.900 rstuvw	3.500 CDEFGHI	4.100 wzyzABCD	3.800 ABCDEFGHI	4.433 uvwxyzAB	4.146
5.		6	5.067 pqrstuv	4.467 tuvwxyzAB	4.733 rstuvwxy	3.733ABCDEFGHI	4.433 uvwxyzAB	4.486
		Mean	7.073	6.040	5.792	4.746	6.006	5.931
S. No	Temperature (^{0}C)	pH	i1	i2	i3	i4	i5	Mean
1.	32	4	8.500 a	2.333 L	6.167 klmn	3.300 EFGHIJK	6.500 ijkl	5.359
2.		4.5	8.500 a	3.133 GHIJK	7.300 efghi	3.400 EFGHIJ	6.267 jklm	5.720
3.		5	8.500 a	3.800 ABCDEFGHI	8.500 a	3.100 GHIJKL	6.267 jklm	6.033
4.		5.5	8.500 a	3.767 ABCDEFGHI	8.000 abcdef	2.333 L	5.867 lmnop	5.693
5.		6	8.500 a	3.767 ABCDEFGHI	8.000 abcdef	3.433 DEFGHI	7.067 ghi	6.153
		Mean	8.5	3.359	7.593	3.112	6.393	5.791
S. No	Temperature (^{0}C)	pH	i1	i2	i3	i4	i5	Mean
1.	34	4	8.500 a	8.167 abcd	8.167 abcd	3.433 DEFGHI	8.500 a	7.353
2.		4.5	8.367 abc	7.667 bcdefg	8.333 abc	4.700 rstuvwxyz	8.500 a	7.512
3.		5	8.500 a	8.133 abcde	8.500 a	4.900 rstuvw	8.500 a	7.706
4.		5.5	8.500 a	8.067 abcdef	8.500 a	4.500 stuvwxyzAB	8.500 a	7.613
5.		6	8.500 a	8.100 abcdef	8.500 a	4.000 xyzABCDEF	8.500 a	7.520
		Mean	8.47	8.026	8.400	4.306	8.5	7.540

	Temp	pH	Iso	Temp*pH	Temp*Iso	pH* Iso	Temp*pH*Iso
SEm	0.049	0.049	0.049	0.109	0.109	0.109	0.244
SEd	0.069	0.069	0.069	0.155	0.155	0.155	0.346

CD (1%) = 0.402 S Em =0.086 S Ed = 0.122

Table 2a. Effect of interaction between Sugar and temperature on the mycelial fresh weight of L. *theobromae*

S. No	Temperature (0C)	Sugar (g/L)	Mycelial Fresh Weight (g) i1		i2	i3	i4	i5	Mean
1.	26	0	5.065 GHIJ	2.300 Y	3.705 TU	3.432 UV	4.277 OPQR		3.7558 j
2.		12	7.554 fgh	7.310 hij	5.220 EFGHI	6.931 klmno	7.548 fgh		6.9126 d
3.		15	6.539 qrstuv	7.541 fgh	5.787 yzA	8.929 ab	8.168 de		7.3928 ab
4.		18	7.667 fg	6.226 wx	6.101 xy	7.514 fgh	8.241 de		7.1498 c
5.		21	7.666 fg	6.226 wx	6.103 xy	7.514 fgh	8.241 de		7.15 c
		Mean	6.8982 c	5.9206 h	5.3832 k	6.864 c	7.295 a		
S. No	Temperature (0C)	Sugar	i1		i2	i3	i4	i5	Mean
1.	28	0	2.614 X	1.553 Z	3.060 W	2.744 X	3.136 W		2.6214 k
2.		12	8.457 cd	6.888 klmnop	7.381 ghi	6.682 nopqrs	7.285 hij		7.3386 b
3.		15	7.016 jklm	5.178 FGHI	8.096 e	7.776 f	7.019 jklm		7.017 d
4.		18	6.560 qrstuv	7.109 ijkl	6.837 klmnopq	8.710 bc	8.303 de		7.5038 a
5.		21	5.756 zA	6.586 pqrstu	8.219 de	7.642 fg	6.446 stuvw		6.9298 d
		Mean	6.0806 g	5.4628 ij	6.7186 d	6.7108 d	6.4378 f		
S. No	Temperature (0C)	Sugar	i1		i2	i3	i4	i5	Mean
1.	30	0	5.114 GHIJ	4.808 JKLM	3.332 VW	3.706 TU	6.803 lmnopqr		4.7526 i
2.		12	6.205 wx	4.213 PQRS	5.684 zA	4.953 IJK	6.616 opqrst		5.5342 f
3.		15	5.834 yz	5.618 zAB	5.204 EFGHI	4.156 RS	6.767 mnopqrs		5.5158 f
4.		18	5.834 yz	4.933 IJK	5.828 yz	4.567 LMNO	5.715 zA		5.3754 g
5.		21	6.101 xy	5.011 HIJK	6.552 qrstuv	5.826 yz	6.941 klmn		6.0862 e
		Mean	5.8176 h	4.9166 m	5.32 k	4.6416 n	6.5684 e		
S. No	Temperature (0C)	pH	i1		i2	i3	i4	i5	Mean
1.	32	0	7.397 ghi	2.456 XY	5.478 ABCDEF	6.346 tuvwx	4.161 QRS		5.1676 h
2.		12	6.204 wx	4.510 MNOP	5.248 DEFGHI	7.654 fg	7.283 hij		6.1798 e
3.		15	9.184 a	6.278 uvwx	6.494 rstuvw	5.868 yz	7.134 ijk		6.9916 d
4.		18	6.336 tuvwx	5.788 yzA	4.326 OPQR	4.818 JKL	6.648 nopqrst		5.5832 f
5.		21	6.699 nopqrs	5.061 GHIJ	4.363 OPQR	2.730 X	6.830 klmnopq		5.1366 h
		Mean	7.164 b	4.8186 m	5.1818 l	5.4832 i	6.4112 f		
S. No	Temperature (0C)	Sugar	i1		i2	i3	i4	i5	Mean
1.	34	0	5.613 zABC	3.631 U	4.487 NOP	4.525 LMNOP	5.323 BCDEFGH		4.7158 i
2.		12	6.630 nopqrst	4.342 OPQR	5.506 ABCDE	5.306 BCDEFGH	4.729 KLMN		5.3026 g
3.		15	5.349 BCDEFG	3.443 UV	5.316 BCDEFGH	5.542 zABCD	6.250 vwx		5.1800 h
4.		18	5.862 yz	3.611 UV	4.821 JKL	4.409 OPQR	5.300 CDEFGH		4.8006 i
5.		21	6.226 wx	3.927 ST	4.476 NOPQ	4.234 PQR	5.150 GHI		4.8026 i
		Mean	5.936 h	3.7908 o	4.9212 m	4.8032 m	5.3504 jk		

	pH	Sugar	Iso	pH*Sugar	Sugar*Iso	pH* Iso	Sugar*pH*Iso
S Em	0.019	0.019	0.019	0.043	0.043	0.043	0.096
S Ed	0.027	0.027	0.027	0.061	0.061	0.061	0.136
CD (1%)	0.350	S Em 0.096			S Ed 0.136		

Mean with same letter(s) are not significantly different according to Duncan Multiple RangeTest (DMRT), = 0.01)

21g). Fresh Weight and Dry Weight were highest at intermediate pH levels (5-5.5) with moderate to high sugar levels (15g-21g) (Fig. 6). To make meaningful comparisons, 18/ g/L sugar were chosen as control, as they consistently supported good fungal growth.

Low sugar concentrations limited growth in all forms, regardless of pH, indicating that sufficient sugar is essential for mycelial development. The ideal conditions varied slightly between metrics, but overall, a combination of moderate pH and high sugar concentration supported the best growth outcomes for fungal mycelium. Interestingly, the isolates demonstrated growth resilience across various sugar concentrations within specific pH combinations, rendering the role of sugar concentration redundant in influencing growth alongside pH.

Conversely, elevated sugar concentration and high pH adversely affected mycelial fresh and dry weight, possibly due to imposed stress on fungal cells and nutrient acquisition challenges, as suggested by Fernandes *et al.* (2017).

Effect of interaction between temperature and sugar on the growth of L. theobromae

The radial growth of *L. theobromae* varied with changes in sugar and temperature on PDA medium. Statistical analysis indicated significant differences in fungal growth across treatments. The results indicated that temperature and sugar

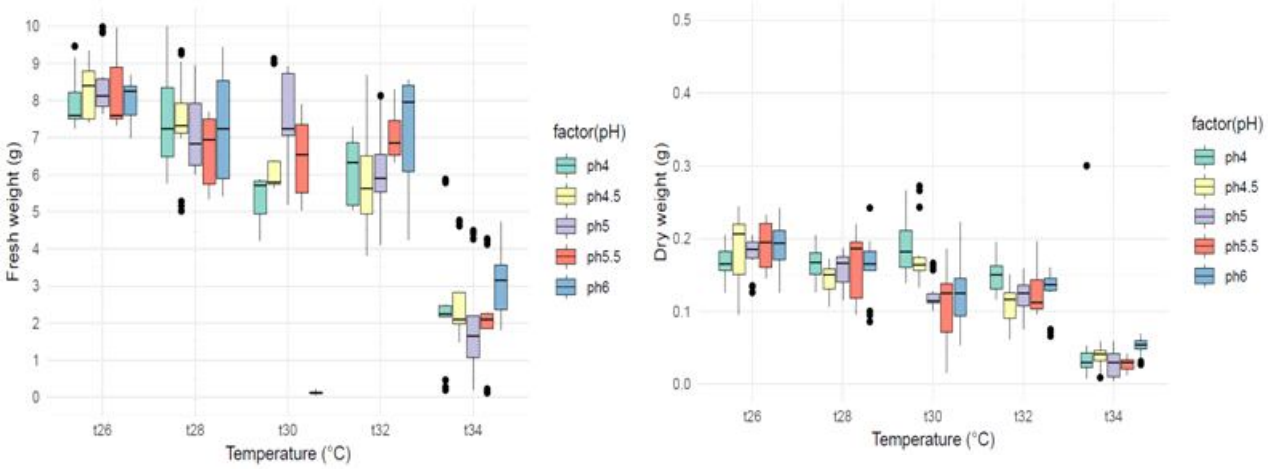

Fig. 4: Box plot of pH and temperature on mycelial fresh wt. (top) and dry wt. (bottom) of L.theobromae

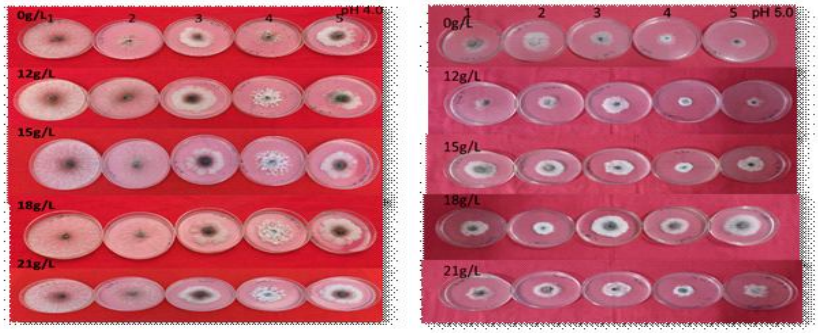

Fig.5: Growth of L.theobromae at different sugar concentrations and pH

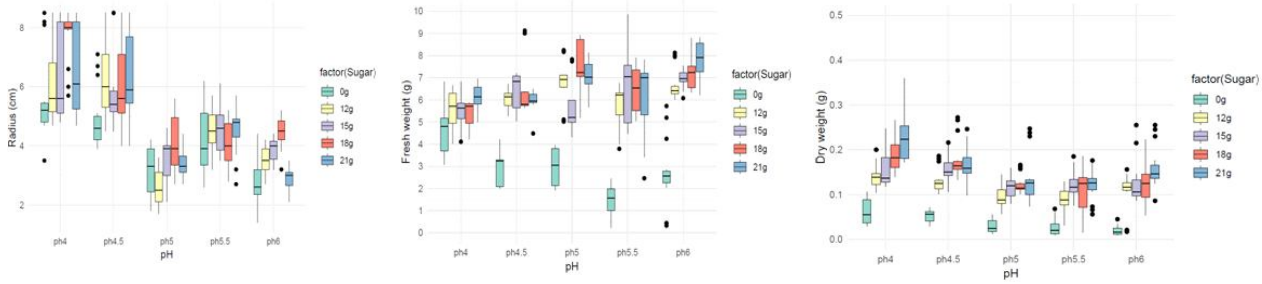

Fig. 6: Box plot of pH and sugar on radial growth (top left), mycelial fresh wt. (top right) and mycelial dry wt. (bottom) of *L.theobromae*

concentration both affected the radius, with higher temperatures (30°C to 34°C) generally leading to larger radii, though with increased variability. At lower temperatures (26°C), radius was smaller, especially at low sugar levels, but increases as sugar concentration rose. The interaction between sugar concentration and temperature also had a significant effect on the mycelial growth (fresh and dry weight) of *Lasiodiplodia theobromae* isolates. Overall, moderate temperatures (28°C and 30°C) combined with higher sugar concentrations (15–18 g/L) promoted the greatest mycelial fresh and dry weight. Specifically, the highest fresh weight was recorded at 28°C with 18 g/L sugar, while the lowest was at 28°C with no added sugar. Similarly, the dry weight was highest at 34°C with 18 g/L sugar, suggesting this isolate maintained substantial growth even at higher temperatures when sufficient sugar was available. In contrast, the lowest dry weights were observed at 26°C and 28°C with 0 g/L sugar (0.031–0.032 g) **(**Fig.7).These results demonstrated that L. *theobromae* thrives under moderate to slightly elevated temperatures with increased sugar availability, and growth is significantly reduced at low sugar concentrations regardless of temperature. (Table 2a, b and Fig. 8). While sugar

Table 2b. Effect of interaction between Sugar and temperature on the mycelial dry weight of L. *theobromae*

S. No	Temperature (⁰C)	Sugar (g/L)	Isolates i1	i2	i3	i4	i5	Mean
1.	26	0	0.034 QRST	0.040 QRS	0.034 QRST	0.031 RST	0.020 ST	0.031 m
2.		12	0.170 jklmnopq	0.114 CDEFGHIJ	0.066 OP	0.126 yzABCDEF	0.094 IJKLMN	0.114 j
3.		15	0.207 gh	0.171 jklmnopq	0.130 xyzABCDE	0.183 ijk	0.136 uvwxyzABC	0.165 c
4.		18	0.214 gh	0.120 ABCDEFGH	0.138 uvwxyzAB	0.212 gh	0.123 zABCDEFG	0.161 cde
5.		21	0.221 fg	0.140 tuvwxyzA	0.114 CDEFGHIJ	0.148 rstuvwxy	0.122 zABCDEFG	0.149 f
		Mean	0.169 c	0.117 i	0.0964 kl	0.14 ef	0.099 jk	
S. No	Temperature (⁰C)	Sugar	i1	i2	i3	i4	i5	Mean
1.	28	0	0.030 ST	0.054 PQ	0.029 ST	0.026 ST	0.024 ST	0.032 m
2.		12	0.177 ijklmn	0.081 LMNO	0.142 stuvwxyzA	0.109 EFGHIJ	0.101 HIJKLM	0.122 ij
3.		15	0.156 nopqrstuv	0.093 JKLMN	0.108 EFGIHJ	0.164 klmnopqrs	0.137 uvwxyzAB	0.131 gh
4.		18	0.124 zABCDEFG	0.167 jklmnopqr	0.158 nopqrstu	0.150 qrstuvwx	0.181 ijklm	0.156 ef
5.		21	0.173 jklmnop	0.126 yzABCDEF	0.214 gh	0.153 opqrstuvw	0.139 tuvwxyzA	0.161 cde
		Mean	0.132 gh	0.1042 jk	0.130 h	0.120 i	0.116 i	
S. No	Temperature (⁰C)	Sugar	i1	i2	i3	i4	i5	Mean
1.	30	0	0.086 KLMNO	0.035 a	0.036 QRS	0.102 GHIJKL	0.052 PQR	0.032 m
2.		12	0.177 ijklmn	0.140 tuvwxyzA	0.141 tuvwxyzA	0.121 ABCDEFGH	0.114 CDEFGHIJ	0.122 ij
3.		15	0.187 ij	0.197 hi	0.136 uvwxyzABC	0.132 wxyzABCD	0.123 zABCDEFG	0.131 gh
4.		18	0.257 de	0.152 pqrstuvwx	0.186 ijk	0.208 gh	0.161 lmnopqrst	0.156 ef
5.		21	0.224 fg	0.186 ijk	0.174 jklmno	0.350 a	0.250 de	0.161 de
		Mean	0.132 g	0.104 jk	0.130 h	0.120 i	0.116 i	
S. No	Temperature (⁰C)	pH	i1	i2	i3	i4	i5	Mean
1.	32	0	0.114 CDEFGHIJ	0.024 ST	0.014 T	0.144 stuvwxyz	0.024 ST	0.064 l
2.		12	0.054 PQ	0.054 PQ	0.114 CDEFGHIJ	0.121 ABCDEFGH	0.094 IJKLMN	0.087 k
3.		15	0.124 zABCDEFG	0.094 IJKLMN	0.114 CDEFGHI	0.134 vwxyzABCD	0.144 stuvwxyz	0.122 ij
4.		18	0.144 stuvwxyz	0.104 FGHIJK	0.144 CDEFGHIJ	0.144 sABCDEFGH	0.084 KLMNO	0.124 hi
5.		21	0.244 de	0.094 IJKLMN	0.124 zABCDEFG	0.034 QRST	0.095 IJKLMN	0.118 ij
		Mean	0.136 fgh	0.074 m	0.102 jk	0.115 i	0.088 l	
S. No	Temperature (⁰C)	Sugar (g/L)	i1	i2	i3	i4	i5	Mean
1.	34	0	0.161 lmnopqrst	0.082 LMNO	0.094 INKLMN	0.040 QRS	0.079 MNO	0.091 k
2.		12	0.236 ef	0.114 CDEFGHIJ	0.142 stuvwxyzA	0.121 ABCDEFGH	0.124 zABCDEFG	0.147 f
3.		15	0.294 c	0.075 NO	0.297 c	0.095 IJKLMN	0.183 ijk	0.188 b
4.		18	0.334 ab	0.116 BCDEFGHI	0.252 c	0.109 EFGHIJ	0.184 ijk	0.199 a
5.		21	0.324 b	0.142 stuvwxyzA	0.183 ijk	0.113 DEFGHIJ	0.158 nopqrstu	0.184 b
		Mean	0.297 a	0.111 j	0.218 b	0.109 j	0.162 cd	

	pH	Sugar	Iso	pH*Sugar	Sugar*Iso	pH* Iso	Sugar*pH*Iso
S Em	0.001	0.001	0.001	0.003	0.003	0.003	0.006
S Ed	0.002	0.002	0.002	0.004	0.004	0.004	0.009
CD (1%)	0.024		S Em 0.06		S Ed 0.09		

Mean with same letter(s) are not significantly different according to Duncan Multiple Range Test (DMRT) ρ=0.01

Fig.7. Mycelial growth of *L. theobromae* under different sugar concentrations and at 28°C and 30°C

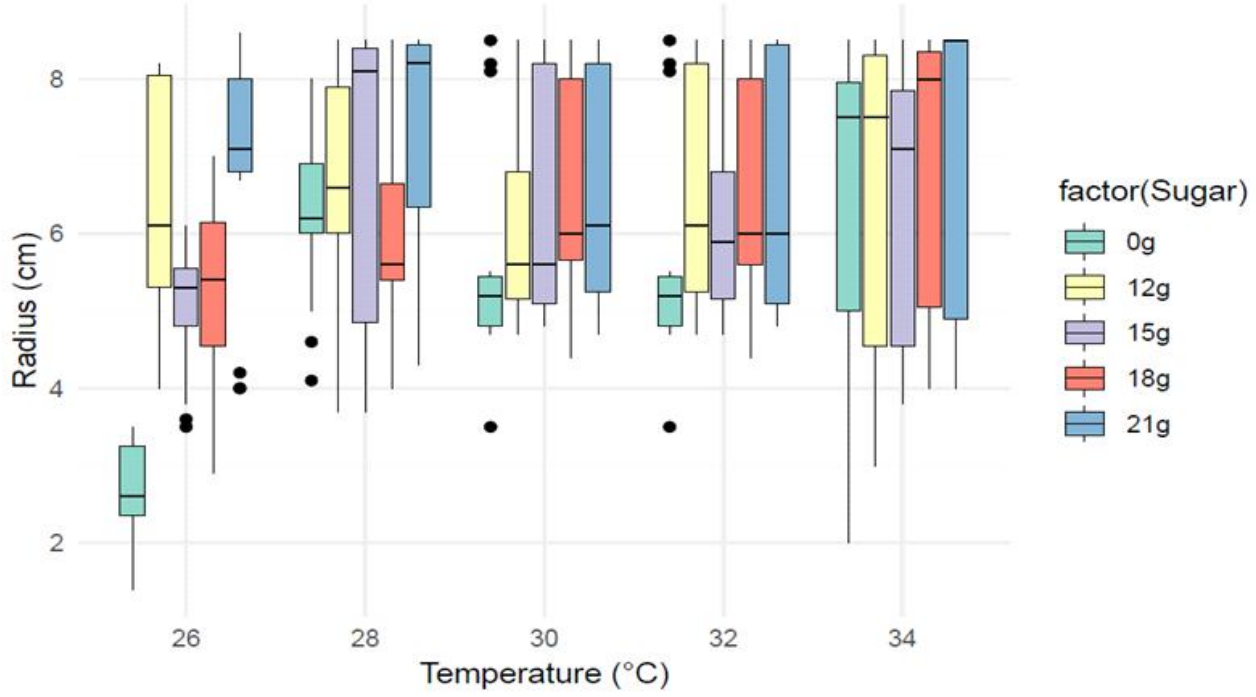

Fig.8 Box plot of temperature and sugar concentration for radial growth of *L. theobromae*.

concentration up to 18g/L positively influenced growth, higher concentrations had minimal impact, possibly due to extracellular polysaccharide production and increased viscosity.

Our results are in close agreement of those reported by Alam *et al.* (2001) who studied the growth of L. *theobromae* and Labuda *et al.* (2012) who found that increase in sugar concentration up to 35g/l significantly reduced the fungal biomass.

CONCLUSION

This study found that *Lasiodiplodia theobromae* growth was significantly affected by temperature, pH, and sugar concentration. Optimal mycelial growth occurred at moderate temperatures (26–30!) with acidic conditions (pH 4.0–5.0) and sugar concentrations of 18–21g/L. Higher temperatures (above 32!) and sugar levels beyond 18g/L did not further enhance growth, indicating a threshold for sugar utilization. L. *theobromae* thrives in acidic, high-sugar environments typical of Northeast India's climate. Rising temperatures due to climate change may further favor the pathogen, increasing the risk of tree bean dieback. However, for better understanding on effect of pH, temperature and sugar on *L. theobromae*, the results have to be evaluated under field conditions. Hence further research can be taken up on host pathogen interaction at different pH, temperature and nutritional level *in vivo*.

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DECLARATION

Conflict of Interest. Authors declare no conflict of Interest.

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