

Effect of Different Media, Temperature, PH, Carbon Source and Nitrogen Source on Mycelial Growth of *Lasiodiplodia theobromae* Causing Crown Rot of Banana.

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ABSTRACT

In this study, the effect of different media, temperature, pH, Carbon source and Nitrogen source was tested against growth of *Lasiodiplodia theobromae* under *in vitro* conditions. Among the solid media tested, Potato Dextrose Agar Medium was most suitable for the growth of *L. theobromae* which recorded 90 mm mycelial growth. Among the different liquid media tested, Potato dextrose broth found to be most suitable which recorded maximum mycelia dry weight (231.52 mg). Maximum radial growth (90 mm) and mycelial dry weight (327.43 mg) recorded at 30°C. pH 7.0 was found to be most suitable which is recorded maximum radial growth, Maximum mycelial dry weight and good pycnidial production. Among tested carbon sources, dextrose recorded the highest mycelial growth (90 mm) and the highest dry weight of (371.60 mg) with excellent pycnidial production. Among the tested nitrogen sources, peptone recorded the maximum growth of mycelium of about 88.66 mm and mycelial dry weight of about 364.76 mg.

Keywords: Crown rot, *Lasiodiplodia*, Banana, PDA, dextrose, Peptone

Introduction:

Banana is the fourth important fruit crop of the world and delivers an important source of starch, particularly in Asia and Africa (Muhammad Ather et al. 2018). Several fungal diseases reduce the quality and post harvest shelf life of this fruit crop (Win et al. 2007). Post harvest diseases cause a wide loss and damage to all the fruit crops and vegetable crops. Among the post harvest diseases in banana, the most common one is Crown rot of banana caused by *Lasiodiplodia theobromae* (Rattanakreetakul 2013). Extensive damage caused by this disease remains a potential problem to the native exporters. Crown rot of banana disease symptoms initially appears on the cut surface of the crescent shaped crown and the tissues infected become soft and black in colour. Also on the

infected tissues, grayish white colour hyphal growth appears and the tissue decays. Further the disease decreases the banana quality with the damage and the drop of the fingers (Sangeetha et al. 2013). The present study was undertaken to study the effect of different media, temperature, pH, Carbon source and Nitrogen source on mycelial growth, mycelial dry weight and Pycnidial production of *L. theobromae* causing crown rot of banana

Effect of different media on the mycelial growth of *L. theobromae* in vitro

Growth on Liquid medium

Growth and sporulation of the pathogen was studied in beet juice broth, carrot sucrose broth, potato sucrose broth, beetroot sucrose broth, corn meal broth, czapek's dox broth, rose bengal broth, peas sucrose broth. Broths were prepared in 250 ml Erlenmeyer flasks containing 50 ml of each broth. Mycelial discs of size 8mm was taken from the seven days old culture of *L. theobromae* with the help of cork borer was inoculated into the flasks containing the broth. The flasks were incubated for upto seven days at $28\pm 2^{\circ}\text{C}$ in BOD incubator. The mycelial mat was filtered through Whatman No. 41 filter paper of known weight. The filter paper along with the mycelial mat was dried in hot air oven at 105°C for 48 hours and mycelial dry weight was recorded. Each treatment was replicated thrice.

Linear growth

Growth and pycnidial production of the pathogen was studied in beet juice agar, carrot sucrose agar, potato sucrose agar, beetroot sucrose agar, corn meal agar, czapek's dox agar, rose bengal agar, peas sucrose agar. Fifteen ml of each medium were taken and dispensed into 90mm of the sterile Petri plates. Mycelial discs of size 8mm was taken from the seven days old culture of *L.theobromae* with the help of cork borer was inoculated into the Petri plates containing the medium. The inoculated plates were incubated at room temperature ($28\pm 2^{\circ}\text{C}$) for seven days and the mycelia growth of the pathogen was measured in each case after 7 days of incubation. Each plate was replicated thrice.

Effect of different temperature levels on the mycelial growth of *L.theobromae* under in vitro

Growth on Liquid medium

Erlenmeyer flasks (250 ml) containing 50 ml of Potato dextrose broth were sterilized and 8 mm culture disc from the seven days old culture of *L. theobromae* was inoculated in the flask. The inoculated plates were incubated at different temperatures viz., 5, 10, 15, 20, 25, 30, 35 and 40°C . for 7 days in BOD incubator. At the end of the incubation period, the mycelial mat was filtered

through Whatman No. 41 filter paper. The mycelial mat along with the filter paper was dried in hot air oven at 105°C for 48 hours and the mycelial dry weight was measured. Each flask was replicated thrice.

Linear growth

A quantity of 15 ml potato dextrose medium was poured into the 90 mm Petri plates and allowed to solidify. An 8 mm culture disc from the seven days old culture of *L. theobromae* was inoculated in the Petri plate and it was incubated at different temperatures viz., 0, 5, 10, 15, 20, 25, 30, 35 and 40°C. The mycelia growth of the pathogen was measured after 7 days of the incubation period. Each plate was replicated thrice.

Effect of certain pH levels on the mycelial growth of *L. theobromae* under *in vitro*

Growth on Liquid medium

Potato dextrose broth were prepared with different pH levels viz., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 in conical flasks and sterilized. The conical flasks were inoculated with 8mm culture disc from the seven days old culture of *L. theobromae*. The flasks were incubated for 7 days at 28±2°C in BOD incubator. At the end of the incubation period, the mycelial mat was filtered through Whatman No. 41 filter paper. The mycelial mat along with the filter paper was dried in hot air oven at 105°C for 48 hours and the mycelial dry weight was measured. Each flask was replicated thrice.

Linear growth

A quantity of 15 ml potato dextrose medium of different pH levels viz., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 was poured into 90 mm Petri plate. An 8 mm culture disc from the seven days old culture of *L. theobromae* was inoculated in the Petri plate for 7 days. The mycelia growth of the pathogen was measured at the end of the incubation period.

Effect of different carbon source on the mycelial growth of *L. theobromae* under *in vitro*

Growth on Liquid medium

The *in vitro* growth of the fungus was tested with seven different carbon sources viz., dextrose, fructose, glucose, mannitol, sucrose, maltose and lactose. Czapek's broth was taken as the basal medium for the study. In the Czapek's broth, sucrose was replaced with various carbon sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the

medium was adjusted to 7.0. After that, the medium was inoculated with 8 mm mycelial disc of pathogen obtained from 7 days old culture grown on PDA. The inoculated media were incubated for 7 days at room temp ($28 \pm 2^\circ\text{C}$). At the end of the incubation period the mycelial mats were filtered through previously dried and weight filter paper (Whatman No.41) and dried in hot air oven at 105°C until the constant mycelial dry weight was calculated. Three replications were maintained for each treatment.

Linear growth

Czapek's agar medium was amended with various carbon sources on equivalent weight basis and were dispensed in sterile Petri plate at fifteen ml quantities. After cooling they were inoculated with 8 mm mycelial disc of the pathogen obtained from 7 days old culture grown on PDA in Petri plates and incubated for 7 days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

Effect of different nitrogen source on the mycelial growth of *L.theobromae* under *in vitro*

Growth on Liquid medium

The *in vitro* growth of the fungus was tested with six different nitrogen sources viz., sodium nitrate, peptone, ammonium sulphate, ammonium chloride, sodium nitrite and potassium nitrate. Czapek's broth was taken as the basal medium for the study. In the Czapek's broth, Sodium nitrate was replaced with various nitrogen sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. After that, the medium was inoculated with 8 mm mycelial disc of pathogen obtained from 7 days old culture grown on PDA. The inoculated media were incubated for 7 days at room temp. ($28 \pm 2^\circ\text{C}$). At the end of the incubation period the mycelial mats were filtered through previously dried and weight filter paper (Whatman No.41) and dried in hot air oven at 105°C for 48 hrs and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Linear growth

Czapek's agar medium was amended with various nitrogen sources on equivalent weight basis and were dispensed in sterile Petri plate at fifteen ml quantities. After cooling they were inoculated with 8 mm mycelial disc of the pathogen obtained from 7 days old culture grown on PDA in petri plates and incubated for 7 days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

Result and Discussion

Effect of different culture media on the growth of L. theobromae (Lt₈)

Studies on the mycelia growth of *L. theobromae* in different culture media revealed that maximum mycelial growth (90mm) was found in potato dextrose agar medium after seven days of incubation period followed by beetroot sucrose agar (86.35mm), carrot sucrose agar (85.27 mm), bean juice agar (82.29 mm) in the decreasing order of merit. The minimum mycelial growth was found in Czapek's dox agar medium of about 34.83 mm (Table 1). Similar results were observed by Gowri Shankar et al. (2016) where potato dextrose agar medium served as the best medium to produce maximum mycelial growth. Achmad and Arshintia (2014) reported that potato dextrose agar medium was identified to be the best medium for the growth and sporulation of *Botrydiplodia* sp. causing dieback of Jabon seedling. Similar reports were also observed by Deepan et al. (2017) and Dheepa et al. (2018) where the colony spreading rate was also found to be higher with the potato dextrose agar medium.

Effect of different liquid media on the mycelial dry weight and sporulation of L. theobromae (Lt₈)

Mycelial dry weight and sporulation of the *L. theobromae* isolate on different liquid media revealed that maximum mycelial dry weight (231.52 mg) was supported by potato dextrose broth after seven days of incubation followed by beetroot sucrose agar (224.99 mg), carrot sucrose agar (179.25 mg) in the decreasing order of merit (Table 2). Sridevi (2018) reported that potato dextrose broth supported the maximum mycelial dry weight of *L. theobromae*. Similar results were also observed by Gowri Shankar et al. (2016) where potato dextrose broth supported the growth of *L. theobromae* and the maximum mycelial dry weight followed by Richard's broth (RB) and Czapek's Dox broth (CDB).

Effect of different temperature levels on the mycelia growth, dry weight and sporulation of L. theobromae (Lt₈)

Among the different temperature levels tested, 30°C was found to be more favourable for the mycelial growth of *L. theobromae* (90 mm) and showed the highest mycelial dry weight of about 327.43 mg under *in vitro* conditions. It was followed by 25°C which showed the mycelial growth of about 72.41 mm and mycelial dry weight of about 271.8 mg and decreases with further decrease in the temperature (Table 3). There was no mycelial growth found at 5, 10°C. The pycnidial production also showed the same trend as that of mycelial growth with respect to the temperature change. The results obtained by Latha et al. (2013) were also similar which states that the maximum

mycelial growth of *L. theobromae* was observed at 30°C followed by 35°C and the mycelial growth was drastically reduced below 20°C. similar results were also obtained by Nandhini (2015) and Lokeshwari (2019). Gowri Shankar et al. (2016) reported that the highest mycelial dry weight of *B. theobromae* was obtained at 25°C to 30°C which was considered as an optimum for the vegetative growth which was similar to the present study. Similarly Joseph Djeugap Fovo et al. (2017) reported that the optimum temperature for sporulation of *L. theobromae* was at 23°C.

Effect of different pH levels on the mycelia growth, dry weight and sporulation of L. theobromae (Lt₈)

Among the different pH levels tested, pH 7.0 recorded highest mycelial growth (90 mm) and the mycelial dry weight of about (321.01 mg) with good pycnidial production. This was followed by pH 6.5 (82.32 mm and 294.93 mg) and pH 7.5 (75.64 mm and 292.29mg) in the decreasing order of merit. Increase or decrease in pH less than 6.5 and higher than 7.5 were not favourable for the growth and sporulation of the pathogen (Table 4). It was concluded from the present study that the acidic pH levels were found to be inhibitory for the growth of the pathogen because at lower pH the cell membranes became saturated with the hydrogen ions which limit the passage of cations. Sridevi (2018) reported that maximum mycelial growth of *L. theobromae* with highest mycelial dry weight was obtained at pH (7.0). Similar results were also recorded by Lokeshwari (2019); Nandhini (2015); Dheepa et al. (2018) where the maximum mycelial growth, biomass production with a good sporulation of *L. theobromae* was observed at pH 7.0.

Effect of carbon sources on the mycelia growth, dry weight, growth characteristics and sporulation of L. theobromae (Lt₈)

Among the different carbon sources tested, dextrose containing media recorded the highest mycelial growth (90 mm) and the highest dry weight of (371.60 mg) and excellent pycnidial production. This was followed by sucrose (86.54 mm and 372.14 mg), glucose (79.68 mm and 340.92 mg), fructose (82.31 mm and 353.47 mg) in the decreasing order of merit. The pycnidial production was good in the media containing fructose, glucose and sucrose as carbon source (Table 5). Durgadevi (2011) and Nandhini (2015) observed that sucrose recorded the maximum mycelial growth, dry weight and sporulation of *L. theobromae*. The maximum mycelial growth and the excellent pycnidial production of *L. theobromae* was recorded on sucrose supplement media followed by glucose supplemented media (Sridevi 2018). Lokeshwari (2019) observed that dextrose served as the effective medium for the growth of the pathogen *L. theobromae* which recorded

maximum mycelial growth, dry weight and sporulation.

Effect of nitrogen sources on the mycelia growth, dry weight, growth characteristics and sporulation of L. theobromae (Lt₈)

Among the different nitrogen sources tested, peptone recorded the maximum growth of mycelium of about 88.66 mm and mycelial dry weight of about 364.76 mg. This was followed by potassium nitrate (85.49 mm and 359.71 mg), ammonium sulphate (76.81 mm and 342.38 mg), ammonium chloride (75.24 mm and 335.04 mg) in the decreasing order of merit. Excellent pycnidial production was observed in the media containing peptone as the nitrogen source. Moderate pycnidial production was observed in ammonium sulphate, ammonium chloride and potassium nitrate (Table 6). Similarly Chaudhuri et al. (2017) reported that the radial growth of *L. theobromae* was higher in case of medium containing aspartic acid and a combination of sodium nitrate and ammonium sulphate. Sridevi (2018) also reported the maximum mycelial growth and excellent pycnidial production of *L. theobromae* was recorded on ammonium di hydrogen phosphate followed by peptone and potassium nitrate. Similarly Lokeshwari (2019) reported that peptone containing medium served as the best medium and recorded the maximum mycelial growth, dry weight and the pycnidial production

Table 5. Effect of different solid media on mycelial growth and pycnidial production of *L. theobromae* (Lt₈)

S.no	Name of the medium	Mycelial growth (mm)	Pycnidial production
1	Bean juice agar	82.29 ^b	+++
2	Carrot sucrose agar	85.27 ^a	+++
3	Potato dextrose agar	90.00 ^a	++++
4	Beetroot sucrose agar	86.35 ^a	++++
5	Corn meal agar	35.83 ^e	-
6	Czapek's dox agar	33.83 ^e	-
7	Rose Bengal agar	58.13 ^d	-
8	Peas sucrose agar	63.37 ^c	++

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the

DMRT method ($p=0.05$)

- : No pycnidia production
- + : Poor pycnidia production
- ++ : Moderate pycnidia production
- +++ : Good pycnidia production
- ++++ : Excellent pycnidia production

Table 6. Effect of liquid media on mycelial dry weight and sporulation of *L. theobromae* (Lt8)

S.no	Name of the broth	Mycelial weight(mg)	Sporulation
1	Bean juice agar	174.26 ^b	+++
2	Carrot sucrose agar	179.25 ^b	+++
3	Potato dextrose agar	231.52 ^a	++++
4	Beetroot sucrose agar	224.99 ^a	++
5	Corn meal agar	133.89 ^d	-
6	Czapek's dox agar	114.02 ^e	-
7	Rose Bengal agar	131.41 ^d	-
8	Peas sucrose agar	149.79 ^c	++

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method ($p=0.05$)

- : No pycnidia production
- + : Poor pycnidia production
- ++ : Moderate pycnidia production
- +++ : Good pycnidia production
- ++++ : Excellent pycnidia production

Table 7. Effect of temperature on mycelial growth and pycnidial production of *L. theobromae* (Lt8)

Temperature	Mycelial growth (mm)	Mycelial dry weight (mg)	Pycnidial production
5	-	-	-
10	-	-	-
15	13.22 ^f	31.61 ^f	-
20	28.50 ^d	89.74 ^e	-
25	72.41 ^b	271.80 ^b	+
30	90.0 ^a	327.43 ^a	+++
35	44.52 ^c	178.14 ^c	++
40	15.25 ^e	151.52 ^d	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the

DMRT method ($p=0.05$)

- : No pycnidia production

+ : Poor pycnidia production

++ : Moderate pycnidia production

+++ : Good pycnidia production

++++ : Excellent pycnidia production

Table 8. Effect of pH on mycelial growth and pycnidial production of *L. theobromae* (Lt8)

pH	Mycelial growth (mm)	Mycelial dry weight (mg)	Pycnidial production
5.5	22.43 ^f	137.89 ^g	-
6.0	41.36 ^e	171.99 ^f	-
6.5	82.32 ^b	294.93 ^b	++
7.0	90.00 ^a	321.01 ^a	+++
7.5	75.64 ^c	292.29 ^b	++

8.0	65.80 ^d	233.97 ^c	+
8.5	40.72 ^e	211.74 ^d	-
9.0	14.00 ^g	197.82 ^e	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method ($p=0.05$

- : No pycnidia production

+ : Poor pycnidia production

++ : Moderate pycnidia production

+++ : Good pycnidia production

++++ : Excellent pycnidia production

Table 9. Effect of carbon source on mycelial growth, dry weight, growth characters and pycnidial formation of *L. theobromae* (Lt8)

S.No	Carbon source	Mycelial growth (mm)	Dry weight (mg)	Growth characters	Pycnidial formation
1	Dextrose	90.0 ^a	371.60 ^a	Colonies were initially white later black	++++
2	Fructose	82.31 ^b	353.47 ^b	Colonies were initially white later grey	+++
3	Glucose	79.68 ^c	340.92 ^c	Colonies were initially white later black	+++
4	Mannitol	48.61 ^e	250.48 ^d	Colonies were initially white later grey	++
5	Sucrose	86.54 ^a	372.14 ^a	Colonies were initially white later black	+++
6	Maltose	41.95 ^g	181.08 ^e	Colonies were initially white later grey	++

7	Lactose	53.53 ^d	255.34 ^d	Colonies were initially white later grey	+
8	control	45.36 ^f	246.26 ^d	Colonies were irregular	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method ($p=0.05$)

- : No pycnidia production

+ : Poor pycnidia production

++ : Moderate pycnidia production

+++ : Good pycnidia production

++++ : Excellent pycnidia production

Table 10. Effect of nitrogen source on mycelial growth, dry weight, growth characters and pycnidial formation of *L. theobromae* (Lt8)

S.no	Nitrogen source	Mycelial growth (mm)	Dry weight (mg)	Growth characters	Pycnidial formation
1	Sodium nitrate	47.80 ^d	263.04 ^c	Colonies were irregular	+
2	Peptone	88.66 ^a	364.76 ^a	Colonies were initially white later black	++++
3	Ammonium sulphate	76.81 ^c	342.38 ^b	Colonies were initially white later grey	++
4	Ammonium chloride	75.24 ^c	335.04 ^b	Colonies were initially white later grey	++
5	Sodium nitrite	46.74 ^{de}	263.18 ^c	Colonies were initially white later black	+
6	Potassium nitrate	85.49 ^b	359.71 ^a	Colonies were initially white later black	++

7	Control	44.10 ^e	182.31 ^d	Colonies were irregular	-
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Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method ($p=0.05$)

- : No pycnidia production
 - +
 - ++
 - +++
 - ++++
- : Poor pycnidia production
- : Moderate pycnidia production
- : Good pycnidia production
- : Excellent pycnidia production

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