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Morpho-cultural and molecular characterization of pathogen causing early blight disease of tomato

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Abstract
Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop of tropical and subtropical countries, which is grown in almost all the states of India. Its cultivation is affected by many diseases, among which early blight disease caused by *Alternaria solani* is the major one. Considering the importance of crop and the losses caused by the pathogen, investigations have been conducted on cultural, morphological and molecular characterization of the pathogen. Among the eight different culture media tested for the growth of *Alternaria solani*, potato dextrose agar medium recorded the maximum radial growth of 90.00 mm in all four isolates, followed by oat meal agar (85.18 to 90.00 mm). While, the least radial growth was recorded in yeast extract agar (19.32 to 22.06 mm). Colony growth was found to be flat or fluffy with regular to irregular margins and light to dark gray pigmentation. Conidia produced by all the four isolates in PDA medium were pale to olivaceous brown in colour, oblong to muriform in nature with size ranging from 81.47 to 95.61 µm in length and 14.21 to 18.85 µm in width, containing 5-9 transverse and 2-3 longitudinal septa. Molecular characterization of four pathogen isolates were done through the CTAB DNA extraction method, followed by PCR amplification using universal primers ITS1 and ITS4. PCR amplification yielded bands at 550 bp in all four isolates. Sequencing and BLAST analysis demonstrated over 98 per cent sequence similarity to *Alternaria solani*. This confirms that the four isolated pathogens are *Alternaria solani*. Corresponding accession numbers were obtained by depositing the sequences of all four isolates in NCBI GenBank. This comprehensive molecular and morphological characterization underscores the precise identification of the pathogen causing early blight disease of tomato.

Keywords: Early blight, morphological, cultural, molecular, characterization and tomato

1. Introduction
Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and it is also known as *pomo d'oro* (golden apple) in Italian, *pomme d'amour* (love apple) in French and "Poor man's apple" because of its nutritional, medicinal properties and consumer affordable price. The name "tomato" is derived from the Spanish word tomate, which comes from the Nahuatl word tomatl, meaning "swelling fruit" (Harper, 2024) [8]. The wild species are known to be originated in the Andes Mountains of South America, mainly in Peru and Ecuador and are domesticated in pre-Columbian Mexico (Mutschler *et al.*, 2006) [11].

With a global production of 186.82 million tonnes and an average yield of 36.97 tonnes per hectare (FAOSTAT, 2022) [6] tomatoes are predominantly grown in China, which accounts for 36 per cent of global production, followed by India with 11 per cent (FAOSTAT, 2023) [7]. In India, during the year 2023-24 tomato was grown over an area of 8,53,990 ha with an annual production of 21,323,220 MT and productivity of 24.97 MT per ha. Madhya Pradesh is the largest tomato producing state with an area of 1,19,670 ha under cultivation and 34,98,260 MT production which alone accounts for 16 per cent of total production. The other major tomato growing states are Andhra Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Maharashtra, Tamil Nadu, Bihar and Chhattisgarh. Andhra Pradesh had highest productivity of 45.00 MT per ha. In Karnataka tomato crop is grown on 62,430 ha area with a production of 20,07,330 MT and productivity of 32.15 MT per ha (Anon., 2024) [2]. The major tomato growing districts of Karnataka are Kolar, Belagavi, Chikkaballapura, Haveri, Chikkamagaluru and Bengaluru rural. Kolar is the leading producer in Karnataka (Anon., 2021) [1].

Tomato cultivation is constrained by different biotic and abiotic factors which have impeding effects on its production and productivity. Keeping abiotic factors apart, among different biotic factors, diseases are the most significant, which have a direct impact on the yield and quality of tomato fruits. There have been reports of more than two hundred diseases infecting tomato in the world (Atherton and Rudich, 1986) [4]. Among them, the early blight disease caused by *Alternaria solani* is regarded as the most crucial and catastrophic disease occurring worldwide (Praveen *et al*., 2019) [12]. *Alternaria solani* is both airborne and soil-inhabiting and is accountable for producing symptoms like early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981) [5]. It produces conidia with transverse and longitudinal septa, multinucleate and dark-colored (melanised) cells. This melanin guards the pathogen against harmful environmental conditions, such as resistance to microorganisms and hydrolytic enzymes (Rotem, 1994) [14]. This study has been conducted to understand the cultural, morphological and molecular characteristics of *Alternaria solani* isolates.

2. Material and methods

Isolation and purification of *Alternaria solani* culture was done for four isolates *viz.*, Machagondanahalli (Alt-1) isolate, Hiriyur (Alt-2) isolate, Kommanalu (Alt-3) isolate and Srinivasapura (Alt-4) isolate from fresh infected leaf of tomato and purified by the hyphal tip method. They were stored at 4 °C on PDA slants for further study.

2.1 Cultural characterization of *Alternaria solani*

The cultural characters of *Alternaria solani* such as colony diameter (mm), type of growth, pigmentation and sporulation were studied on different solid media *viz*., Potato dextrose agar, Czapek's Dox agar, Corn meal agar, Host leaf extract agar, Oat meal agar, Yeast extract agar, Sabouraud's dextrose agar and Richard's agar. 20 mL of each medium listed above was poured into Petri plates of 90 mm diameter and allowed to solidify. Such plates were inoculated with 5 mm discs from 9 days old *Alternaria solani* cultures which were cut by using a cork borer and a single disc was placed upside down at the centre of the plate. Each set was replicated thrice and the plates were incubated at 28 ± 1 ˚C. Observations on the Colony diameter (mm), Type of growth, Pigmentation and Sporulation on different solid media were recorded when the maximum growth was attained in any one of the media tested.

Using a transparent plastic scale linear growth of the colony was measured in millimetre. In addition, the sporulation was observed from 10 days old culture of each isolate by making the spore suspension. A single block of 5 mm diameter was cut out from the fungal colony near the margin by sterilized cork borer. It was transferred to 5 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on a slide and the average spore count of three microscopic fields under low power (10X) objective of the microscope. The sporulation was graded as follows (Table 1).

Table 1: Sporulation index

Sl. No.	Score	Grade	Conidia/ microscopic field (10X)
1.	++++	Excellent	> 75
2.	+++	Good	51-75
3.	++	Fair	26-50
4.	+	Poor	1-25
5.	-	No sporulation	0

2.2 Morphological characterization of *Alternaria solani*

The morphological characters of the fungus were studied on potato dextrose agar (PDA) medium. 20 ml of sterilized and cooled PDA medium was poured into each Petri plate aseptically and allowed to solidify. Such plates were inoculated with 5 mm discs of the pathogen cut from the periphery of the actively growing culture and incubated at room temperature (28 ± 1 ˚C) for a period of 12 days. Each treatment was replicated thrice. The morphological characters like length and width of conidia, number of transverse and longitudinal septa, presence/ absence of beak were recorded.

2.3 Molecular characterization of *Alternaria solani*

2.3.1 DNA Extraction

Total genomic DNA of *A. solani* isolates was extracted using the standard CTAB method (Murray and Thompson, 1980) [10] with slight modifications. Two to three grams of fungal mat grown on potato dextrose broth was homogenized in a mortar and pestle. One ml of lysis buffer was added and incubated at 60 °C for one hour. The homogenate was then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (1:1:1) and centrifuged at 10,000 rpm for 15-20 minutes at 4 °C. The supernatant was treated with RNase and proteinase K and incubated for 30 minutes at room temperature. DNA was precipitated using chilled isopropanol, centrifuged, washed with 70 per cent ethanol, air-dried and finally dissolved in 500 µl of $T_{10}E_1$ buffer. The purity and concentration of DNA were determined by agarose gel electrophoresis. Sambrook *et al.* (1989) [15].

2.3.2 PCR Amplification, Gel Electrophoresis and Sequencing

The extracted DNA was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. Each 20 µl PCR reaction contained template DNA, forward and reverse primers, dNTPs, 10X buffer with $MgCl_2$, Taq DNA polymerase and nuclease-free water. The PCR conditions were as follows: initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 45 seconds, extension at 72 °C for 1.30 minutes and a final extension at 72 °C for 10 minutes. Amplified products were visualized under UV light after gel electrophoresis. The PCR products were separated using 1.5 per cent agarose gel prepared in 1X TAE buffer with ethidium bromide. Two µl of loading dye was added to each sample and electrophoresis was carried out at 70 volts for 60 minutes. The resulting DNA bands were observed under a UV transilluminator and documented. Amplified products were further sequenced and the obtained sequences were compared using BLAST on the NCBI database. Relevant sequences and accession numbers of closely related isolates were retrieved from GenBank for confirmation of the pathogen identity.

3. Results

3.1 Cultural characteristics of *Alternaria solani isolates*

The cultural characteristics have been studied for all four isolates on eight different solid media. All the media are basically energy source for different microorganisms. Not all the media support the equal growth of the fungi. So, eight different solid media were studied to evaluate the

growth and cultural characteristics of all four isolates of *A. solani*.

3.1.1 Cultural characterization of *Alternaria solani* isolates on different solid media with emphasis on colony diameter and pigmentation

Among the eight different solid media tested, notable differences were observed in the growth and pigmentation of all four isolates of *A. solani*. The results revealed that potato dextrose agar (PDA) was the most favorable medium for mycelial growth, as all four isolates reached the maximum colony diameter of 90.00 mm within the incubation period of 15 days. Colonies on PDA appeared light gray, except Srinivasapura (Alt-4) isolate, which showed a dark gray pigmentation. Oat meal agar also supported substantial growth, with colony diameters ranging from 85.18 mm to 90.00 mm and colonies showing light gray to whitish gray pigmentation, suggesting good nutrient support and moderate pigmentation. Corn meal agar and Richard's agar showed moderately reduced colony diameter ranging from 75.94 mm to 86.74 mm, with colonies displaying predominantly dark gray pigmentation except Kommanalu (Alt-3) isolate. On Czapek's Dox agar, colony diameter was lower, especially for Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates (58.51 mm and 45.01 mm, respectively) and pigmentation varied from dark gray to light gray with whitish margins. Sabouraud's dextrose agar showed a similar trend with comparatively lower colony diameters (40.42 mm to 61.71 mm) and mostly light gray colonies, often with white margins. The poorest growth was observed on host leaf extract agar and yeast extract agar, where colony diameters were significantly reduced, especially on yeast extract agar (as low as 19.32 mm for Kommanalu isolate) and colonies appeared brownish black in pigmentation (Table 2, Plate 1).

Table 2: Cultural characterization of *Alternaria solani* isolates on different solid media with emphasis on colony diameter and pigmentation

Tr. No.	Media	Colony diameter (mm) of the isolates				Pigmentation of the isolates			
		Alt-1	Alt-2	Alt-3	Alt-4	Alt-1	Alt-2	Alt-3	Alt-4
T_1	Potato dextrose agar	90.00*	90.00*	90.00*	90.00*	Light gray	Light gray	Light gray	Dark gray
T_2	Oat meal agar	85.18	85.27	90.00	86.77	Light gray	Light gray	Whitish gray	Light gray
T_3	Corn meal agar	83.52	83.80	86.48	86.74	Dark gray	Dark gray	Dark gray	Dark gray
T_4	Richard's agar	82.38	75.94	80.81	63.91	Dark gray	Dark gray	Light gray	Dark gray
T_5	Czapek's Dox agar	79.09	70.94	58.51	45.01	Dark gray with whitish margin	Dark gray	Light gray	Dark gray
T_6	Sabouraud's dextrose agar	40.42	61.71	53.20	44.27	Light gray with white margin	Light gray	Light gray	Light gray
T_7	Host leaf extract agar	32.68	48.56	36.24	40.02	Black	Black	Black	Black
T_8	Yeast extract agar	20.15	20.47	19.32	22.06	Brownish black	Brownish black	Brownish black	Brownish black
S.Em. ±		0.19	0.36	0.27	0.35				
CD @ 1%		0.57	1.08	0.83	1.06				

*Mean of three replications; Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate

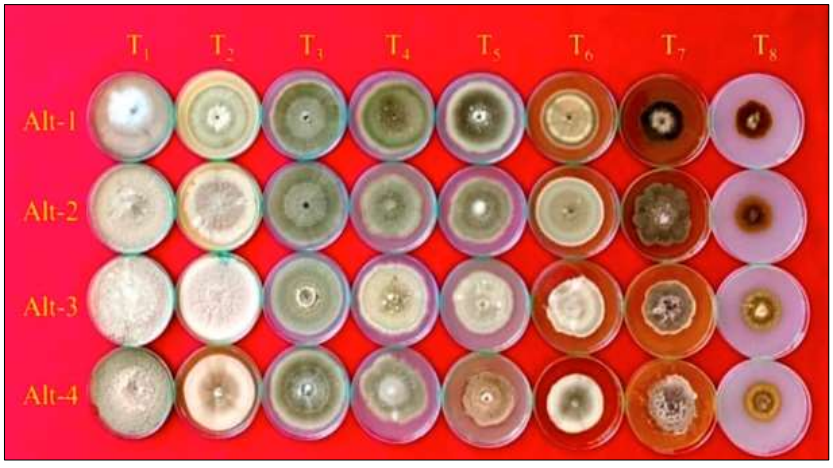


Plate 1: Cultural characteristics of *Alternaria solani* isolates on different solid media

3.1.2 Cultural characterization of *Alternaria solani* isolates on different solid media with emphasis on type of growth and sporulation

In addition to differences in colony diameter and pigmentation, variation was also observed in the type of growth and sporulation across the different media. On potato dextrose agar, all four isolates exhibited flat to fluffy growth with regular margins and showed good sporulation, making it the most suitable medium for both vegetative and reproductive growth. On oat meal agar, Machagondanahalli (Alt-1) and Hiriyur (Alt-2) isolates produced flat growth with regular margins, whereas Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates showed fluffy growth and all isolates exhibited fair sporulation. On corn meal agar, flat growth was observed in Machagondanahalli (Alt-1) and Hiriyur (Alt-2) isolates, while Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates showed fluffy growth and sporulation was fair in Machagondanahalli (Alt-1) and

Kommanalu (Alt-3) isolates and poor in Hiriyur (Alt-2) and Srinivasapura (Alt-4) isolates. On Richard's agar, Machagondanahalli (Alt-1) and Hiriyur (Alt-2) isolates showed flat growth with regular margins, whereas Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates produced fluffy growth with regular and irregular margins respectively. Sporulation was fair in Machagondanahalli (Alt-1), Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates and poor in Hiriyur (Alt-2) isolate. Czapek's Dox agar supported flat growth with regular margins in Machagondanahalli (Alt-1) and Hiriyur (Alt-2) isolates and fluffy growth in Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates, all with regular margins; however, only Machagondanahalli (Alt-1) isolate showed poor sporulaton, while the other three isolates did not sporulate. On Sabouraud's dextrose agar, all isolates exhibited either flat or fluffy growth with regular margins, but none of them showed any sporulation. On host leaf extract agar, Machagondanahalli (Alt-1) and Hiriyur (Alt-2) isolates showed flat growth, while Kommanalu (Alt-3) and Srinivasapura (Alt-4) isolates exhibited fluffy growth and Hiriyur (Alt-2) and Srinivasapura (Alt-4) isolates showed irregular margins and all isolates demonstrated fair sporulation. On yeast extract agar, all four isolates showed flat to fluffy growth with regular margins and exhibited poor sporulation. These results confirm that although most media supported vegetative growth and sporulation was significantly influenced by media composition, with PDA being the most effective for promoting abundant conidia production in all isolates of *A. solani* (Table 3, Plate 1).

Table 3: Cultural characterization of *Alternaria solani* isolates on different solid media with emphasis on type of growth and sporulation

Tr. No.	Media	Type of growth of the isolates				Sporulation of the isolates			
		Alt-1	Alt-2	Alt-3	Alt-4	Alt-1	Alt-2	Alt-3	Alt-4
T_1	Potato dextrose agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	+++	+++	+++	+++
T_2	Oat meal agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	++	++	++	++
T_3	Corn meal agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	++	+	++	+
T_4	Richard's agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with irregular margin	++	+	++	++
T_5	Czapek's Dox agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	+	-	-	-
T_6	Sabouraud's dextrose agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	-	-	-	-
T_7	Host leaf extract agar	Flat with regular margin	Flat with irregular margin	Fluffy growth with regular margin	Fluffy growth with irregular margin	++	++	++	++
T_8	Yeast extract agar	Flat with regular margin	Flat with regular margin	Fluffy growth with regular margin	Fluffy growth with regular margin	+	+	+	+

Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate

3.2 Morphological characteristics of *Alternaria solani* isolates on potato dextrose agar medium

The mycelial characteristics of the four isolates of *A. solani* revealed distinct differences in mycelial colony appearance. Two of the isolates collected from Machagondanahalli (Alt-1) and Hiriyur (Alt-2) exhibited flat growth with regular margins and light gray pigmentation (Plate 8). In contrast, the isolates from Kommanalu (Alt-3) and Srinivasapura (Alt-4) showed fluffy growth with regular margins (Plate 9); the Kommanalu (Alt-3) isolate had light gray pigmentation, whereas the Srinivasapura (Alt-4) isolate displayed a darker gray color. Despite these differences all four isolates showed good sporulation under laboratory conditions (Table 4).

Among the isolates, Machagondanahalli (Alt-1) isolate produced the longest conidia, measuring 95.61 µm in length and 15.34 µm in width, with 3 longitudinal and transverse septa. The Hiriyur (Alt-2) isolate had conidia measuring 88.64 µm in length and 18.85 µm in width, with 2 longitudinal and 5 transverse septa (Plate 2). In Kommanalu (Alt-3) isolate, the conidia were 81.47 µm long and 16.24 µm wide, with 2 longitudinal and 5 transverse septa. The Srinivasapura (Alt-4) isolate had conidia measuring 84.25 µm in length and 14.21 µm in width, with 3 longitudinal and 7 transverse septa (Plate 2). All conidia of four isolates exhibited the presence of a beak. Despite these minor differences in conidial size and septation, the isolates were found to be morphologically quite similar in their conidial characteristics (Table 5).

Table 4: Mycelial characters of *Alternaria solani* isolates

Isolates code	Mycelial characters		
	Type of growth	Pigmentation	Sporulation
Alt-1	Flat with regular margin	Light gray	+++
Alt-2	Flat with regular margin	Light gray	+++
Alt-3	Fluffy growth with regular margin	Light gray	+++
Alt-4	Fluffy growth with regular margin	Dark gray	+++

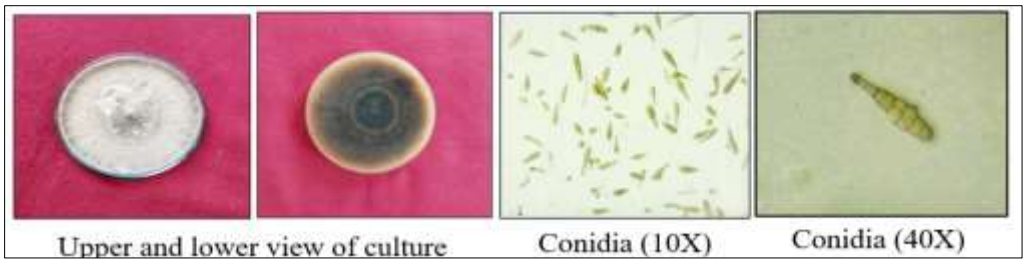
Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate

Table 5: Conidial characters of *Alternaria solani* isolates

Isolates code	Conidial characters				
	Length of conidia (µm)	Width of conidia (µm)	No. of longitudinal septa	No. of transverse septa	Presence of beak
Alt-1	95.61*	15.34*	3*	9*	Yes
Alt-2	88.64	18.85	2	5	Yes
Alt-3	81.47	16.24	2	5	Yes
Alt-4	84.25	14.21	3	7	Yes

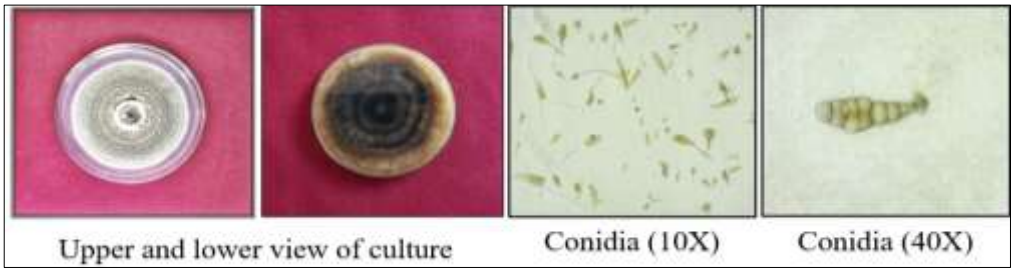
Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate

Machagondanahalli (Alt-1) isolate



Hiriyur (Alt-2) isolate

Kommanalu (Alt-3) isolate



Srinivasapura (Alt-4) isolate

Plate 2: Morphological characteristics of *Alternaria solani* isolates on potato dextrose agar media

3.3 Molecular characterization of the isolated pathogens

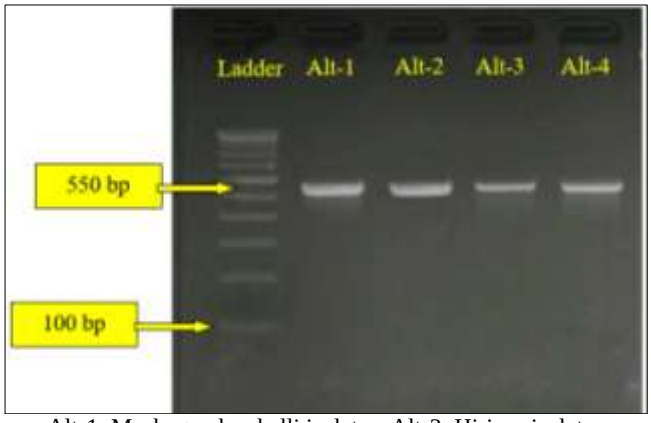
The genomic DNA of the fungus was extracted by the CTAB method. The DNA obtained was observed on 1.5 per cent agarose gel electrophoresis. Polymerase chain reaction was performed to amplify DNA by using ITS 1 and ITS 4 as forward and reverse primers, respectively. Then the amplicon was observed at 550 bp for all four isolates (Plate 3). The PCR product was sent for sequencing at Biokart India Pvt. Ltd., Bengaluru. The obtained DNA sequences of all four isolates are mentioned below.

DNA sequences of all four isolates were compared using the bioinformatics tool NCBI (National Centre for Bioinformatics) blast program. Based on sequence comparison, nucleotide sequences of the ITS region of the ribosomal DNA of all isolates had above 98 per cent homogeneity with isolates available in the NCBI. Thus, the sequences were deposited in the NCBI GeneBank and obtained accession number for all four isolates. This study confirms that the early blight disease of tomato is caused by *Alternaria solani* (Table 6).

Table 6: Molecular characterization of *Alternaria* solates from early blight of tomato

Isolate code	Name of the isolate	Organism	Accession No.	Per cent homology
Alt-1	Machagondanahalli	*Alternaria solani*	PV761050	98.89
Alt-2	Hiriyur	*Alternaria solani*	PV761051	98.72
Alt-3	Kommanalu	*Alternaria solani* f. sp. *lycopersici*	PV761052	98.43
Alt-4	Srinivasapura	*Alternaria solani*	PV761053	99.26

Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate



Alt-1: Machagondanahalli isolates; Alt-2: Hiriyur isolate; Alt-3: Kommanalu isolate; Alt-4: Srinivasapura isolate

Plate 3: Gel picture showing amplified ITS rDNA region of four *Alternaria solani* isolates

4. Discussion

4.1 Cultural characterization of *Alternaria solani isolates*

Cultural characteristics of all four isolates of *A. solani* were studied on eight different media. Among those eight media used for growth and sporulation of *A. solani*, maximum radial growth of 90.00 mm was recorded on potato dextrose agar medium (PDA) for all four isolates. Kommanalu isolate (Alt-3) recorded maximum growth of 90.00 mm on both PDA and oat meal agar. Whereas, least radial growth range of 19.32 - 22.06 mm was recorded on yeast extract agar. PDA medium along with maximum radial growth, it also showed good sporulation in all four isolates and showed light to dark gray pigmentation with flat or fluffy growth and regular margins. These results were in accordance with the findings of Koley and Mahapatra (2015) [9] who reported that potato dextrose agar and oat meal agar media were the best for the growth of the *A. solani* and Arunakumara (2008) [3] reported that the PDA medium contains sucrose as its sole carbon source and nitrate as its sole nitrogen source which makes the pathogen to grow well in this medium.

4.2 Morphological characterization of *Alternaria solani isolates*

Morphological characters such as length, breadth, septation and pigmentation were studied on potato dextrose agar medium. This study showed that the mycelium was septate, brownish, flat or fluffy growth with regular margins and with light to dark gray pigmentation. Conidia were multicelled, oblong to muriform conidia, pale or olivaceous brown in colour. Their size ranged from 81.47 to 95.61 µm in length and 14.21 to 18.85 µm in width at the broadest part, containing 5-9 transverse and 2-3 longitudinal septa. All the four isolates showed good sporulation in potato dextrose agar medium. These results were similar with the findings of Shekhar *et al.* (2022) who reported mycelium of *A. solani* is septate and brownish to black in colour and conidia of *A. solani* varied in length (90-160 µm), width (18-22 µm) and number of horizontal (2-10) and vertical (3-4) septa.

4.3 Molecular characterization of the isolated pathogens

The four different *Alternaria* isolates isolated from different regions of central dry and southern transition zones of Karnataka were used for amplification by PCR (Polymerase chain reaction). The pathogen isolates which were characterized as *Alternaria solani* based on cultural and morphological characteristics, were subjected for molecular confirmation by PCR using ITS rDNA technique. The PCR amplification using the universal fungal primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') yielded a single band at 550 bp. The identity of the pathogen as *Alternaria solani* was molecularly confirmed for all four isolates through sequence analysis in the NCBI database, showing homogeneity of more than 98 per cent. The sequences of all four isolates were deposited in GenBank and accession numbers were obtained. The results obtained were in agreement with the results obtained by earlier workers Ragupathi *et al.* (2020) [13] who isolated *Alternaria* from tomato causing early blight disease.

5. Conclusion

Cultural studies showed potato dextrose agar medium was found to be superior for growth of the pathogen. Conidia produced by all the four isolates in PDA medium were oblong to muriform in nature with size ranging from 81.47 to 95.61 µm in length and 14.21 to 18.85 µm in width, containing 5-9 transverse and 2-3 longitudinal septa. Molecular characterization of all four isolates of pathogen causing early blight disease of tomato identified as *Alternaria solani* by polymerase chain reaction (PCR) assay using ITS rDNA.

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