J. Hortic. Sci.
Vol. 21(1), 2026

https://doi.org/10.24154/jhs.v21i1.3790

Original Research Paper

Characterization of *Alternaria polianthi* causing leaf blight disease in tuberose and standardization of its growth parameters

Poojitha S.R.[1], Bharathi T.U.[1], Nandeesha P.[2], Sonavane P.[3*], Azeez S.[2], Kumar R.[1], Venugopalan R.[4], Dhananjaya M.V.[5] and Tejaswini P.[1]

[1]Division of Flower and Medicinal Crops, [2]Division of Basic Sciences, [3]Division of Crop Protection, [4]Division of Social Sciences, [5]Division of Vegetable Crops
ICAR-Indian Institute of Horticultural Research, Bengaluru - 560 089, India
[*]Corresponding author Email: priti9883@gmail.com

ABSTRACT

Tuberose is an ornamental crop cultivated for its long-lasting spikes and alluring scent. The incidence of leaf blight disease caused by *Alternaria polianthi* is the most widespread and severe, resulting in reduced growth and significant yield losses. In the present study, a pure culture was isolated from the infected leaves of tuberose and based on typical symptoms on the foliage, microscopic observation and cultural traits of the fungus, it was identified as *Alternaria polianthi,* which was further confirmed by molecular characterization showing 100% similarity with *Alternaria* sp. infecting only tuberose host. Ten different media were tested to analyze the growth parameters, among them the mean colony diameter was found highest on corn meal agar (9 cm), potato dextrose agar (8.80 cm), potato carrot agar (8.27 cm) and other colony parameters were greatly influenced by the type of the growth media. Excellent sporulation was observed on corn meal agar, potato dextrose agar and water agar. This is the first kind of study on molecular characterization of *Alternaria polianthi* on tuberose in India.

Keywords: *Alternaria polianthi*, characterization, media, leaf blight, tuberose

INTRODUCTION

Tuberose (*Agave amica* (Medik.) Thiede & Govaerts) is one of the most important commercial flower crops in India, valued for its fragrant white flowers and cultivated for loose flowers, cut flowers and essential oil extraction (Bharathi et al., 2023). Tuberose is commercially cultivated in several countries worldwide, including India, Hawaii, China, Brazil, Italy, Iran, the UK and the USA. In India, major tuberose-growing states include West Bengal, Karnataka, Maharashtra, Tamil Nadu, Haryana, Punjab, Gujarat, Rajasthan, Andhra Pradesh and Assam, covering approximately 389,000 ha with an annual production of 3,046,000 MT of loose flowers and 971,000 MT of cut flowers (Anon., 2024-25). However, the cultivation of tuberose is significantly hindered by several fungal diseases, of which leaf spot caused by *Alternaria polianthi* is a major and economically important disease. It was first reported from Coimbatore, Tamil Nadu, India (Mariappan et al., 1977). The leaf symptoms appeared as small, circular to irregular spots of 2-4 mm later turning from light brown to dark brown patches, the spots enlarge, leading to complete drying and browning of the leaves.

The genus *Alternaria* are filamentous fungi that are widely distributed across the globe and are commonly present in both natural and human-dominated ecosystems. It comprises endophytic, saprophytic and pathogenic species that are frequently found in air, soil and plant debris, and many *Alternaria* species can survive for long periods as mycelium or conidia on plant residues (Schmey et al., 2024). The genus includes nearly 299 species, many of which are significant plant pathogens affecting a broad range of hosts. These are challenging to manage due to their broad host range, high genetic variability among pathogenic isolates and prolonged active phase in their disease cycles. These traits make *Alternaria* an adaptable and persistent pathogen in agricultural systems (Chohan et al., 2015).

Growth and sporulation of *A. polianthi* vary markedly by culture medium, temperature and humidity, hindering timely screening of large germplasm collections. Existing management relies on repeated fungicide applications, which are costly and environmentally unsustainable. *Alternaria polianthi* was previously identified solely based on morphological characteristics without molecular

evidence and there is limited information on its morphological traits. Hence, the present investigation focuses on the isolation and identification of the pathogen based on morphological and molecular characterization, and to study the effects of different cultural media on fungal growth, sporulation, and colony characteristics.

MATERIALS AND METHODS

The experiment was conducted in the Division of Flower and Medicinal Crops and Division of Crop Protection, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India. Tuberose plants exhibiting typical leaf blight symptoms were collected from infected fields at ICAR-IIHR, Bengaluru, in July 2023 for pathogen isolation and identification. Infected leaves were washed with tap water, surface-dried, and cut into 5 mm bits having both healthy and diseased tissue. Bits were surface-sterilized in 1% sodium hypochlorite (NaOCl) for 30 seconds, then rinsed three times in sterile distilled water to eliminate NaOCl traces. Excess moisture was blotted on sterile filter paper, followed by air-drying. Four bits per plate were aseptically placed on potato dextrose agar (PDA) in petri dishes, sealed with parafilm, and incubated at 26±1°C in a BOD incubator for 7-10 days. Fungal growth was monitored, subcultured to obtain pure cultures, and maintained on PDA slants at 4°C for future use (Singh et al., 2021).

Morphological and molecular characterization of the fungus

Fungal colonies resembling *Alternaria polianthi* were subcultured on PDA for purification. For morphological characterization, pure cultures were flooded with sterile distilled water, gently scraped to disperse conidia, and diluted based on conidial density to prevent overcrowding in microscopic fields. Spores were observed using a ZEISS AXIO Imager A2 light microscope (Germany) at 20X magnification. The cultural and morphological characters recorded were-colony appearance, conidial shape, colour, size, and septation, compared with the dimensions for tuberose leaf blight pathogenic fungi reported by earlier workers (Mariappan et al., 1977 and Ambesh et al., 2014).

For further confirmation, genomic DNA was extracted from the mycelial mat using the cTAB method (Doyle, 1990). The DNA was then amplified with ITS1 and ITS4 primers (White et al., 1990) following a standard PCR protocol (Sonavane et al., 2023). The resulting amplicons were analyzed on an agarose gel, purified, and sequenced using the Sanger method with an automated DNA sequencer at Eurofins Genomics India Pvt. Ltd., Bengaluru.

Media used for growth and sporulation of *Alternaria polianthi*

The fungus was grown on different media using agar plate technique to examine its growth and cultural traits. Ten different media (Table. 1) were poured separately into sterilized petri plates of the same size. After solidification of media, the plates were inoculated with a 5 mm mycelial bit of ten-day-old culture at the centre of each plate and the plates were incubated at 25±1°C in BOD (biochemical oxygen demand) incubator in inverted position for 10 days. The experiment was conducted using completely randomized design with three replications for each media.

Data collection and analysis

The observations on colony diameter, degree of sporulation, colony shape, margin, topography, concentric rings, culture colour, reverse colour and mycelial texture were recorded after 10 days of inoculation. The experiment data was analysed in ANOVA using IBM SPSS Statistics v. 22 software. The ITS sequence of the isolate (deposited in the NCBI gene bank) was subjected to BLAST in the NCBI database (www.ncbi.nlm.nih.gov) and a maximum likelihood phylogenetic tree with 1000 bootstrap replicates was constructed using MEGA X. v. 11 (Tamura et al., 2021).

RESULTS AND DISCUSSION

Isolation and morphological characterization

The pure culture of *Alternaria polianthi* colony was uniform and fluffy with dark olive green black, cottony mycelium. The conidia from the culture were single, muriform, light to dark brown, tapered at apex with beak ranging from 8.83 µm to 41.35 µm. The length and breadth of the conidia ranged between 10.15-95.23 µm and 7.75-16.12 µm, respectively. Furthermore, it had 5-7 horizontal and 0-2 vertical septation (Fig. 1). Based on the cultural characteristics as per Mariappan et al. (1977) the isolate was identified as *Alternaria polianthi.* Similar conidial characters were observed by Ambesh et al. (2014) and Pawar et al. (2021) in tuberose.

Table 1 : Composition of different culture media

Nutrient media	Composition
Corn meal agar (CMA)	Corn meal: 50 g, agar: 15 g, distilled water: 1000 mL
Potato dextrose agar (PDA)	Peeled potato: 250 g, dextrose: 20 g, agar: 20 g, distilled water: 1000 mL
Potato carrot agar (PCA)	Potato: 200 g, carrot: 20 g, agar: 20 g, distilled water: 1000 mL
Carrot agar (CA)	Carrot: 200 g, dextrose: 20 g, agar: 20 g, distilled water: 1000 mL
Yeast peptone dextrose adenine medium (YPDA)	Yeast extract: 10 g, peptone: 20 g, dextrose: 20 g, agar: 20 g
Water agar (WA)	Agar: 20 g, distilled water: 1000 mL
Oat meal agar (OMA)	Oat meal: 60 g, agar: 12.5 g, distilled water: 1000 mL
V-8 juice agar (V8)	V-8 juice: 8.3 g, l-asparagine: 10 g, yeast extract: 2 g, CaCO*f* : 2 g, glucose: 2 g, agar: 20 g, distilled water: 1000 mL
Richard's synthetic agar (RSA)	FeCl*f* : 0.02 g, sucrose: 50 g, agar: 15 g, distilled water: 1000 mL
Czapek's dox agar (CDA)	Sucrose: 30 g, $NaNO_3$: 2 g, K, $HPO_{,,}$: 1 g, $MgSO_{,,}$: 0.5 g, KCl: 0.5 g, $FeSO_{,,}$: 0.01 g and agar: 15 g, distilled water: 1000 mL

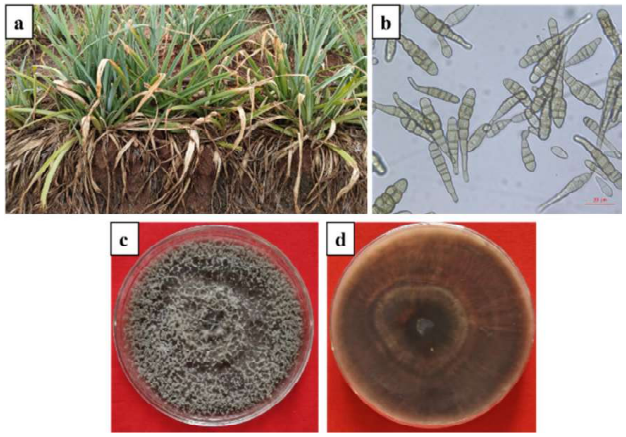

Fig. 1 : a. Severe damage of leaf blight in the field, b. Conidia of *A. polianthi* (20X), c. Dorsal, and d. Ventral side of culture of *A. polianthi* on PDA

Molecular characterization

The two *Alternaria* isolates namely IIHR-1 and IIHR-2 were amplified with ITS 1 and ITS 4, yielded an amplicon size of 484 bp and 479 bp, respectively. Their sequences, deposited in NCBI under accession numbers PQ530981 and PQ530980, respectively, showed 99-100% similarity to tuberose-specific *Alternaria* isolates *via* BLAST and clustered in a distinct phylogenetic clade (Fig. 2).

Thus, based on conidial morphology and molecular phylogeny, it confirms that these isolates causing leaf blight in tuberose are *Alternaria polianthi*. To our best knowledge, the molecular characterization of *Alternaria polianthi* has not been reported in any previous studies in India.

Effect of different culture media on growth and sporulation of *A. polianthi*

A. polianthi growth varied significantly across ten different media after 10 days of incubation (Table 1, Fig. 3). Corn meal agar (CMA) supported maximum radial growth (9.0 cm), followed by PDA (8.8 cm) and WA (7.97 cm), while YPDA (4.0 cm) and OMA (4.37 cm) recorded lowest colony growth. Thereafter, the media *viz*., PCA, CA, YPDA, WA, V8, RSA, CDA showed the colony diameter of 8.27, 7.27, 7.97, 4.37, 6.00, 5.97, and 7.87 cm, respectively. Profuse sporulation occurred on CMA, PDA, and WA. Morphological traits of the fungus varied across media due to differences in components and nutrient bioavailability for mycelium development. Similar results were reported for *A. polianthi* by Pawar et al. (2021), and for other *Alternaria* spp. by Koley & Mahapatra (2015), Ginoya & Gohel (2015), Gupta et al. (1987) and Arunakumara (2006).

In the present study, *Alternaria polianthi* sporulated well on corn meal agar, potato dextrose agar and water agar. Previous studies have highlighted the influence of nutritional factors on fungal reproduction, where the sporulation was triggered by lower sugar concentration (Rajderkar, 1966). Present results aligned with the findings of Ginoya & Gohel (2015) and Zhu et al. (1985), who reported profuse sporulation of *A. solani* on CMA; Su et al. (2012) noted high sporulation on WA, while, Krishna et al. (2018) found good sporulation of *A. dauci* on PDA. In contrast, Koley & Mahapatra (2015) observed

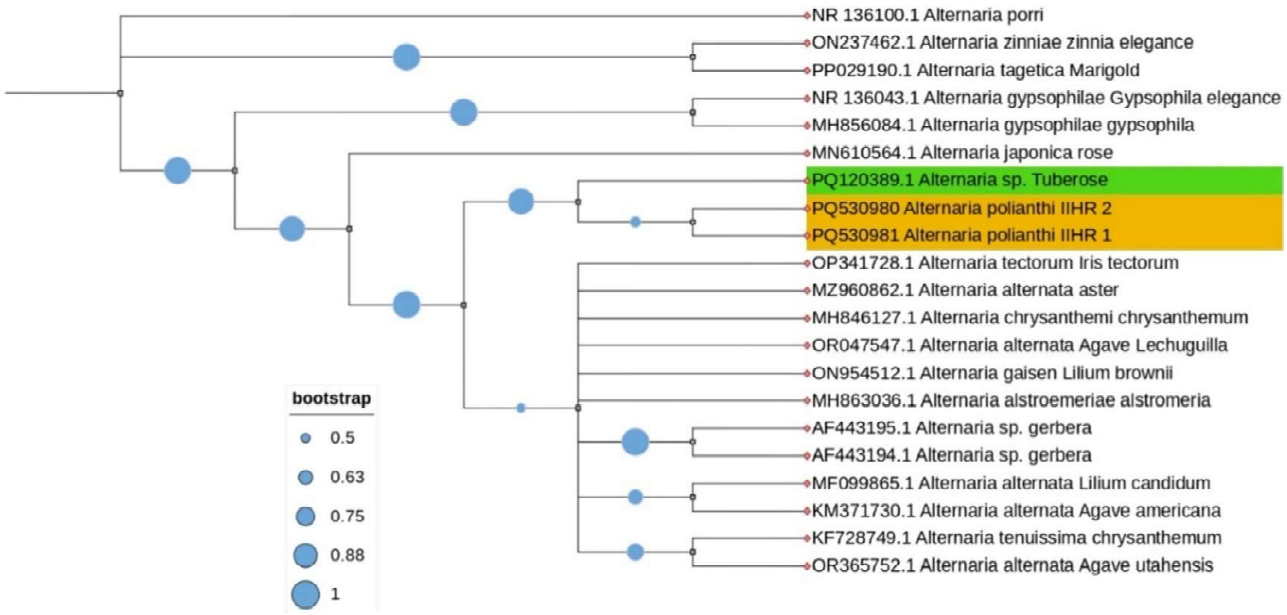

Fig. 2 : Phylogenetic analysis using maximum likelihood method with 1000 bootstraps using MEGA X with ITS

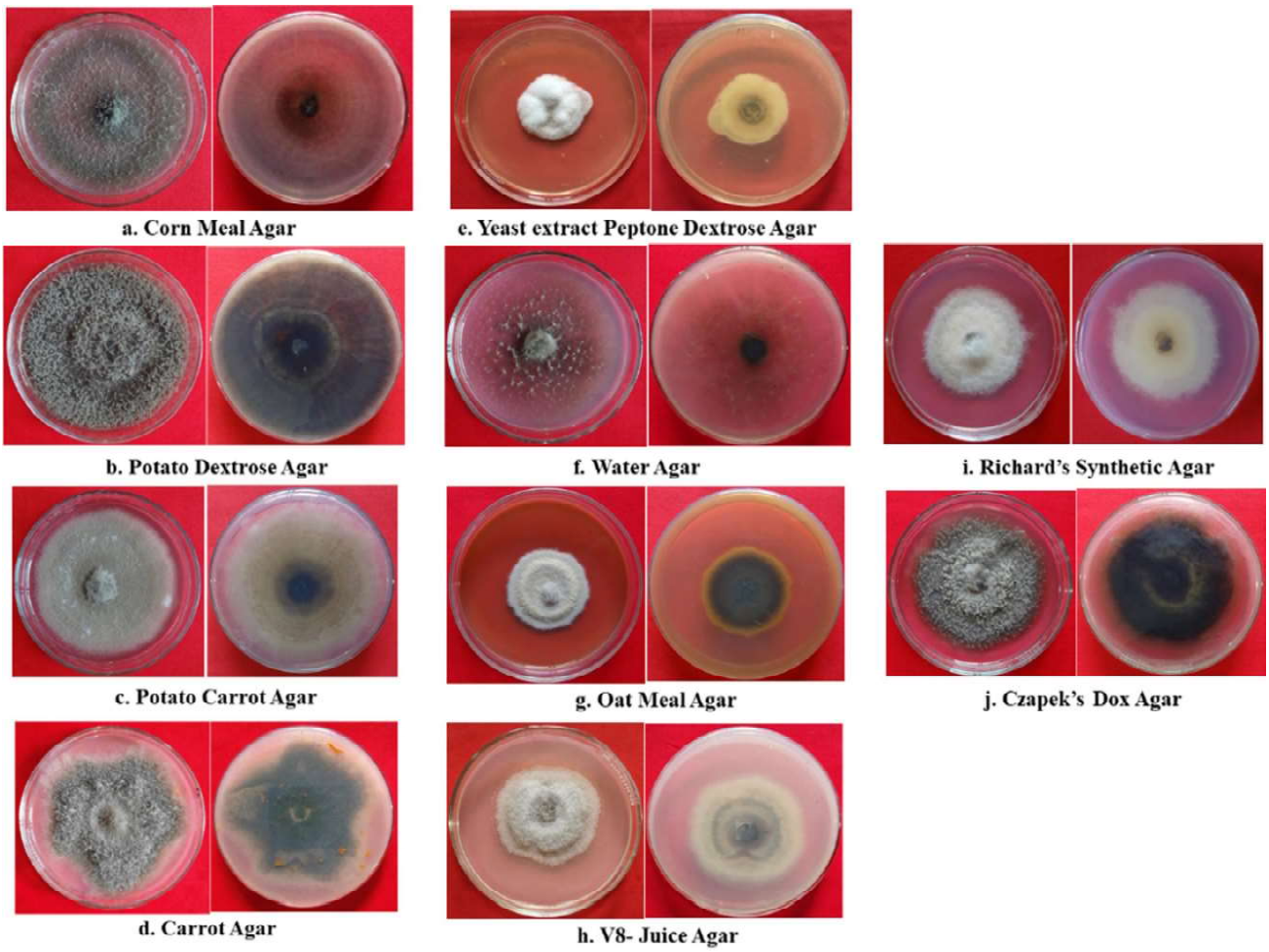

Fig. 3 : Colony morphology of *A. polianthi* on different media

Table 2 : Cultural characteristics of *Alternaria polianthi* on different media

Media	Average diameter of culture	Sporulation	Colony shape	Margin	Elevation	Concentric rings	Culture colour	Reverse colour	Mycelial texture
Corn meal agar (CMA)	9.00[a]	+++	Circular	Filiform	Flat	Present	Greyish black	Greyish black	Cottony
Potato dextrose agar (PDA)	8.80[ab]	+++	Circular	Filiform	Umbonate	Present	Olive green	Black	Cottony
Potato carrot agar (PCA)	8.27[bc]	+	Circular	Filiform	Umbonate	Absent	Brownish grey	Brownish grey	Cottony
Carrot agar (CA)	7.27[d]	+	Irregular	Filiform	Flat	Present	Greyish black	Olivaceous green	Cottony
Yeast peptone dextrose adenine (YPDA)	4.00[f]	+	Circular	Filiform	Raised	Present	White	Off white	Cottony
Water agar (WA)	7.97[cd]	+++	Filamentous	Filiform	Flat	Present	Greyish black	Black	Cottony
Oat meal agar (OMA)	4.37[f]	+	Circular	Filiform	Raised	Present	White	Grey	Velvety
V-8 juice agar (V8)	6.00[e]	++	Irregular	Filiform	Raised	Present	Greyish black	Greyish black	Velvety
Richard's syntheticagar (RSA)	5.97[e]	+	Circular	Filiform	Umbonate	Present	White	White	Cottony
Czapek's dox agar (CDA)	7.87[cd]	+	Irregular	Filiform	Raised	Present	Greyish black	Black	Cottony
CD ($P \leq 0.05$)	0.702								
SE(m)±	0.238								
CV (%)	5.933								

*Sporulation, +++: > 50 conidia; ++: 10- 50 conidia; + : <10 conidia

profuse sporulation of *A. solani* on oat meal agar. These indicate media composition significantly influences growth and sporulation of *Alternaria* spp.

Cultural characters of *Alternaria polianthi*

Observations pertaining to colony colour and characteristic growth pattern of *Alternaria polianthi* on different media on 10th day of incubation are presented in the Table 1. Significant variation was observed in colony colour, margin of the colony, elevation, zonation and mycelial texture. Colony colour showed varied shades of green, black and white. Whereas, in reverse side shades of black colour was observed. Circular growth margin was found prominent and on the other hand, an irregular growth margin was recorded in CA, V8, and CDA while a filamentous margin was seen in the WA. Mycelial topography was flat in CMA, WA and CA whereas umbonate in PDA, RSA, and CDA. The raised mycelia were found in PCA, YPDA, OMA and V8. Zonation or the concentric rings were present in all the media but not PCA and WA media.

Mycelial texture was cottony in all the media expect OMA and V8 which was velvety in texture. The findings are close conformity with the results of Pawar et al. (2021) who noticed that the colour of *A. polianthi* varied from creamy ash white to greenish ashy white on different media, while Apet et al. (2014) observed colonies of *Alternaria alternata* as greenish grey to olivaceous black. Similar variation was observed by Pipaliya & Jadeja (2008) and Kumar et al. (2015).

CONCLUSION

Based on the results, the pathogen causing leaf blight in tuberose was identified as *Alternaria polianthi* through morphological and molecular characterization. Among ten media tested, corn meal agar (CMA), potato dextrose agar (PDA), and water agar (WA) proved most effective, yielding the highest radial growth (up to 9 cm on CMA) and profuse sporulation. These findings demonstrate that the composition of the culture medium strongly influences the growth and sporulation of *A. polianthi*, making these media suitable for further research, understanding its behaviour, and developing better management practices.

ACKNOWLEDGEMENT

The authors thank the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru, India, for facilitating this research work and senior author acknowledges the PG School, ICAR- Indian Institute of Agricultural Research, New Delhi for granting fellowship for her Ph.D. program.

REFERENCES

Ambesh, B. S., Ngomle, S., Marak, T., & Das, S. (2014). Morphological and biochemical variation of some *Alternaria* species infected on different floricultural plants. *The Ecoscan*, *Special Issue*, 361-368.

Anonymous. (2024–25). *Horticulture statistics at a glance 2024-25*. Ministry of Agriculture and Farmers' Welfare, Government of India, New Delhi.

Apet, K. T., Jagdale, J. S., Baig, M. F., Wagh, S. S., & Chavan, P. G. (2014). Effect of various culture media on cultural and morphological characteristics of *Alternaria alternata*. *Trends in Biosciences*, *7*(21), 3383-3385.

Arunakumara, K. T. (2006). *Studies on Alternaria solani (Ellis and Martin) Jones and Grout causing early blight of tomato*. M.Sc. (Agri.) thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.

Bharathi, T. U., Kumar, R., Nair, S. A., Umamaheswari, R., Sonavane, P., Kalaivanan, D., & Rao, V. K. (2023). Evaluation of tuberose genotype IIHR 17-23SP-08 (IC0642158) for flower yield, quality, and response to biotic stress. *Journal of Horticultural Science*, *18*(1), 60-66. https://doi.org/10.24154/jhs.v18i1.2148

Chohan, S., Perveen, R., Abid, M., Naz, M. S., & Akram, N. (2015). Morpho-physiological studies, management, and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Agriculture and Biology*, *17*(1), 111-118.

Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*, 13-15.

Ginoya, C. M., & Gohel N. M. (2015). Cultural and morphological variability among the isolates of *Alternaria alternata* (Fr.) Keissler, Incitant of Fruit Rot of Chilli. *International Journal of Plant Protection*, *8*(1): 118–125. https://doi.org/10.15740/HAS/IJPP/8.1/118-125.

Gupta, R. P., Pathak, V. N. & Verma O. P. (1987). Morphological, cultural and pathogenic variation in *Alternaria porri* (Ellis) Ciff., the incitant of purple blotch of onion. *Zentralblatt für Mikrobiologie*, *142*, 155–162.

Koley, S., & Mahapatra, S. (2015). Evaluation of culture media for growth characteristics of *Alternaria solani* causing early blight of tomato. *Journal of Plant Pathology and Microbiology*, *2*(1), 1-5. https://doi.org/10.4172/2157-7471.S1-005.

Krishna, V. P., Suryawanshi, A. P., Prajapati, S., & Surekha, S. (2018). Effect of different culture media on growth and sporulation of *Alternaria dauci* causing carrot leaf blight. *Journal of Pharmacy and Phytochemistry*, *7*(6), 1789-1792.

Kumar, V., Kumar K. P., & Kumar K. M. (2015). Study of variability and sporulation by isolates of *Alternaria solani* of *Lycopersicon esculentum* (Mill.). *Asian Journal of Science and Technology*, *6*(2), 1264–1270.

Mariappan, V., Kochu Babu, M., & Kandasamy, T. K. (1977). A leaf spot disease of tuberose (*Polianthes tuberosa* L.) caused by a new species of *Alternaria polyanthi*. *Current Science*, *46*, 131.

Pawar, P. B., Kakade, D. S., & Vavare, K. B. (2021). Morphological and cultural characteristics of *Alternaria polianthi*. *The Pharma Innovation*, *10*(10), 1053-1056.

Pipaliya, B. H., & Jadeja K. B. (2008). Cultural variability and mancozeb sensitivity of different isolates of *Alternaria burnsii* L. *Journal of Indian Mycology and Plant Pathology*, *38*(1), 121–122.

Rajderkar, N. R. (1966). Certain chemical requirements for growth and sporulation of *Alternaria* species. *Mycopathologia et Mycologia Applicata*, *30*(2), 172-176. https://doi.org/10.1007/BF02130367

Singh, P., Nema, S., & Tantwai, K. (2021). Morphological and molecular characterization of *Alternaria* species isolated from different plants. *Journal of Experimental Agriculture International*, *43*(6), 61–71. https://doi.org/10.9734/JEAI/2021/v43i630703

Schmey, T., Tominello-Ramirez, C. S., Brune, C., & Stam, R. (2024). Alternaria diseases on potato and tomato. *Molecular Plant Pathology*, *25*(3), e13435

Sonavane, P., Venkataravanappa, V., Krishna Reddy, M., & R. S. P. (2023). Occurrence of *Phytophthora nicotianae* causing collar and root rot disease of Chrysanthemum in India. *Environmental Conservation Journal*, *24*(1), 217-224. https://doi.org/10.36953/ecj.11922310

Su, Y. Y., Qi, Y. L., & Cai, L. (2012). Induction of sporulation in plant pathogenic fungi. *Mycology*, *3*(3), 195-200. https://doi.org/10.1080/215012

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*, 3022-3027.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols*: *A Guide to Methods and Applications* (pp. 315-322). Academic Press.

Zhu, Z. Y., Huang, X. M., & Li, Y. H. (1985). An efficient technique for inducing profuse sporulation of *Alternaria solani* in pure culture. *Journal of Phytopathology*, *4*(3), 180-184.

(Received : 23.11.2024; Revised : 13.2.2026; Accepted : 20.2.2026)