

Original Research Article

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Physiological and Nutritional Requirements of *Colletotrichum capsici* [Syd. (Butler and Bisby)] Causing Anthracnose of Betelvine (*Piper betle* L.)

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ABSTRACT

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Anthracnose caused by *Colletotrichum* sp. is a major disease of betelvine under humid cultivation conditions. The present investigation was conducted to study the physiological and nutritional requirements of the pathogen and to evaluate effective management strategies under *in vitro* conditions. Among the solid media tested, Oat meal agar supported maximum mycelial growth (45.16 mm at 3 days and 79.66 mm at 6 days) with excellent sporulation, followed by Potato dextrose agar, while minimum growth and no sporulation were observed on Sabouraud's dextrose agar. Optimum fungal growth was recorded at 25°C, pH 7.5 and 95–100% relative humidity. Glucose and maltose were the most suitable carbon sources, whereas potassium nitrate and ammonium nitrate supported maximum growth among nitrogen sources.

Introduction

Betelvine (*Piper betle* L.) is an important perennial cash crop cultivated extensively in tropical and subtropical regions of India under specially constructed shade structures known as barejas. India is one of the largest producers and consumers of betel leaves and the crop plays a vital role in rural economies by providing regular income and employment opportunities to small and marginal farmers. Betel leaves are widely used for masticatory, medicinal and cultural purposes and their

trade contributes significantly to local and regional markets (Guha and Jain, 1997; Majumdar and Mandal, 2019). Owing to its high market value and year-round demand, betelvine cultivation is considered economically profitable when disease incidence is low.

However, the warm, humid and shaded microclimatic conditions required for successful betelvine cultivation also favour the development of several fungal diseases, which adversely affect leaf quality, yield and market acceptability. Among these, anthracnose caused by

Colletotrichum sp. is one of the most destructive diseases of betelvine. The disease manifests as necrotic lesions on leaves and stems, leading to blight, drying and premature leaf fall. Severe infection results in substantial yield losses and reduction in leaf quality, thereby directly affecting growers' income (Biswas, 2001; Majumdar and Mandal, 2019).

The growth, sporulation and pathogenic behaviour of *Colletotrichum* sp. are strongly influenced by physiological and nutritional factors such as culture media, temperature, pH, relative humidity and availability of carbon and nitrogen sources. Understanding these factors is essential for elucidating pathogen biology and for developing effective disease management strategies. Previous studies have shown that *Colletotrichum* spp. exhibit optimum growth under moderate temperatures, near-neutral pH and high humidity, while appropriate nutritional sources significantly influence fungal development (Chandra and Tandon, 1962; Naik *et al.*, 1988; Kumar and Rawal, 2008).

In this context, the present investigation was undertaken to study the physiological and nutritional requirements of *Colletotrichum* sp. under in vitro conditions.

Materials and Methods

Physiological and nutritional requirements of the pathogen

Effect of culture media on mycelial growth and sporulation

The effect of different solid media on mycelial growth and sporulation of the test fungus was evaluated under in vitro conditions. Seven culture media, namely Malt Extract Agar (MEA), Oat Meal Agar (OMA), Waksman's Agar, Richard's Agar, Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar (SDA) and Czapek's Dox Agar (CDA), were prepared as per standard compositions and sterilized at 121.6°C and 15 psi for 20 min.

Approximately 20 ml of molten medium was poured into 90 mm sterile Petri plates. A 5 mm mycelial disc taken from the margin of an actively growing seven-day-old culture was placed at the centre of each plate. The plates were incubated at 25 ± 2°C with three replications per

treatment. Radial mycelial growth and sporulation were recorded at 3 and 6 days after inoculation (DAI).

Effect of temperature on mycelial growth and sporulation

To determine the optimum temperature, PDA plates were inoculated with a 5 mm mycelial disc and incubated at 10, 15, 20, 25, 30, 35 and 40°C. Each treatment was replicated thrice. Radial mycelial growth was measured at 3 and 6 DAI, while sporulation was assessed by counting conidia per microscopic field.

Effect of pH on mycelial growth and sporulation

The effect of pH on fungal growth was studied by adjusting the pH of Potato Dextrose Broth to 4.0, 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 using 0.1 N NaOH or 0.1 N HCl. Two per cent agar was added and the medium was sterilized and poured into Petri plates. Plates were inoculated with a 5 mm mycelial disc and incubated at 25 ± 2°C with three replications. Linear growth and sporulation were recorded at 3 and 6 DAI.

Effect of relative humidity on mycelial growth

The effect of relative humidity (RH) on mycelial growth was studied using desiccators maintained at 40, 60, 80, 85, 90, 95 and 100 per cent RH with different concentrations of anhydrous sodium chloride solutions. Inoculated plates were incubated at 25 ± 2°C and radial growth was recorded at 3 and 6 DAI.

Effect of carbon sources on mycelial growth

Carbon source utilization was studied using Richard's medium as the basal medium. Sucrose was replaced with glucose, dextrose, fructose, sucrose, maltose, lactose and mannitol, while potassium nitrate was maintained as a constant nitrogen source. Carbon was supplied at 21.053 g per litre based on molecular weight. Medium without carbon source served as control. Plates were incubated at 25 ± 2°C and colony diameter was recorded at 3 and 6 DAI.

Effect of nitrogen sources on mycelial growth

Nitrogen source utilization was studied by replacing potassium nitrate in Richard's medium with ammonium nitrate, ammonium sulphate, ammonium chloride,

leucine, tyrosine and histidine. Sucrose was used as a fixed carbon source and nitrogen was supplied at 1.385 g per litre. Medium without nitrogen served as control. The inoculated plates were incubated at $25 \pm 2^\circ\text{C}$ and radial growth was recorded at 3 and 6 DAI.

Results and Discussion

Physiological nutritional requirements of *Colletotrichum* sp.

Effect of different solid media on mycelial growth and sporulation

The effect of seven different solid media on the mycelial growth and sporulation of *Colletotrichum* sp. was evaluated and the results are presented in Table 4.5 and Fig. 4.2. After 3 days of incubation, maximum mycelial growth was recorded on Oat meal agar (45.16 mm), followed by Potato dextrose agar (43.16 mm). Moderate growth was observed on Richard's agar (38.66 mm), Wakman's agar (36.16 mm), Czapek's agar (34.50 mm) and Malt extract agar (31.33 mm), whereas minimum growth was recorded on Sabouraud's dextrose agar (18.50 mm). After 6 days, Oat meal agar (79.66 mm) and Potato dextrose agar (76.83 mm) supported significantly higher mycelial growth compared to other media. Sabouraud's dextrose agar recorded the lowest growth (32.00 mm). Excellent sporulation was observed on Oat meal agar and Richard's agar, good sporulation on Potato dextrose agar, fair sporulation on Czapek's and Malt extract agar, poor sporulation on Wakman's agar and no sporulation on Sabouraud's dextrose agar. Similar observations were reported by Biswas (2001), Deshmukh (2012), Malabannavar (2019) and Majumdar and Mandal (2019).

Effect of temperature on mycelial growth and sporulation

The effect of temperature on mycelial growth and sporulation was studied at seven temperature levels (10–40°C) (Table 4.6, Fig. 4.3). After 3 days, maximum mycelial growth was recorded at 25°C (42.00 mm), followed by 30°C (40.83 mm) and 35°C (39.50 mm). Minimum growth was observed at 15°C (8.50 mm) and 40°C (9.00 mm). After 6 days, maximum growth was observed at 25°C (75.33 mm), followed by 30°C (73.00 mm) and 35°C (69.00 mm). Excellent sporulation occurred at 25°C and 30°C, good sporulation at 20°C,

fair sporulation at 35°C, poor sporulation at 40°C and no sporulation at 10°C and 15°C. These findings are in agreement with Kumara and Rawal (2008), Thangamani (2011), Sangeetha and Rawal (2009), Pandey (2012) and Prajapati (2020).

Effect of pH on mycelial growth and sporulation

The pathogen exhibited significant variation in growth at different pH levels (Table 4.7, Fig. 4.4). After 3 and 6 days, maximum mycelial growth was recorded at pH 7.5 (45.66 and 74.33 mm), followed by pH 7.0 and 6.5, while minimum growth was observed at pH 4.0 (8.50 and 17.16 mm).

Excellent sporulation was observed at pH 6.5 and 7.0, good sporulation at pH 7.5, fair sporulation at pH 5.0, 6.0 and 8.0 and poor sporulation at pH 4.0. These results are in conformity with the findings of Biswas (2001) and Thangamani (2011).

Effect of relative humidity on mycelial growth and sporulation

Relative humidity had a pronounced effect on mycelial growth and sporulation (Table 4.8, Fig. 4.5). Maximum growth was recorded at 100% relative humidity (75.16 mm at 3 days and 88.33 mm at 6 days), followed by 95% and 90% RH. Minimum growth was observed at 40% RH (10.33 and 21.33 mm).

Excellent sporulation occurred at 95–100% RH, good sporulation at 90–85% RH, fair sporulation at 80–60% RH and poor sporulation at 40% RH. These findings corroborate the reports of Chung and Lee (1986) and Mishra and Gupta (1994).

Nutritional requirements of *Colletotrichum* sp.

Effect of carbon sources on mycelial growth and sporulation

Among the carbon sources tested, glucose supported maximum mycelial growth (41.00 and 81.00 mm) with excellent sporulation, followed by maltose and dextrose. Poor growth was recorded with fructose and lactose, while no growth occurred in the control. These results are in agreement with earlier reports by Chandra and Tandon (1962), Prasad (1965), Naik *et al.*, (1988), Kumara and Rawal (2008) and Kushawaha (2015).

Table.1 Effect of different physiological requirements on the mycelial growth and sporulation of *C. capsici*

| Parameter | Treatment | Mycelial growth (mm)* After 3 days | Mycelial growth (mm)* After 6 days | Sporulation |
|-----------------------|---------------------------|------------------------------------|------------------------------------|-------------|
| Culture media | Malt Extract Agar | 31.33 | 57.50 | ++ |
| | Oat Meal Agar | 45.16 | 79.66 | ++++ |
| | Wakman's Agar | 36.16 | 51.16 | + |
| | Richard's Agar | 38.66 | 66.16 | ++++ |
| | Potato Dextrose Agar | 43.16 | 76.83 | +++ |
| | Sabouraud's Dextrose Agar | 18.50 | 32.00 | - |
| | Czapek's Agar | 34.50 | 64.00 | ++ |
| | CD at 5% | 2.538 | 2.862 | |
| | SE(m) ± | 0.829 | 0.934 | |
| Temperature (°C) | 10 | 0.00 | 0.00 | - |
| | 15 | 8.50 | 13.00 | - |
| | 20 | 34.83 | 62.83 | +++ |
| | 25 | 42.00 | 75.33 | ++++ |
| | 30 | 40.83 | 73.00 | ++++ |
| | 35 | 39.50 | 69.00 | ++ |
| | 40 | 9.00 | 17.33 | + |
| | CD at 5% | 0.642 | 0.345 | |
| | SE(m) ± | 1.967 | 1.057 | |
| pH levels | 4.0 | 8.50 | 17.16 | + |
| | 5.0 | 12.50 | 23.16 | ++ |
| | 6.0 | 26.16 | 47.16 | ++ |
| | 6.5 | 42.16 | 69.83 | ++++ |
| | 7.0 | 42.31 | 72.00 | ++++ |
| | 7.5 | 45.66 | 74.33 | +++ |
| | 8.0 | 40.00 | 67.83 | ++ |
| | CD at 5% | 0.199 | 0.267 | |
| | SE(m) ± | 0.610 | 0.819 | |
| Relative Humidity (%) | 40% | 10.33 | 21.33 | + |
| | 60% | 22.33 | 34.33 | ++ |
| | 80% | 36.66 | 47.50 | ++ |
| | 85% | 46.66 | 57.66 | +++ |
| | 90% | 55.83 | 68.16 | +++ |
| | 95% | 65.33 | 79.66 | ++++ |
| | 100% | 75.16 | 88.33 | ++++ |
| | CD at 5% | 0.696 | 0.819 | |
| | SE(m) ± | 0.227 | 0.267 | |

* Mean of three replications

(Sporulation = +++++ - Excellent, +++ - Good, ++ - Fair, + - Poor and – No sporulation)

Table.2 Effect of different nutritional requirements on the mycelial growth and sporulation of *C. capsici*

| Parameter | Treatment | Mycelial growth (mm)* After 3 days | Mycelial growth (mm)* After 6 days | Sporulation |
|------------------|-------------------|------------------------------------|------------------------------------|-------------|
| Carbon sources | Glucose | 41.00 | 81.00 | ++++ |
| | Dextrose | 36.33 | 75.66 | ++++ |
| | Fructose | 24.50 | 63.50 | ++ |
| | Sucrose | 37.83 | 67.16 | +++ |
| | Maltose | 39.66 | 79.00 | ++++ |
| | Lactose | 30.33 | 56.00 | + |
| | Mannitol | 33.00 | 71.33 | +++ |
| | Control | 0.00 | 0.00 | - |
| | CD at 5% | 0.94 | 0.97 | |
| | SE(m) ± | 0.31 | 0.32 | |
| Nitrogen sources | Ammonium nitrate | 37.66 | 63.50 | ++++ |
| | Potassium nitrate | 38.17 | 67.16 | ++++ |
| | Ammonium sulphate | 0.00 | 0.00 | - |
| | Ammonium chloride | 19.83 | 43.50 | + |
| | Leucine | 27.50 | 51.00 | ++ |
| | Tyrosine | 24.00 | 66.00 | + |
| | Histidine | 35.83 | 63.16 | +++ |
| | Control | 0.00 | 0.00 | - |
| | CD at 5% | 0.94 | 0.81 | |
| | SE(m) ± | 0.31 | 0.27 | |

* Mean of three replications

(Sporulation = +++++ - Excellent, +++ - Good, ++ - Fair, + - Poor and - No sporulation)

Plate.1 Mycelial growth of *C. capsici* on different solid media

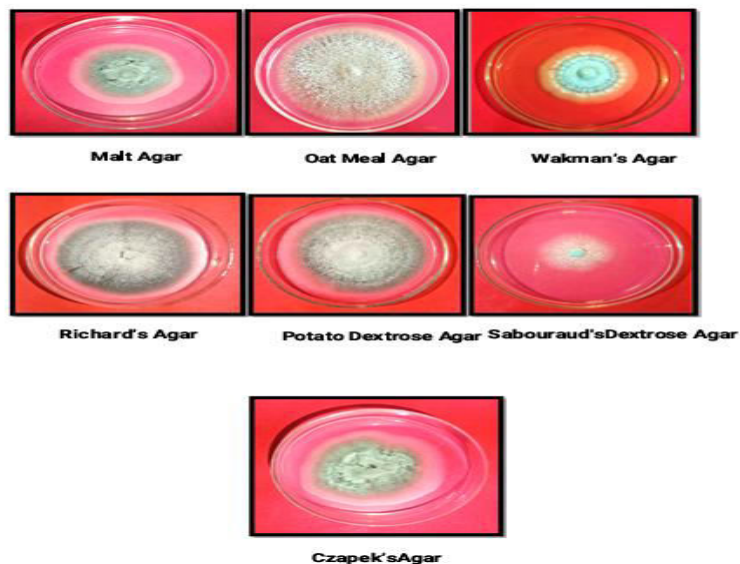


Plate.2 Mycelial growth of *C. capsici* on different temperatures

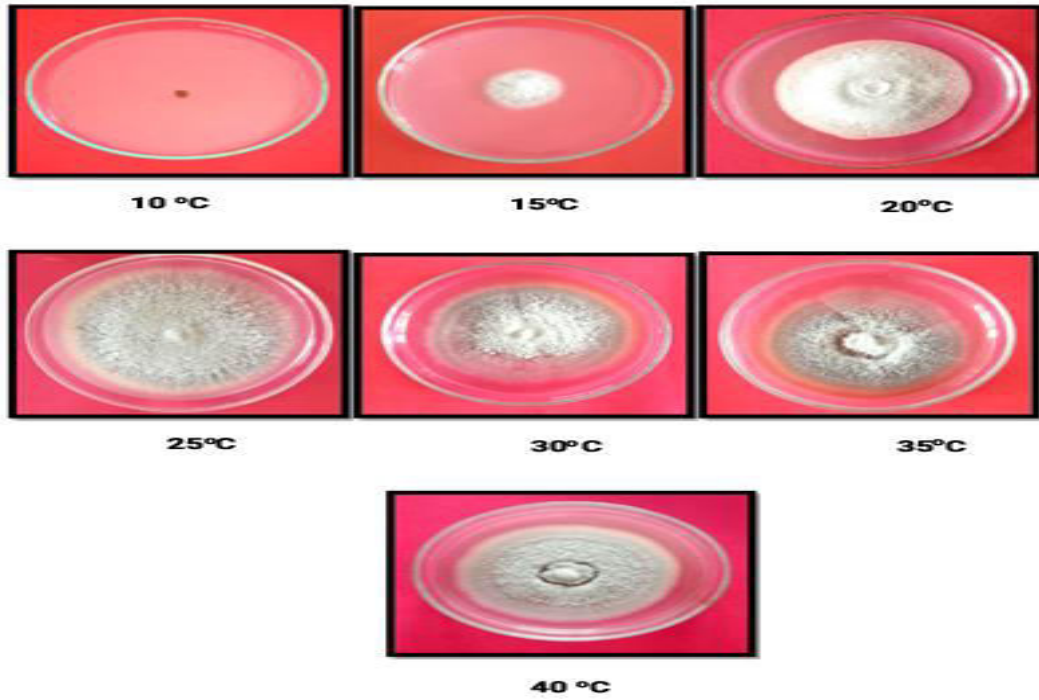


Plate.3 Mycelial growth of *C. capsici* on different pH

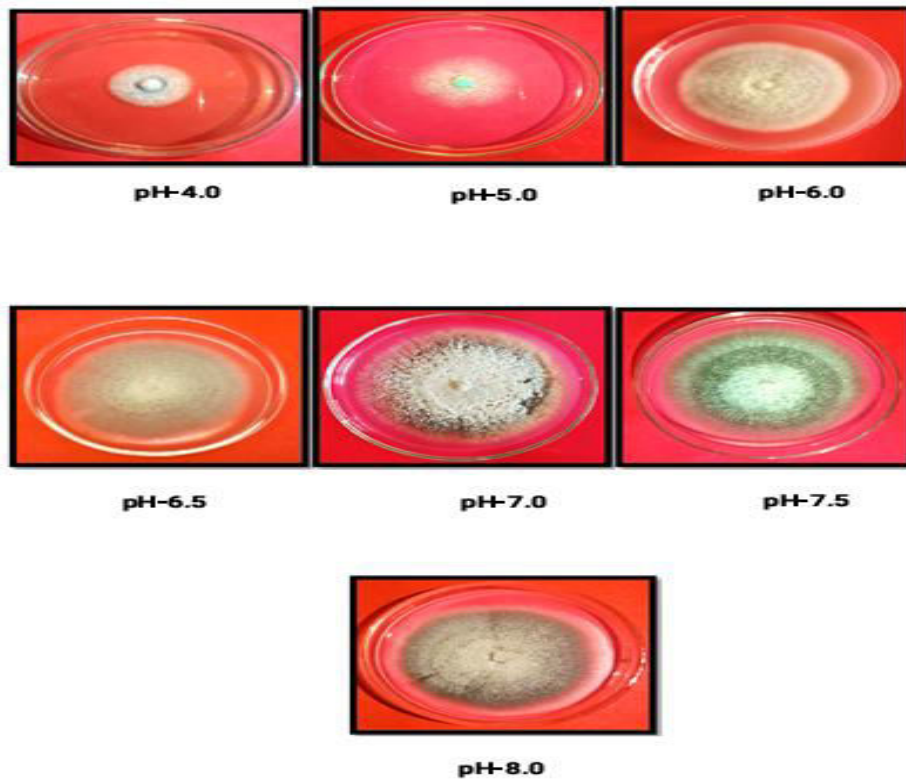


Plate.4 Mycelial growth of *C. capsici* on different pH

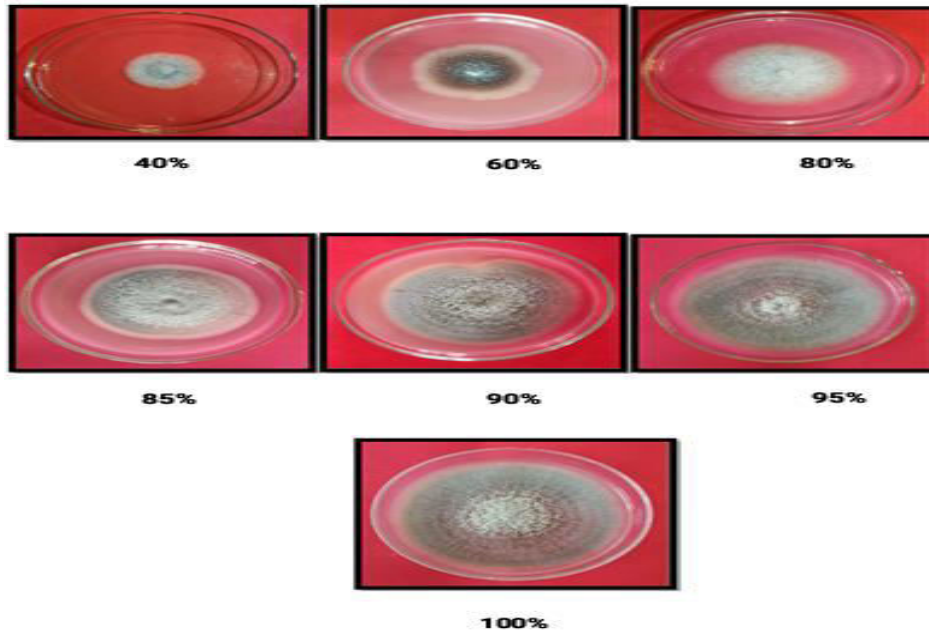


Plate.5 Mycelial growth of *C. capsici* on different carbon sources

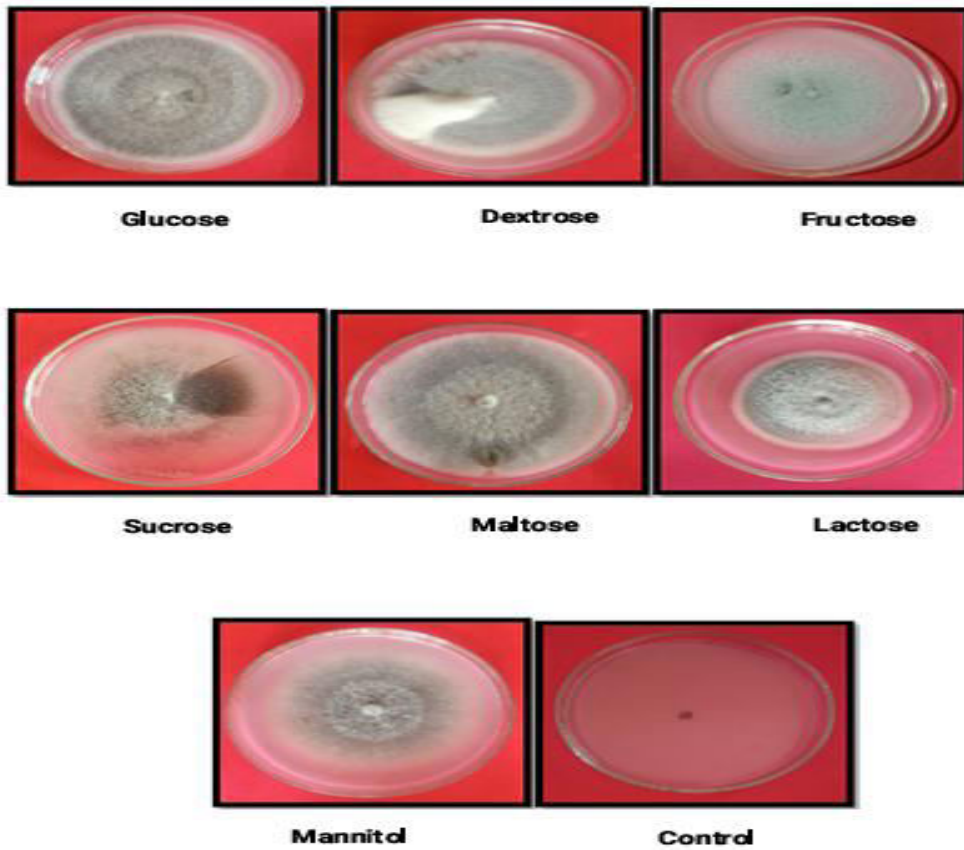


Plate.6 Mycelial growth of *C. capsici* on different nitrogen sources

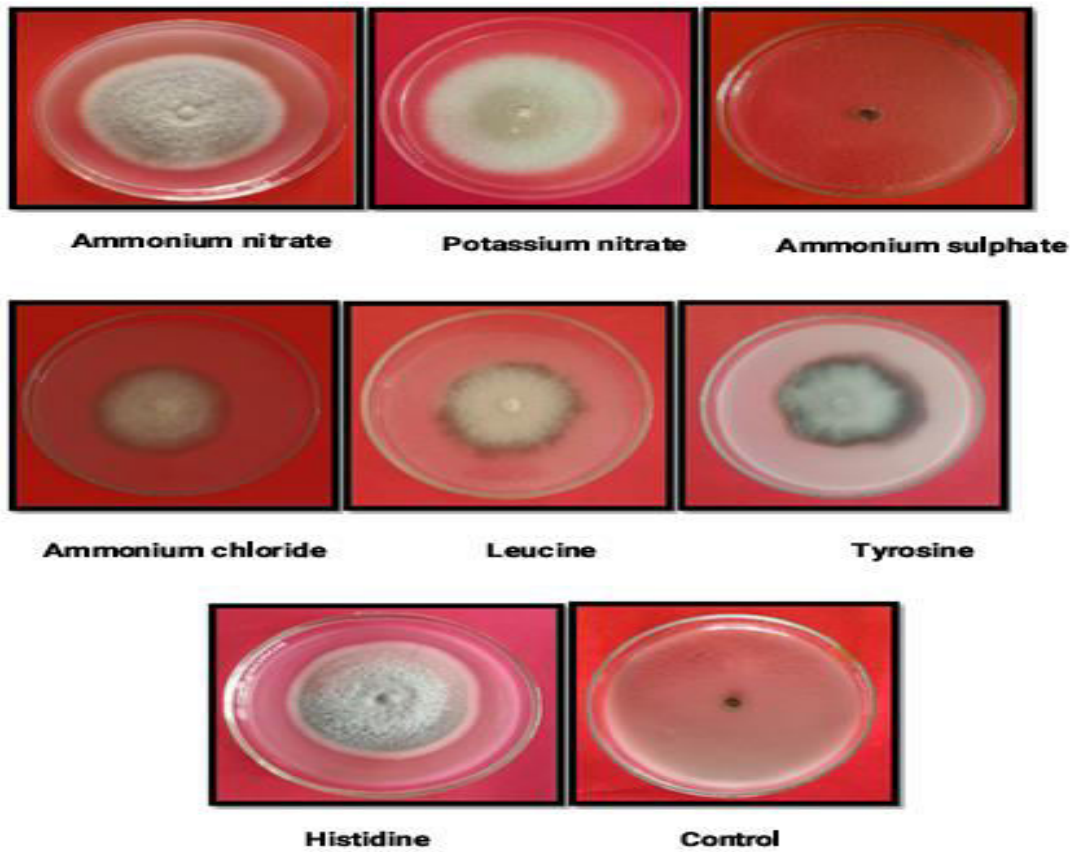


Fig.1 Effect of different media on the mycelial growth (mm) of *C. capsici*

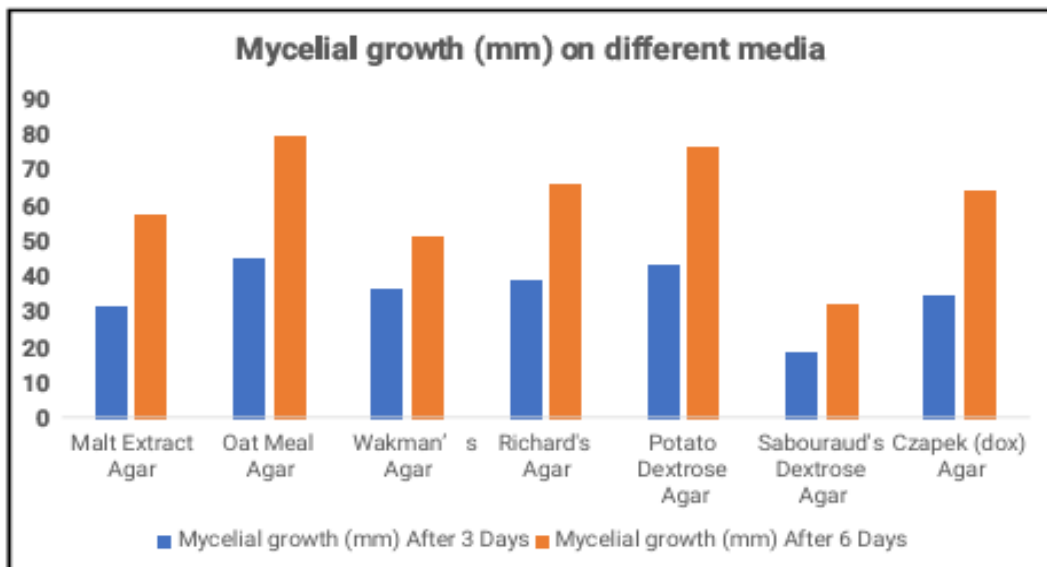


Fig.2 Effect of different temperatures on the mycelial growth (mm) of *C. capsici*

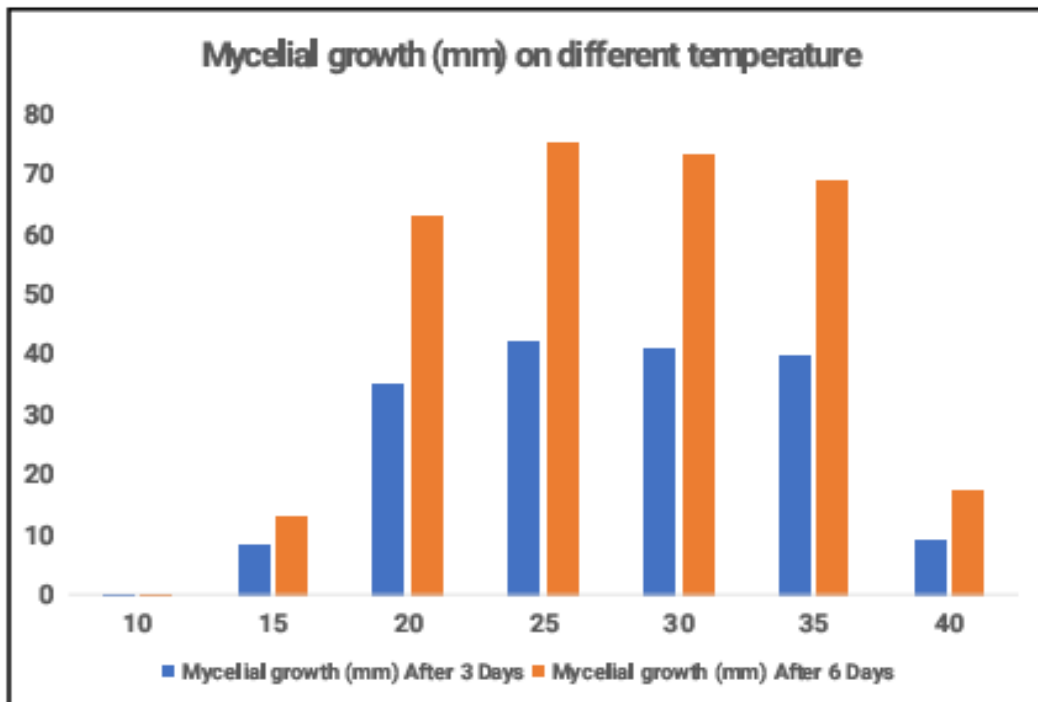


Fig.3 Effect of different pH on the mycelial growth (mm) of *C. capsici*

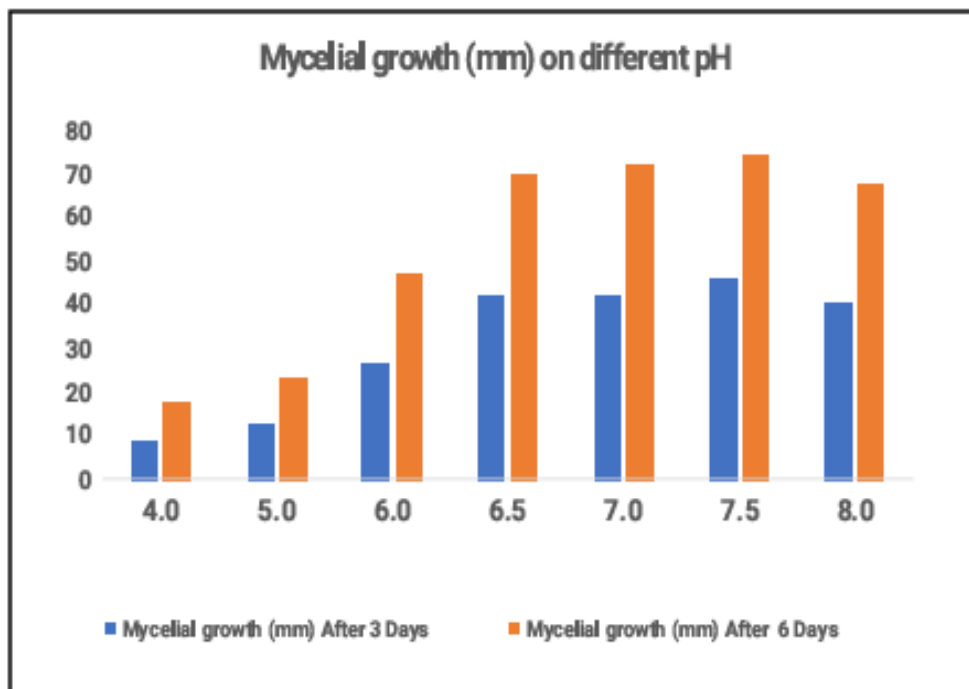


Fig.4 Effect of different RH on the mycelial growth (mm) of *C. capsici*

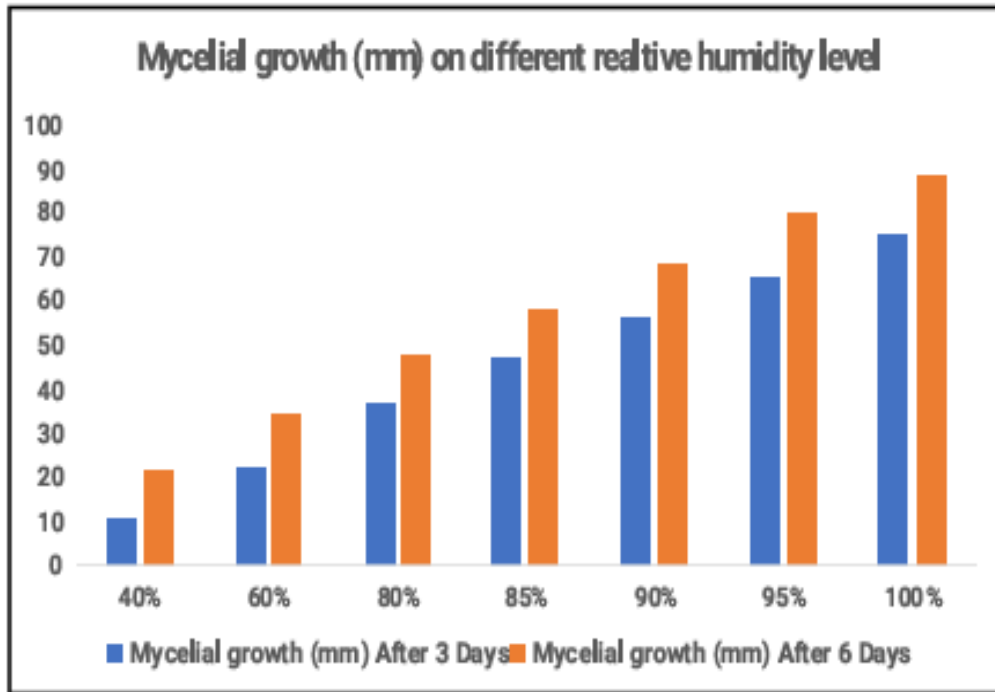


Fig.5 Effect of different carbon sources on the mycelial growth (mm) of *C. capsici*

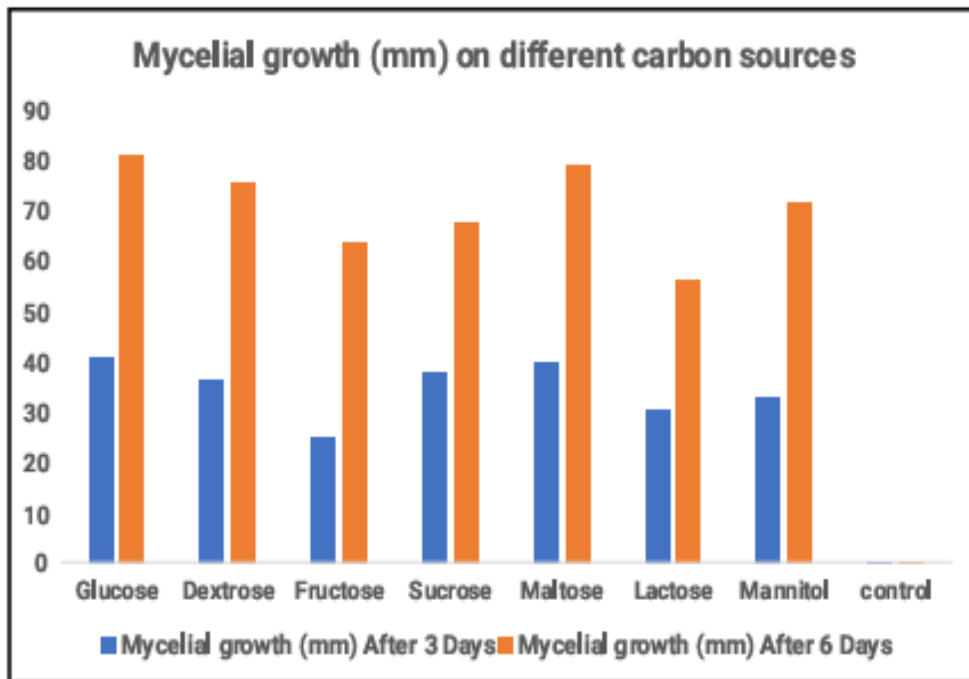
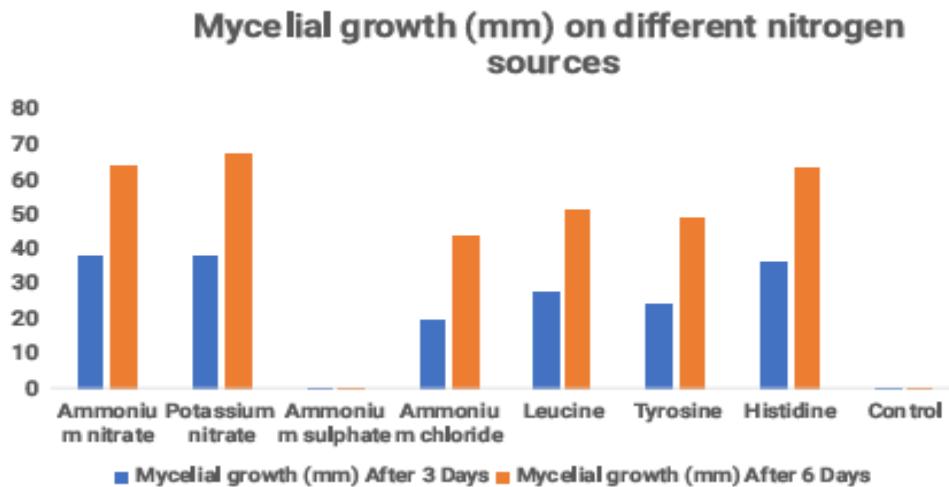


Fig.6 Effect of different nitrogen sources on the mycelial growth (mm) of *C. capsici*



Effect of nitrogen sources on mycelial growth and sporulation

Among nitrogen sources, potassium nitrate supported maximum mycelial growth (38.17 and 67.16 mm), followed by ammonium nitrate and histidine. Poor growth was observed with ammonium chloride and tyrosine, while no growth occurred with ammonium sulphate and control. Excellent sporulation was recorded on potassium nitrate and ammonium nitrate. These findings are supported by Ekbote (1994) and Kumara and Rawal (2008).

In conclusion, the study revealed that *C. capsici* requires moderate temperature (25–30°C), slightly acidic pH (6.5) and high relative humidity (90–100%) for optimum growth and sporulation. Sucrose and potassium nitrate were the most suitable carbon and nitrogen sources, respectively. Integration of physiological knowledge can play a vital role in controlling anthracnose of betelvine.

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Author Contributions

Pinki: Investigation, formal analysis, writing—original

draft. Susma Nema: Validation, methodology, writing—reviewing. Ravi Regar:—Formal analysis, writing—review and editing.

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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