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RESPONSE OF VARIOUS CULTURE MEDIA AND ABIOTIC FACTORS ON GROWTH OF *Alternaria alternata* CAUSING BROWN LEAF SPOT OF POTATO

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ABSTRACT

Alternaria alternata is an emerging pathogen causing brown leaf spot disease in potato. However, information on its nutritional and environmental requirements for growth and sporulation are minimal, requiring the determination of appropriate culture media and abiotic factors for its growth and maintenance under *in vitro* conditions. The pathogen was isolated from infected potato leaves and identified based on morphological and cultural characteristics and confirmed through ITS sequencing (GenBank accession PX062184). Among the tested culture media, potato dextrose agar exhibited maximum colony growth (90.00 mm), highest growth rate (11.25 mm/day) and excellent sporulation. Maximum colony growth and growth rate were noted at temperature 30°C (85.46 mm; 12.21 mm/day) and pH 6.5 (60.33 mm; 8.62 mm/day) along with excellent sporulation. The results showed that potato dextrose agar, pH 6.5 and temperature 30°C were favorable for growth and sporulation of *A. alternata*, offering appropriate conditions for maintaining its culture and conducting additional biological and disease management research.

***Key words*:** Potato, *A. alternata*, temperature, culture media, pH

Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop cultivated across diverse agro-climatic conditions, however, its productivity constrained by several biotic stresses including diseases caused *Alternaria* spp., (Secor and Gudmestad, 1999). Traditionally, *A. solani* has been recognized as major pathogen infecting potatoes, however, several other *Alternaria* spp. are also associated with potato affecting foliage (Woudenberg *et al*., 2015; Choi *et al*., 2023). In particular, *A. alternata* has been identified as the cause of potato brown leaf spot, underscoring the growing significance of other *Alternaria* spp., in potato disease complexes (Jaffer *et al*., 2025).

The growth and development of *A. alternata* are governed by nutritional and environmental factors, which influence its establishment and development under *in vitro* conditions (Dhingra and Sinclair, 1995; Domsch *et al*., 2007). Variation in the nutritional makeup of culture media can affect mycelial development, colony characteristics and sporulation (Dhingra and Sinclair, 1995; Domsch *et al*., 2007). Similarly, pH and temperature are significant abiotic variables that influence the growth and development of fungi (Dhingra and Sinclair, 1995; Domsch *et al*., 2007). In order to establish consistent cultures and enable additional research on the biology, virulence and management of the pathogen, it is crucial to determine appropriate nutritional and environmental conditions.

In the view of the emerging occurrence *A. alternata* in potato and need for its characterization and additional biological research, it is crucial to comprehend its growing requirements (Choi *et al*., 2023; Jaffer *et al*., 2025). Therefore, the present study was conducted in order to assess the impact of different culture media and specific abiotic variables on the growth of *A. alternata* and to identify favorable circumstances for its optimal growth and maintenance.

Materials and Methods

Isolation and identification of *A. alternata* causing potato brown leaf spot disease

During the *Rabi* season of 2024, potato leaves exhibiting typical symptoms of brown leaf spot were collected, washed with running tap and blotted dry. Small fragments of infected leaves were excised from the edge of lesions, surface sterilized for one to two minutes with 1% sodium hypochlorite, completely rinsed with sterile distilled water and then blotted dry in an aseptic environment. After being sterilized, the tissue fragments were cultured for seven days and incubated at 25 ± 1°C in the dark on water agar supplemented with 100 µg mL^{-1} streptomycin. Emerging fungal colonies were purified using single spore isolation technique (Hansen, 1926). After purification, the isolate was maintained on potato dextrose agar (PDA) and utilized for further studies. In accordance with traditional taxonomic classifications, the pathogen was first identified using morphological traits, such as colony features and microscopic analysis of conidia and conidiophores (Ellis, 1971; Simmons, 2007). Furthermore, the pathogen's identity was then verified through molecular characterisation.

Response of various parameters on growth characteristics of *A. alternata*

Response of culture media:

The growth characteristics of *A. alternata* were assessed on eight different culture media (solid) to identify the most suitable media for mycelial development and sporulation. The media accessed in present investigation include beet root agar, carrot potato agar, cauliflower agar, rye agar, mustard leaf agar, v8 juice agar, oat meal agar and potato dextrose agar (Table 1). Each media was prepared using double distilled water, enriched with antibiotics to prevent bacterial contamination and sterilized in autoclave at 121.6 °C (15 psi) for 15 min. After sterilization, each media was cooled down to 45 °C, which was then poured in 90 mm sterile Petri plates. The center of each plate was inoculated with 5 mm mycelial disc of seven day old culture of *A. alternata* which was then incubated at 25 ± 1 °C. Each treatment was replicated thrice. After seven days of incubation, colony characteristics and sporulation were recorded using hemocytometer.

Response of pH:

The growth characteristics of *A. alternata* were evaluated at different pH levels using potato dextrose agar (PDA) media under *in vitro* conditions. The pH of medium was adjusted from 5.0 to 8.5 at an interval of 0.5 unit using 0.1 N HCl or 0.1 N NaOH using digital pH meter prior to sterilization under autoclave. After sterilization, media adjusted to different pH levels were cooled down to 45 °C, which was then poured in 90 mm sterile Petri plates. The center of each plate was inoculated with 5 mm mycelial disc of seven day old culture of *A. alternata* which was then incubated at 25 ± 1 °C. Each treatment was replicated thrice. After seven days of incubation, colony characteristics and sporulation were recorded using hemocytometer.

Response of temperature:

The growth characteristics of *A. alternata* were evaluated on different temperature range using potato dextrose agar (PDA) media under *in vitro* conditions. Sterilized media was poured in 90 mm sterile Petri plates and inoculated with 5 mm mycelial disc of seven day old culture of *A. alternata* at center. The plates were then incubated at different temperature range (15, 20, 25, 30, 35 and 40 °C) in B.O.D. incubator. Each treatment was replicated thrice. After seven days of incubation, colony characteristics and sporulation were recorded using hemocytometer.

Results and Discussion

Isolation and identification of *A. alternata* causing potato brown leaf spot disease

The fungus isolated from symptomatic potato leaves

Table 1: Composition of different growth media.

S. No.	Growth media	Composition
1	Beet root agar	200 g beet root, 20 g agar, 20 g dextrose and 1000 ml distilled water
2	Carrot potato agar	100 g carrot, 100 g potato, 20 g agar, 20 g dextrose and 1000 ml distilled water
3	Cauliflower agar	200 g cauliflower, 20 g agar, 20 g dextrose and 1000 ml distilled water
4	Rye agar	60 g rye, 20 g sucrose, 15 g agar and 1000 ml distilled water
5	Mustard leaf agar	200 g mustard leaf, 20 g agar, 20 g dextrose and 1000 ml distilled water
6	V8 juice agar	250 g carrots, 500 g tomato, 500 g bunch of spinach, 5 g white onion, 10 g beet root, 2 g garlic cloves, 50 g leaves of Chinese cabbage, 50 g radish leaves, 20 g dextrose, 20 g agar and 1000 ml distilled water
7	Oat meal agar	30 g rolled oats, 15 g agar and 1000 ml distilled water
8	Potato dextrose agar	200 g peeled potato, 20 g agar, 20 g dextrose and 1000 ml distilled water.

Table 2: Response of different culture media on growth characteristics of *A. alternata*.

Culture media	Colony diameter (mm)	Growth rate (mm/day)	Colony colour	Colony margin	Sporulation	Zonation	Elevation
Beet root agar	85.53	10.69	Greyish white	Entire	+++	Present	Raised
Carrot potato agar	86.63	10.83	Greyish white	Entire	+++	Present	Flat
Cauliflower agar	75.50	9.44	Grey	Entire	++	Present	Raised
Rye agar	87.50	10.94	White	Entire	+++	Absent	Flat
Mustard leaf agar	75.90	9.49	Greenish grey	Irregular	++	Present	Sub raised
V8 juice agar	80.11	10.01	Grey	Entire	++	Present	Sub raised
Oat meal agar	61.70	7.71	Greyish white	Entire	+	Present	Flat
Potato dextrose agar	90.00	11.25	Greenish grey	Entire	+++	Present	Flat
C.D. at 5%	4.606	-	-	-	-	-	-
SE(d)	2.154	-	-	-	-	-	-
C.V.	3.268	-	-	-	-	-	-

(-) No sporulation, (+) Poor sporulation, (++) Moderate sporulation, (+++) Excellent sporulation

exhibiting brown leaf spot symptoms exhibited rapid and profuse mycelial growth producing gray to greyish-olivaceous colonies, with a darker center and dense, velvety to powdery aerial growth. The margin of colonies were irregular to slightly uneven and exhibited faint concentric variability in pigmentation. Microscopic analysis revealed solitary, obclavate to pyriform, light brown to olivaceous conidia with tapering apical ends (beaks). Conidia were multicellular, having 2-3 transvers septa. The conidia gradually narrowed towards the beak after becoming wider toward the basal portion. The cultural and morphological characteristics of the isolate were similar to those of *A. alternata*, Similar cultural and morphological characteristics have been reported for *A. alternata* causing brown leaf spot of potato (Choi *et al.*, 2023; Jaffer *et al.*, 2025). Furthermore, ITS sequence analysis confirmed the identity of the isolate as *A. alternata* and the sequence was submitted to GenBank with accession number PX062184.

Response of culture media on growth characteristics of *A. alternata*

The growth characteristics of *A. alternata* on different culture media varied significantly (Table 2) (Fig. 1) (Plate 1). Among the tested media, potato dextrose agar exhibited the maximum colony growth (90.00 mm) with the highest growth rate (11.25 mm/day), followed

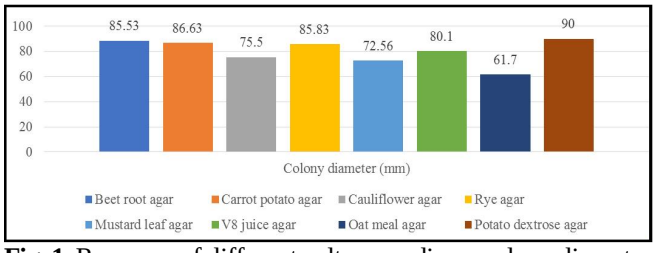


Fig. 1: Response of different culture media on colony diameter of *A. alternata.*

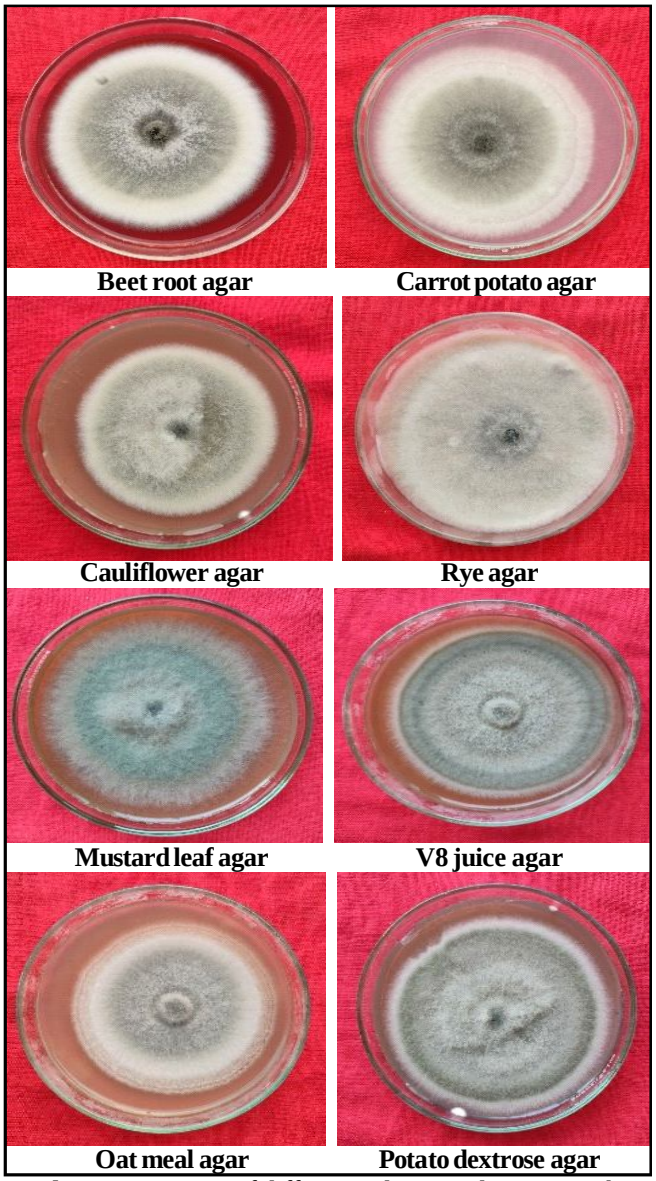


Plate 1: Response of different culture media on growth characteristics of *A. alternata*

Table 3: Response of different pH on growth characteristics of *A. alternata.*

pH	Colony diameter (mm)	Growth rate (mm/day)	Colony colour	Colony margin	Sporulation	Zonation	Elevation
5	46.08	6.58	White	Entire	-	Absent	Flat
5.5	50.40	7.20	White	Entire	+	Present	Sub raised
6	58.80	8.40	White with grey ting	Entire	+++	Present	Raised
6.5	60.33	8.62	Whitish grey	Entire	+++	Present	Raised
7	54.80	7.83	Grey	Irregular	++	Absent	Flat
7.5	52.64	7.52	Grey	Irregular	++	Absent	Flat
8	51.61	7.37	Grey	Irregular	+	Absent	Flat
8.5	41.18	5.88	Greyish black	Irregular	-	Absent	Flat
C.D. at 5%	1.212	-	-	-	-	-	-
SE(d)	0.401	-	-	-	-	-	-
C.V.	1.335	-	-	-	-	-	-
(-) No sporulation, (+) Poor sporulation, (++) Moderate sporulation, (+++) Excellent sporulation							

by rye agar (87.50 mm; 10.94 mm/day) and carrot potato agar (86.63 mm; 10.83 mm/day). The lowest colony diameter and growth rate were recorded on oat meal agar (61.70 mm; 7.71 mm/day). The color of the colonies varied from white to greenish gray, gray and grayish white. Most media produced colonies with an entire margin, while mustard leaf agar exhibited an irregular margin.

Abundant sporulation (+++) was observed on potato dextrose agar, rye agar, carrot potato agar and beet root agar, whereas, moderate sporulation (++) was observed on cauliflower agar, mustard leaf agar and V8 juice agar, while poor sporulation (+) was observed on oat meal agar. Zonation was evident on all media except rye agar. Colony elevation ranged from flat to elevated, with sub-raised colonies observed on mustard leaf and V8 juice agar. The present investigation aligns with previous findings exhibiting notable differences in growth, colony characteristics and sporulation of *A. alternata* on various culture media. Singh *et al.*, (2001) and Saha *et al.*, (2015) reported significant differences in growth and sporulation among media, while Wei *et al.*, (1985) determined that potato dextrose agar is an appropriate medium for sporulation. Additionally, differences in colony color, growth pattern and zonation between different medium were documented by Solanki *et al.*, (2023), while, Tele *et al.*, (2021) observed variations in sporulation, mycelial growth and colony color. The favorable growth and sporulation reported on carrot potato agar in the present investigation were similar to the findings of Balakrishnan *et al.*, (2020).

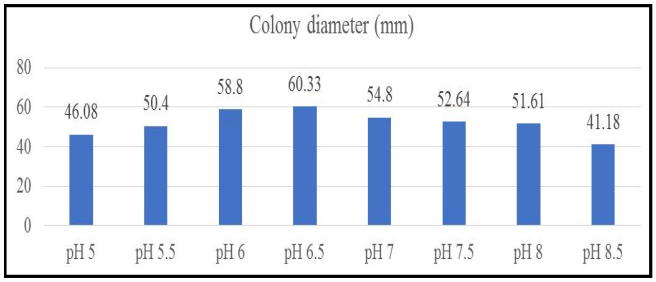


Fig. 2: Response of different pH on growth characteristics of *A. alternata.*

Response of pH on growth characteristics of *A. alternata*

The growth characteristics of *A. alternata* on different pH varied significantly (Table 3) (Fig. 2) (Plate 2). Among the tested pH, maximum colony growth and highest growth rate were observed at pH 6.5 (60.33 mm; 8.62 mm/day) followed by pH 6.0 (58.80 mm; 8.40 mm/day) and pH 7.0 (54.80 mm; 7.83 mm/day). Whereas, at pH 8.5, lowest colony diameter and growth rate was observed (41.18 mm; 5.88 mm/day). Overall, colony growth increases as pH increases from 5.0 to 6.5 and decreased as pH increased further. The colors of the colonies varied from white to grayish black, white with a hint of gray and whitish gray.

At pH 5.0-6.5, colonies exhibited an entire margin,

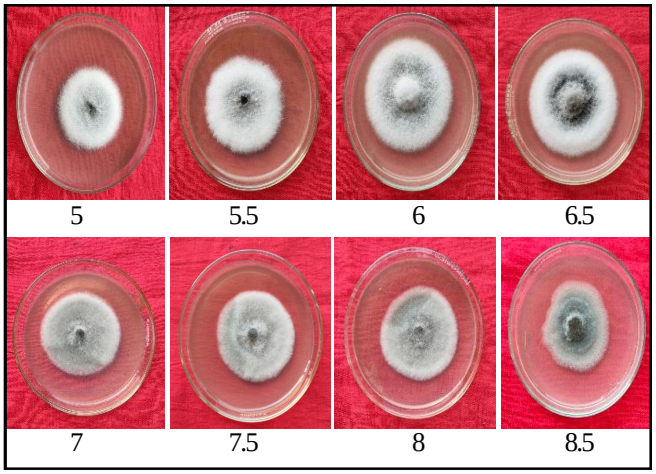


Plate 2: Response of different pH on growth characteristics of *A. alternata*

Table 4: Response of different temperature on growth characteristics of *A. alternata*.

Temperature °C	Colony diameter (mm)	Growth rate (mm/day)	Colony colour	Colony margin	Sporulation	Zonation	Elevation
15	47.83	6.83	Whitish grey	Entire	-	Present	Raised
20	56.66	8.09	Whitish grey	Entire	+	Absent	Raised
25	76.56	10.94	Grey	Irregular	++	Absent	Flat
30	85.46	12.21	Grey	Entire	+++	Present	Flat
35	51.26	7.32	Grey	Entire	+	Present	Sub raised
40	39.21	5.60	Grey to light black	Irregular	-	Absent	Flat
C.D. at 5%	1.096	-	-	-	-	-	-
SE(d)	0.352	-	-	-	-	-	-
C.V.	1.024	-	-	-	-	-	-
(-) No sporulation, (+) Poor sporulation, (++) Moderate sporulation, (+++) Excellent sporulation							

whereas, at pH 7.0-8.5, an irregular margin was observed. At pH 5.0 and 8.5, there was no sporulation, while at pH 5.5 and 8.0, there was poor sporulation (+). At pH 7.0 and 7.5, moderate sporulation (++) was observed, while extensive sporulation (+++) was observed at pH 6.0 and 6.5. Zonation was observed only at pH 5.5, 6.0 and 6.5 respectively. Colony elevation ranged from flat to raised, with sub raised colonies at pH 5.5. The present investigation revealed significant variation in the colony growth and sporulation of *A. alternata* across different pH levels. The maximum colony growth and excellent sporulation at pH 6.5 are consistent with the findings of Maheshwari *et al*., (2000), Singh *et al*., (2001), Kantwa *et al*., (2015) and Fagodiya *et al*., (2022), who reported that favorable growth and sporulation of *A. alternata* was observed at pH 6.5.

Response of temperature on growth characteristics of *A. alternata*

The growth characteristics of *A. alternata* on different temperature varied significantly (Table 4) (Fig. 3) (Plate 3). Among the tested temperature range, maximum colony growth and highest growth rate were observed at 30°C (85.46 mm; 12.21 mm/day), followed by 25°C (76.56 mm; 10.94 mm/day) and 20°C (56.66 mm; 8.09 mm/day). Whereas, the colony diameter and growth rate were lowest at 40°C (39.21 mm; 5.60 mm/day). Overall, colony growth increases as the temperature increases from 15°C to 30°C and then decreases due to further increase in temperature. The colors of the colonies varied from whitish grey to grey and grey to light black. At 15, 20, 30, and 35°C, colonies exhibited an entire margin, whereas, at 25 and 40°C, an uneven margin was observed.

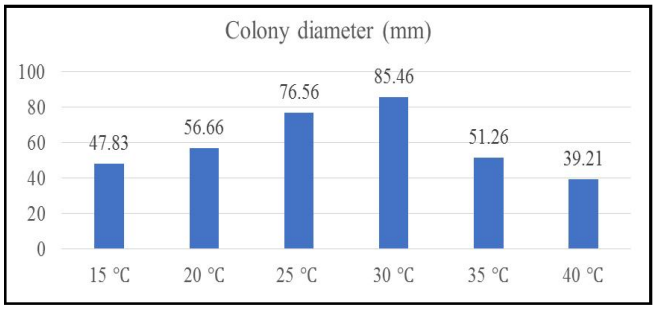


Fig. 3: Response of different temperature on growth characteristics of *A. alternata*.

At 15 and 40°C, there was no sporulation, while at 20 and 35°C poor sporulation (+) was observed. Moderate sporulation (++) was recorded at 25°C, whereas abundant sporulation (+++) was observed at 30°C. Zonation was present at 15, 30 and 35°C but absent at 20, 25 and 40°C. Colony elevation ranged from flat to raised, with sub raised colonies at 35°C. The present investigation aligns with previous findings exhibiting notable differences in growth, colony characteristics and sporulation of *A. alternata* across various temperature range. At 30°C, the maximum colony growth and excellent sporulation are consistent with the findings of Hubballi *et al*., (2010) and Bessadat *et al*., (2014) who reported that ideal temperature for growth and sporulation of *A. alternata* lies between 25 and 30°C.

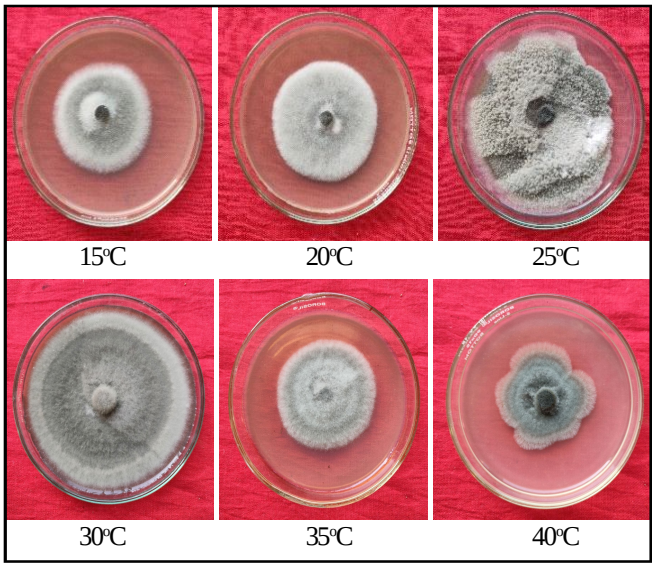


Plate 3: Response of different temperature on growth characteristics of *A. alternata*.

Conclusion

The study confirmed that the growth and sporulation of *A. alternata*, causing potato brown leaf spot varied significantly in response to different culture media and abiotic factors. Among the tested culture media, potato dextrose agar (PDA) exhibited the maximum colony growth and growth rate, along with excellent sporulation. The fungus exhibited optimal growth and sporulation at pH 6.5 and temperature 30°C, suggesting that moderate temperatures and slightly acidic conditions are ideal for its growth. These findings provide appropriate nutritional and environmental conditions for *in vitro* maintenance of *A. alternata* as well as future research focusing on biology and management.

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