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Cultural, Morphological and Biochemical Variability Studies among the Isolates of *Alternaria solani*, the Causal Agent of Early Blight Disease of Tomato

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Abstract

Present experiment was conducted at College of Horticulture, Bengaluru (KA) during year 2017–18 to study the cultural, morphological and biochemical variations among the isolates of the pathogen *Alternaria solani*, the causal agent of early blight disease in tomato. The results revealed variation among the isolates collected from different regions of Karnataka state, India with regard to the colony characteristics *viz*., colony colour, mycelial growth pattern, margin of the colony and zonations. Maximum mycelial growth in terms of diameter (90 mm) was observed in the isolates Bagalkot (BaBG) and Chikkamagaluru (CMH) on Czapek's (Dox) agar medium while the least growth (36.33) was noticed in Bidar (BiHH) isolate. The isolate could grow better on Czapek's (Dox) agar medium as among the 3 media tested Czapek's (Dox) agar medium produced maximum growth of 80.70 mm and the least growth (63.70 mm) was noticed in V-8 juice agar. The morphological studies revealed that all the conidia of various isolates varied in length (25.07–42.90 µm), breadth (10.53–21.52 µm) and number of horizontal septa (2–7), longitudinal septa (0–4). Biochemical studies among the isolates revealed significant variation in their enzyme activities. The peroxidase activity was more in Chikamagaluru (CMH) isolate (81.80 Unit g^{-1} FW) least activity was found in Bidar (BiHH) isolate 11.78 Unit g^{-1} FW whereas the esterase activity was more Bengaluru (BYC) isolate (69.01 Unit g^{-1} FW) least activity was found in Bagalkot (BaBG) isolate 11.78 Unit g^{-1} FW. Existence of variation among the isolates of *Alternaria solani* is evident from the results obtained.

Keywords: *Alternaria solani*, biochemical, cultural, isolates, morphological, variability

1. Introduction

Tomato (*Solanum lycopersicum* L., 2n=24) belongs to the family *Solanaceae*. It ranks next to potato in world acreage and ranks first among the processing crops. Tomato is being exported in the form of whole fruits, paste and in canned form. Tomato is called as "Poor man's orange". The cultivated tomato has a narrow genetic diversity that resulted from its intense selection and inbreeding during period of evolution and domestication thus, these species are more prone to disease epidemics. Among the fungal diseases early blight (EB) is one of the most catastrophic disease which is caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. It may cause damping-off in the seedbed and a stem canker or

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collar rot that is destructive to transplants in the field (Sherf and MacNab, 1986; Walker, 1952)

The fungus is cosmopolitan in nature. It can attack to all parts of the plant. The disease in severe cases can lead to complete defoliation (Peralta et al., 2005). This disease is prevalent and found to be destructive causing the yield losses to the tune of 48–80% (Datar and Mayee, 1984; Mathur and Shekhawat, 1986; Pandey and Pandey, 2002). The disease prevails in the areas of higher humidity, rainfall, and temperature (Sahu et al., 2013). The optimum temperature for fungal growth was 23–28°C and pH 6–8 was found to be optimum (Tong et al., 1994). Penetration can occur at temperatures between 10 to 25°C. This disease epidemics initially progress slowly but accelerate when plants attain maturity. The disease is capable of causing damage to all the aerial parts of tomato, such as stem, leaf and fruit and at all growth stages (Blancard et al., 2012). It is very difficult to manage, due to its broad host range, extreme variability in pathogenic isolates and prolonged active phase of the disease cycle.

This fungus produces unique club-shaped conidia, often beaked with horizontal and often vertical septa that may be produced either individually or in a chain, depending on the species. Hyphal cells are darkly pigmented with melanin, which protect hyphae and spores against environmental stress and allows spores to survive in soil for long periods of time. The fungus overwinters in soil, plant debris, seed and alternate hosts in the form of either conidia or mycelia, which may serve as primary sources of inoculum.

The variability studies are important tools to document the changes occurring in populations and individuals as variability indicate the existence of different pathotypes. *A. solani* reproduces asexually; a sexual stage of this fungus is unknown. Despite having asexual reproduction, the isolates of the pathogen *A. solani* causing early blight disease in tomato exhibit high level of variability within the population in morphological, cultural, biochemical criteria and genetic composition which indicates the possible existence of different patho-types (Van der waals et al., 2004). Such variation may arise out of heterokaryosis or mutation. Because of high variation, *A. solani* can easily adapt to the changing environment and develop resistance to fungicides. Besides, high variation also affects the rate of disease development and induces infection in promising host lines that have implication for stability of cultivar resistance resulting in higher risk of overcoming existing genetic resistance of the host challenging the control of early blight disease using a completely resistant cultivar (Adhikari et al., 2017). The existence of the high level of variability has been reported in many countries (Pryor and Gilbertson, 2002a; Pryor and Michailides, 2002b; Leiminger et al., 2016; Odilbekov et al., 2016; Mohammadi and Bahramikia, 2019; Chaerani et al., 2017). Morphological characterization is the classical approaches to distinguish fungal species that is one of the main requisites of fungal taxonomy. The present investigation was carried out to find a comprehensive understanding of this causal organism with reference to cultural, morphological and biochemical variation among the isolates of *A. solani*, from the major tomato growing areas of Karnataka.

2. Materials and Methods

2.1. Collection and isolation of the isolates

The isolates of *A. solani* were collected from Kolar, Chikkaballapur, Ramanagara, Tumakuru, Bidar, Bagalkot, Chikkamagaluru, Mysuru and Bengaluru districts of Karnataka state during 2017–18. The tomato leaves infected with early blight disease from the above said places were used for the isolation of the fungus that was carried out as per the tissue segment methodology of Rangaswami (1958). The pathogen was purified using single spore isolation method (Riker and Riker, 1936). The identification was done through colony colour, morphology and spore characters. The pure culture of the pathogen was maintained on PDA slants at 27±1°C.

2.2. Designation of collected isolates

The isolates of *Alternaria solani* collected from various sources from Karnataka were designated based on their locations and sources (Table 1). For example, an isolate designated by BiHH means this isolate was collected from Bidar district (Bi), Humanabad Taluk (H), Hallikhed village (H).

2.3. Cultural variability

Cultural variability studies were carried out on Potato dextrose agar, Czapek's (Dox) agar, and V-8 juice agar. The media were prepared as mentioned in the "Ainsworth and Bisby's Dictionary of Fungi" by Ainsworth (1961). The cultural variability among the isolates *viz*., colour of the colony, mycelial growth, type of margin, zonations and growth in terms of colony diameter were studied after 14 days of incubation.

2.4. Morphological variability

For the purpose of studying variation in spore morphology of isolates of the pathogen, each isolate was grown on V-8 juice agar medium and incubated at 27°C for 14 days. The slides of the selected fungal cultures or colony were prepared in order to study the fungal morphology such as conidial length, breadth, number of septations and length of the beak. The prepared slides were observed under microscope. Ocular micrometer was calibrated by use of micrometry (Pramila et al., 2014). Conidia were harvested after 14 days from the V-8 juice agar plates. Conidia were mounted in lacto phenol and measured at 40× magnification with the aid of ocular and stage micrometre in compound microscope (Tuite, 1969).

2.5. Biochemical variations among the isolates of Alterneria solani

2.5.1. Preparations of enzymes extract

2.5.1.1. Culture condition

Isolates were grown in 250 ml conical flask containing 100

Table 1: Details of the isolates of *Alternaria solani* collected from different parts of Karnataka

Sl. No.	Isolates	Sample collected area				Climatic conditions
		District	Taluk	Area	Geographical locations	
1.	BiHH	Bidar	Humnabad	Hallikhed	17˚50'53.693' N Lattitude 77˚16'37.75'' E Longitude	23˚ to 32˚C
2.	BaBG	Bagalkot	Badami	Govanakoppa	15˚0'34.632'' N Lattitude 75˚40'5.332'' E Longitude	22˚ to 34˚C
3.	KSC	Kolar	Srinivaspura	Chowdadenahalli	13˚9'22.657'' N Lattitude 78˚0 '37.716'' E Longitude	18˚ to 25˚C
4.	ChCN	Chikkaballapur	Chintamani	Nandhiganahalli	13˚9 '22.657'' N Lattitude 78˚0 '37.716'' E Longitude	20˚ to 28˚C
5.	TGJ	Tumakuru	Gubbi	Jyothinagar	13 ˚1 '25.334'' N Lattitude 77˚27'32.341'' E Longitude	21˚ to 31˚C
6.	RMT	Ramanagara	Magadi	Tavarikeri	13˚ 1 ' 25.334'' N Lattitude 77˚ 27'32.341'' E Longitude	19˚ to 30˚C
7.	CMH	Chikkamagaluru	Mudigere	Horticulture campus	13˚ 8' 0.001'' N Lattitude 75˚ 38' 30.906'' E Longitude	14˚ to 22˚C
8.	BYC	Bengaluru	Yelahanka	Chikka bettahalli	12˚ 59 ' 29.089'' N Lattitude 77˚ 27 ' 56.863'' E Longitude	18˚ to 28˚C
9.	MNY	Mysuru	Nanjangud	Yedahalli	12˚ 24' 47.627'' N Lattitude 76 ˚ 30 ' 27.624'' E Longitude	20˚ to 27˚C

ml potato dextrose broth. Each flask was inoculated with 2 mycelial discs, each of 5 mm diameter cut from the advancing margin of five-day old culture grown on potato dextrose agar at 25±1°C. The inoculated flask was incubated at 25±1°C for seven days. Then mycelia mat was harvested by filtering through Whatman No 1 filter paper, washed with Phosphate buffer (pH 7.0) and damp dried and frozen overnight at -20°C.

2.5.1.2. Enzyme preparations

The enzyme was extracted by grinding 1 g of freeze-dried mycelium by using pestle and mortar in liquid nitrogen then powder was dispensed and vortexed in 2 ml of 0.1M phosphate buffer (pH 7.0) (Chowdappa and Chandramohanan, 1995). Further centrifuged for 45 min at 10,000 rpm and a supernatant was collected. This supernatant was used for enzyme analysis.

2.5.1.3. Peroxidase (POD) assay

Peroxidase activity was determined using the guaiacol oxidation method by Lin and Kao (2001). The reaction mixture contained 0.3 ml of 20 mM guaiacol, 2 ml of 100 mM potassium phosphate buffer (pH 7.0) and 0.2 ml enzyme extract. The reaction was initiated by the addition of 0.5 ml of H_2O_2 (1%). Increase in absorbance at 470 nm was recorded in 30 sec interval and upto three minute using UV-visible spectrophotometer. The POD activity was calculated by using an absorption coefficient (26.6 mM-cm) at 470 nm for the tetra guaiacol. Enzyme activity was expressed as µmoles of guaiacol oxidized/min /gram fresh wait (Unit g^{-1} FW).

2.5.1.4. Esterase assay

For esterase activity measurement, naphthyl acetate were used as broad spectrum substrate for esterase according to Burlina and Galzigna (1972). The esterase activity was determined spectrophotometrically at room temperature (23 °C) by measuring the increase in Absorbance at 420 nm. The reaction solution contained 1.5 ml 0.1 M Tris/HCl pH 7.4 and 30 µL 100 mM naphthyl acetate dissolved in absolute methanol. For each measurement 200 µL of crude extract was used. Measurements were performed in 1.0 cm cuvettes every 15 seconds over a three-min-period. The esterase activities were corrected for spontaneous hydrolysis of naphthyl acetate. The activities were calculated using the extinction coefficient of naphthol (420 nm)=2,222 M^{-1} cm^{-1} (Rudnicka and Kochman, 1984). The blank contained the buffer and corresponding naphthyl acetate. The activity was expressed as µmol of hydrolysed substrate per minute and per gram of fresh weight (µmol min^{-1} g^{-1} FW).

2.6. Data analysis

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a statistical software MSTAT-C (Freed and Scott, 1986).

3. Results and Discussion

3.1. Cultural variability

3.1.1. Colony characters on potato dextrose agar

The colonies of the BiHH and TGJ isolates were whitish and pinkish white with cottony mycelial growth without any

zonations (Table 2). The colonies of BaBG, KSC, RMT and CMH isolates appeared greyish with former three isolates showing cottony mycelial growth with zonation while the latter also had cottony growth but without zonations. The ChCN, BYC and MNY isolates produced light grey coloured colonies with the former two isolates showing cottony types of mycelial growth without zonations whereas the later had compact growth with slight zonations (Figure 1).

Colony characters on Czapek's (Dox) agar: Except the BaBG isolate all other isolates did not show zonations (Table 3). The BiHH isolate produced grey coloured colonies with cottony mycelial growth whereas the CMH isolate colonies appeared dark grey in colour colonies with cottony mycelial growth. The colonies of the ChCN isolate appeared dull grey while it was whitish grey in RMT isolate with the former had cottony type of mycelial growth whereas the later has slightly compact growth (Figure 2).

Table 2: Colony characteristics of different isolates of *Alternaria solani* on PDA medium

Sl. No.	Isolate	Colony characteristics			
		Colony colour	Mycelial growth	Margin of the colony	Zonation
1.	BiHH	Whitish with grey center	Cottony	Round with white margin	No zonations
2.	BaBG	Grey with dark grey at centre	Slightly cottony	Irregular with grey margin	Zonations
3.	KSC	Grey with a wide whitish margin	Cottony	Irregular white margin	Zonations
4.	ChCN	Light 1 grey surrounded by whitish margin	Cottony growth	Regular	No zonations
5.	TGJ	Pinkish white with grey center	Cottony	Round white margin	No zonations
6.	RMT	Grey colour	Slightly cottony	Irregular	Slight zonations
7.	CMH	Grey with dark grey at centre sur-rounded by pinkish margin	Cottony growth	Regular	No zonations
8.	BYC	Light grey	Cottony growth	Regular	No zonations
9.	MNY	Light Grey with raised white center	Compact	Regular with grey margin	Slight zonations

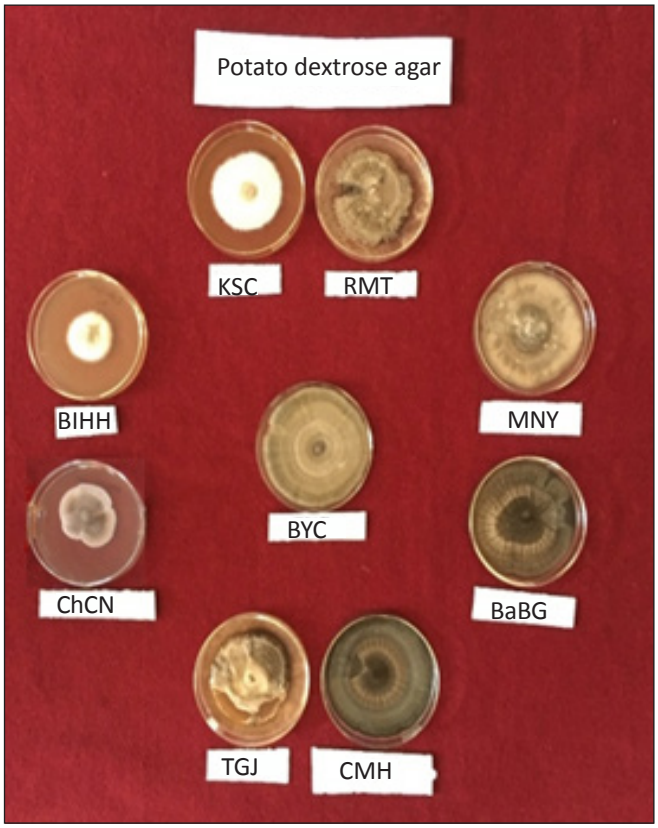

Figure 1: Colony characters of the isolates of isolates of *Alternaria solani* on Potato dextrose agar medium

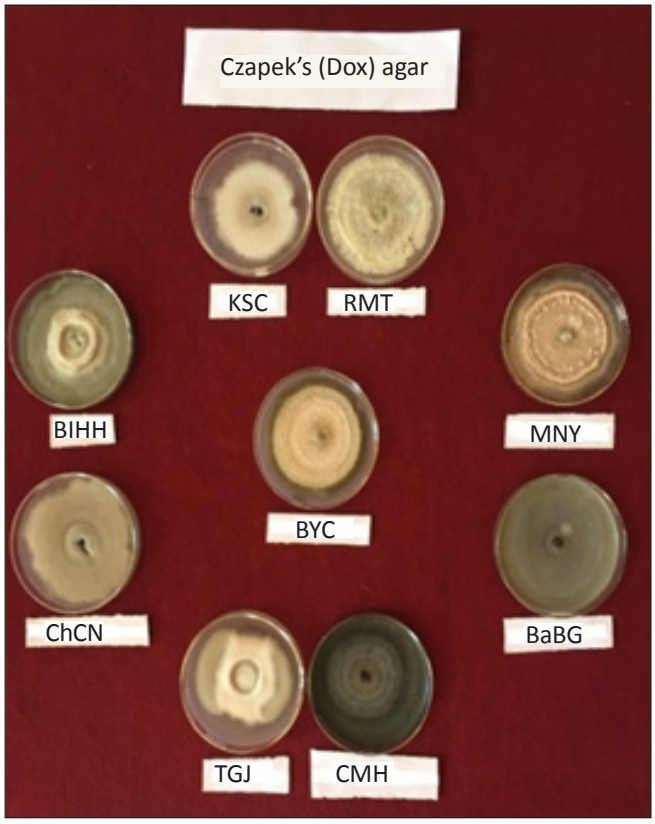

Figure 2: Colony characters of the isolates of *Alternaria solani* on Czapek's (Dox) agar medium

Table 3: Colony characteristics of different isolates of *Alternaria solani* on Czapek's (Dox) agar medium

Sl. No.	Isolate	Colony characteristics			
		Colony colour	Mycelial growth	Margin of the colony	Zonation
1.	BiHH	Grey colour with raised pinkish white at the center	Cottony	Regular	No zonations
2.	BaBG	Purple colour surrounded by a wide grey colour mar-gin	Slightly cottony	Regular	Zonations
3.	KSC	Bright white	Cottony	Irregular	No zonations
4.	ChCN	Dull grey colour	Cottony growth	Irregular	No zonations
5.	TGJ	Pinksih white surrounded by grey margin	Cottony	Regular	No zonations
6.	RMT	Whitish grey	Slightly compact	Regular	No zonations
7.	CMH	Dark grey with purplish center	Cottony	Irregular	No zonations
8.	BYC	Pinkish white surrounded by greenish white margin	Compact	Regular	No zonations
9.	MNY	Pink surrounded by grey margin	Compact	Regular	No zonations

Colony characters on V-8 juice agar: The studies showed that all the isolates had no zones except BaBG and CMH isolates (Table 4). The BiHH isolate produced pink coloured colonies with cottony mycelial growth whereas the colonies of BaBG and CMH appeared pinkish white with zonations. The KSC, TGJ and Bangalore isolates produced grey coloured colonies without any zonations. The colonies of ChCN and MNY isolates appeared dull white in colour with no zonations while the RMT isolate had bright white colonies without any zonations (Figure 3).

In the present studies, variability in cultural characteristics of different isolates of *A. solani* was noticed on three different solid media. Devi et al. (2017) found variability among the 16 isolates of *A. alternata* and four isolates of *A. solani*. Similar results obtained by Anil et al. (2017); Yadav et al. (2016); Waghunde et al. (2018) and Banne et al. (2021) which support our present findings.

Table 4: Colony characteristics of different isolates of *Alternaria solanion* V-8 juice agar medium

Sl. No.	Isolate	Colony characteristics			
		Colony colour	Mycelial growth	Margin of the colony	Zonation
1.	BiHH	Pink colour surrounded by dull grey mar-gin with raised dull white center	Cottony	Regular	No zonations
2.	BaBG	Pinkish white to dull white with dark grey colour at the center	Compact	Regular	Zonations
3.	KSC	Grey with a black margin	Slightly com-pact	Irregular pinkish grey margin	No zonations
4.	ChCN	Dull white with grey colour at the center	Cottony growth	Slightly irregular	No zonations
5.	TGJ	Grey surrounded pinkish grey margin	Compact	Regular	No zonations
6.	RMT	Bright white with pinkish white center	Cottony	Regular	No zonations
7.	CMH	Pinkish white surrounded by dull grey margin	Compact	Irregular	Zonations
8.	BYC	Grey colour surrounded by pinkish grey at the center	Compact	Regular	No zonations
9.	MNY	Dull white surrounded by pinkish white margin	Compact	Regular	No zonations

3.2. Variability in radial growth of the isolates of A. solani on different media

The results on the variability in radial growth of different isolates of the pathogen are presented in Table 5 and the results on individual medium are explained hereunder.

Among different isolates, maximum growth of 87.67 mm

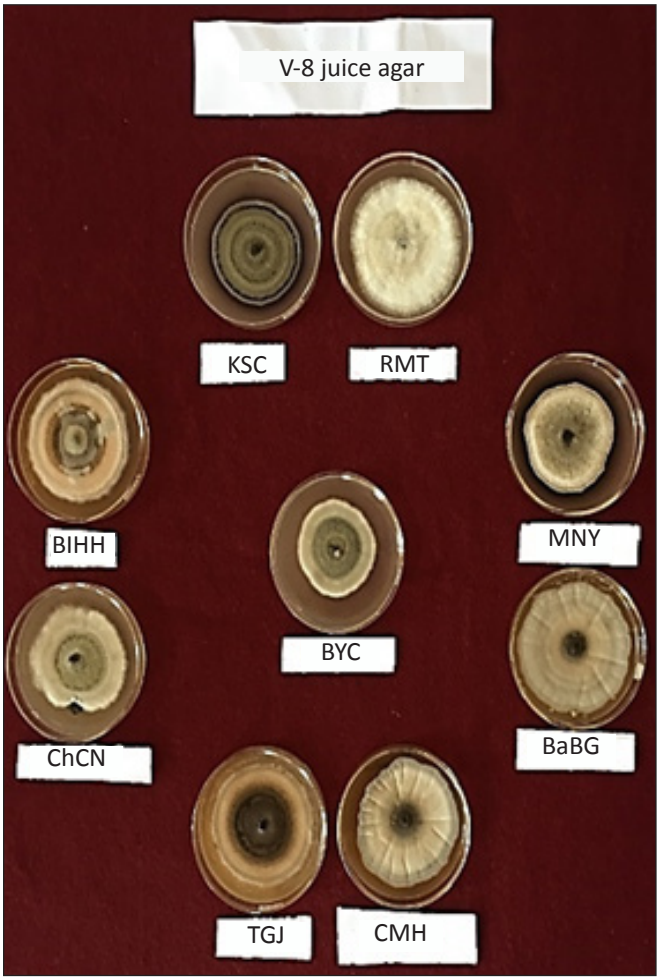

Figure 3: Colony characters of the *Alternaria solani* on V-8 juice agar medium

was noticed in CMH isolate on potato dextrose agar which was statistically significant over the growth of other isolates followed by BaBG (83.67 mm), BYC (78.33 mm), MNY (75.67 mm), RMT (70.00 mm) and TGJ (68.33 mm) isolates. The least growth was noticed in BiHH isolate (36.33 mm) followed by ChCN (54.00 mm) and KSC (55.67 mm) isolates.

On Czapek's (Dox) agar medium, maximum growth of 90.00 mm was recorded in the isolates CMH and BaBG which were statistically superior over the growth of other isolates followed by BiHH (87.67 mm), RMT (86.67 mm), KSC (87.00 mm), ChCN (80.33 mm) and BYC (77.33 mm). Minimum growth (62.67 mm) was observed in TGJ followed by MNY (64.67 mm) isolates.

In V-8 juice agar medium, maximum growth (75.67 mm) was recorded in BaBG isolate which was statistically significant over the growth of other isolates followed by TGJ (74.00 mm), BiHH (70.00 mm), RMT (69.33 mm), KSC and MNY isolates (62.67 mm). The least growth (46.67 mm) was recorded in CMH isolate followed by Bangalore isolate (52.00 mm).

From the mean data of different media, it was observed that

Table 5: Variability in growth of different isolates of *Alternaria solani*

Sl. No.	Isolate	Radial growth of isolates on different solid media (mm Ø)		
		Potato dextrose agar	Czapek's (Dox) agar	V-8 Juice agar
1.	BiHH	36.33	87.67	70.00
2.	BaBG	83.67	90.00	75.67
3.	KSC	55.67	87.00	62.67
4.	ChCN	54.00	80.33	60.33
5.	TGJ	68.33	62.67	74.00
6.	RMT	70.00	86.67	69.33
7.	CMH	87.67	90.00	46.67
8.	BYC	78.33	77.33	52.00
9.	MNY	75.67	64.67	62.67
Mean		67.74	80.70	63.70
SEm±		0.254	0.174	0.124
CD (*p*=0.01)		1.032	0.709	0.506

the medium Czapek's (Dox) agar was superior as maximum growth (80.70 mm) of the pathogen irrespective of the isolates was noticed in this medium followed by Potato dextrose agar (67.74 mm). The least growth of the pathogen was noticed in V-8 juice agar (63.70 mm).

In the present study, a lot of variability was found with respect to the radial growth of the different isolates of the pathogen *A. solani*. Mohsin et al. (2016) worked on variation studies in twenty-seven isolates of *Alternaria porri* and revealed that the isolates varied in colony diameter, colony and substrate colour, margin, topography, zonation, pigmentation and sporulation on different culture media. Similarly, Loganathan et al. (2016) observed variability in seventeen isolates of *Alternaria* spp based on culture colour and conidial dimensions. Results obtained by Banne et al. (2021), Mohsin et al. (2016), Aung et al. (2020); Luo et al. (2018); singh et al., 2014 and Pandey et al. (2021) are confirmatory to our present findings.

3.3. Morphological variability

Nine isolates of *Alternaria solani* showed morphological variability in respect of conidial length, conidial width, beak length and number of septa (Table 6). Average conidial length varied from 27.55 to 39.32 µm (range: 25.07–42.90 µm). Maximum conidial length (42.90 µm) was noticed in ChCN isolate whereas minimum was observed in TGJ isolate (25.07 µm). The average conidial width varied from 15.21 to 19.93 µm (range: 10.53–21.52 µm). Maximum conidial width (21.52 µm) was recorded in ChCN isolate while the minimum was observed in BiHH isolate (10.53 µm). Average beak length varied from 7.64 to 12.73 µm (range: 2.4 to 20.36 µm). Maximum beak length (20.36 µm) was observed in CMH

Table 6: Variability in spore morphology of different isolates of *Alternaria solanion* V-8 Juice agar

Sl. No.	Isolate	No. of transverse septation	Average	No. of longitudinal septation	Average	Length of conidia with beak (µm)	Average	Beak length (µm)	Average	Width of conidia (µm)	Average
1.	BiHH	3–4	3.7	1–2	1.9	30.02–32.23	32.34	3.4–14.30	9.58	10.53–17.90	15.21
2.	BaBG	3–5	4.2	1–3	2.2	33.00–35.04	34.09	2.7–12.32	8.13	12.02–18.23	17.12
3.	KSC	2–5	3.5	1–3	2.3	28.25–36.02	33.13	2.4–11.52	7.64	15.50–20.36	19.93
4.	ChCN	4–6	5.2	2–3	2.7	35.07–42.90	39.32	2.5–18.63	12.65	13.26–21.52	19.39
5.	TGJ	3–4	3.8	1–2	1.7	25.07–28.03	27.55	3.5–14.56	10.03	13.29–18.35	17.82
6.	RMT	2–3	2.8	2–4	3.2	28.80–32.25	31.52	5.2–17.25	12.25	15.30–17.03	16.35
7.	CMH	3–5	4.2	0–3	1.8	30.23–38.32	35.75	3.1–20.36	12.73	10.95–19.68	17.31
8.	BYC	4–7	5.8	2–3	2.8	33.25–38.32	36.85	4.23–13.85	10.40	12.23–18.60	16.45
9.	MNY	3–5	4.3	1–4	2.7	30.25–41.02	36.36	2.59–18.35	11.47	14.69–17.36	16.25
	SEm±		0.416		0.294		1.551		1.050		1.462
	CD (*p*=0.01)		1.692		1.197		6.313		4.273		5.951

isolate and minimum was observed KSC isolate (2.4 µm). The average number of transverse septa varied from 2.8 to 5.8 (range: 2–7). Maximum number of transverse septa (7) was noticed in BYC isolate and minimum (2) was noticed in RMT and KSC isolates. The average number of longitudinal septa varied from 1.8 to 3.2 (range: 0–4). Maximum number of longitudinal septa (4) was noticed in RMT and MNY isolates whereas longitudinal septum was not noticed in CMH isolate. Thus, the studies on spore morphology revealed variation in morphology of the pathogen. The smallest size of conidia was observed in TGJ isolate whereas, that of largest size was noticed in ChCN isolate.

In the present investigations, nine single-spore cultures of *Alternaria solani* showed morphological variability in respect of conidial length, conidial width, beak length and number of septa. The studies on spore morphology revealed that the smallest size of conidia was observed in TGJ isolate whereas, the largest size of conidia was noticed in ChCN isolate. Tymon et al. (2016) observed variability in conidial dimension and septa of *Alternaria* species associated with potato in the U.S. Northwest. Similar morphological variations among *A. solani* isolates had also been studied enormously by many researchers (Alhussaen, 2012; Singh et al., 2014; Verma et al., 2007; Kumar et al., 2008; Roopa et al., 2016; Parvin et al., 2021 and Pandey et al., 2021)

3.4. Biochemical variability

The enzymes peroxidase and esterase assist in overcoming the stress conditions in pathogens created by climatic factors or chemical which are used in the management practices. Peroxidase detoxifies the H_2O_2 and esterase detoxifies chemicals. The primary defence response in the host plant against a pathogen involves the rapid generation of reactive oxygen species (ROS), also known as oxidative burst. ROS include reduced and chemically reactive molecules, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and hydroperoxyl radical (HO_2) (Lehmann et al., 2015). ROS play an important role during the early stages of pathogen infection, which involves direct antimicrobial action, lignin formation, phytoalexin production, and SAR onset (Lamb and Dixon, 1997). The balance between ROS production and anti-oxidation is important for maintaining a healthy biological system (Davies, 2000). To mitigate the cell damage caused by ROS, plants express enzymes that scavenge excess ROS produced in cells under stressed conditions. These enzymes include superoxide dismutase, peroxidase, ascorbate peroxidase and catalase. Esterase helps in modification of cell wall during pathogen attack.

In present studies, diversity of pathogen was assessed by estimating the peroxidase and esterase activity using

spectrophotometer. Significant difference was found in the activity of these two enzymes in the isolates collected from different parts of Karnataka (Table 7; Figure 1). Peroxidase activity was higher in CMH isolate i.e., 81.80 Unit g^{-1} FW and least activity was found in BiHH isolate 11.78 Unit g^{-1} FW. Esterase activity was higher in BYC isolate 69.01 Unit g^{-1} FW and least activity was found in BaBG isolate 6.80 Unit g^{-1} FW. Similar results obtained by Shahbazi et al. (2010) on biochemical characterization of *Alternaria solani* isolates which showed variation with respect to peroxidase isoenzymes in different isolates collected from Iran. Similarly, Upadhyay et al. (2018) assessed the diversity among *Alternaria solani* isolates in tomato from India. Petrunak and Christ (1992) also noticed isozyme variability in isolates of *Alternaria solani* and *Alternaria alternate.*

Table 7: Esterase and peroxidase activity assay for different isolates of *Alternaria solani*

Sl. No.	Isolates	Esterase activity (Unit g^{-1} FW)	Peroxidase activity (Unit g^{-1} FW)
1.	BiHH	18.79	11.78
2.	BaBG	6.80	56.7
3.	KSC	58.97	54.10
4.	ChCN	25.27	49.60
5.	TGJ	21.38	45.20
6.	RMT	18.46	67.50
7.	CMH	52.67	81.80
8.	BYC	69.01	46.80
9.	MNY	49.25	79.00
	SEm±	0.441	0.568
	CD (*p*=0.01)	1.795	3.705

4. Conclusion

Nine isolates of *Alternaria solani* infecting tomato exhibited variation with respect to colour of the colony, mycelial growth, margin and zonations on diffent media. Among the media Czapek's (Dox) agar supported maximum mycelial growth. All the nine isolates differed morphologically with respect to conidial length, conidial width, beak length and number of septa. In addition, isolates exhibited varying capacity to produce esterase and peroxidase. The Esterase activity was more in Bengaluru isolate whereas peroxidase activity was more in Chikkamagaluru isolate.

5. References

Adhikari, P., Oh, Y., Panthee, D., 2017. Current status of early blight resistance in tomato: An Update. International Journal of Molecular Sciences 18(10), 1–22.

Ainsworth, G.C., 1961. Dictionary of fungi. common wealth mycological institute, Kew, Swerry. England, (5th Edn.) 54. Bib ID-849169.

Alhussaen, K.M., 2012. Morphological and physiological characterization of *Alternaria solani* isolated from tomato in jordan valley. Research Journal of Biological Sciences 7(8), 316–319.

Anil, G.H., Ashtaputre, S.A., Rao, M.S.L., 2017. Studies on morphological and cultural variability of *Alternaria* spp. causing leaf blight in cotton. International Journal of Plant Protection 10(2), 281–290.

Aung, S.L.L., Liu, H.F., Pei, D.F., Lu, B.B., Oo, M.M., Deng, J.X., 2020. Morphology and molecular characterization of a fungus from the *Alternaria alternata* Species Complex Causing Black Spots on *Pyrus sinkiangensis* (Koerle pear). Mycobiology 48(3), 233–239.

Banne, S.N., Sunita, J.M., Shruti, S.K., Suryawanshi, A.P., 2021. Cultural and morphological characterization of isolates of *Alternaria alternata* (Fr.) Keissler, causing chrysanthemum leaf blight. The Pharma Innovation Journal 10(1), 625–630.

Blancard, D., Laterrot, H., Marchoux, G., Candresse, T., 2012. A colour handbook-Tomato Diseases: identification, biology and control. Manson Publishing Limited (2nd Edn.) London UK, 688.

Burlina, A., Galzigna, L., 1972. A new and simple procedure for serum arylesterase. Clinica Chimica Acta 39(1), 255–257.

Chaerani, M., Kardin, K., Suhardi, Sofiari, E., Ginkel, R.V.V., Groenwolt, R., Voorrips, R.E., 2017. Variation in aggressiveness and aflp among *Alternaria solani* isolates from Indonesia. Indonesian Journal of Agricultural Science 18(2), 51–62.

Chowdappa, P., Chandramohanan, R., 1995. Electrophoretic protein patterns of three species of *Phytophthora* associated with black pod disease of cocoa (*Theobroma cacao* L.). Journal of Biosciences 20(5), 637–644.

Datar, V.V., Mayee, C.D., 1984. Assessment of loss in tomato yield due to early blight. Indian Phytopathology 34(2), 191–195.

Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses and damage removal, repair, and replacement systems. IUBMB Life 50(4–5), 279–289.

Devi, P.R., Prasadji, J.K., Srinivas, T., Rani, Y.A., Rao, G.R., 2017. Cultural, morphological and pathogenic variability in Alternaria spp. causing early blight, of tomato in Andhra Pradesh. Journal of Mycology and Plant Pathology 47(2), 176–192.

Freed, R.D, Scott, D.E., 1986. MSTATC crop and soil science department, Michigan State University, MI, USA.

Kumar, V., Haldar, S., Pandey, K.K., Singh, R.P., Singh, A.K., Singh, P.C., 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. World Journal of Microbiology and Biotechnology 24(7), 1003–1009.

Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Annual Review of Plant Physiology and Plant Molecular Biology 48**,** 251–275. https://doi.

org/ 10.1146/annurev.arplant.48.1.251

Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S.E., Metraux, J.P., 2015. Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry 112, 54–62. http://dx.doi.org/10.1016/j.phytochem.2014.08.027

Leiminger, J.H., Auinger, H.J., Wenig, M., Bahnweg, G., Hausladen, H., 2016. Genetic variability among *Alternaria solani* isolates from potatoes in southern germany based on RAPD-profiles. Journal of Plant Diseases and Protection 120, 164–172. https://doi.org/10.1007/BF03356470

Lin, C., Kao, C.H., 2001. Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedling. Plant and Soil 230(1), 135–143.

Loganathan, M., Venkataravanappa, V., Saha, S., Rai, A.B., Tripathi, S., Rai, R.K., Pandey, A.T., Chowdappa, P., 2016. Morphological, pathogenic and molecular characterizations of *Alternaria* species causing early blight of tomato in northern India. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 86(2), 325–330.

Luo, H, Xia, Z.Z, Chen, Y.Y., 2018. Morphology and molecular characterization of *Alternaria argyranthemi* on *Chrysanthemum coronarium* in China. Mycobiology 46(3), 278–282.

Mathur, K., Shekhawat, K.S., 1986. Chemical control of early blight in *kharif* tomato. Indian Journal of Mycology and Plant Pathology 16(2), 235–238.

Mohammadi, A., Bahramikia, S., 2019. Molecular identification and genetic variation of *Alternaria* species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods. Current Medical Mycology 5(2), 1–8.

Mohsin, S.M., Islam, R., Ahmmed, A.N.F., Nisha, H.A.C., Hasanuzzaman, M., 2016. Cultural, morphological and pathogenic characterization of *Alternaria porri* causing purple blotch of onion. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 44(1), 222–227.

Odilbekov, F., Edin, E., Garkava-Gustavsson, L., Hovmalm, H.P., Liljeroth, E., 2016. Genetic diversity and occurrence of the F129L substitutions among isolates of *Alternaria solani* in south-eastern Sweden. Hereditas 153, 1–10. https://doi.org/10.1186/s41065-016-0014-0

Pandey, P.K., Pandey, P.K., 2002. Field screening of different tomato germplasm lines against *Septoria, Alternaria* and bacterial disease complex at seedling stage. Indian Journal of Mycology and Plant Pathology 32(2), 234–235.

Pandey, I., Mondal, C., Sultana, S., Sultana, N., Aminuzzaman, F.M., 2021. Pathological survey on early leaf blight of tomato and *In Vitro* effect of culture media, temperature and ph on growth and sporulation of *Alternaria solani*. Open Access Library Journal 8(3), 1–17.

Peralta, I.E., Knapp, S., Spooner, D.M., 2005. New species of wild tomatoes (*Solanum lycopersicon*: Solanaceae) from Northern Peru. Systematic Botany 30, 424–434.

Petrunak, D.M., Christ, B.J., 1992. Isozyme variability in *Alternaria solani* and *Alternaria alternate*. Phytopathology 82(11), 1343–1347.

Pramila, P.G., Tasleem, M., Taj, G., Mal, R., Kumar, A., 2014. Morphological, cultural, pathogenic and molecular variability amongst Indian mustard isolates of *Alternaria brassicae* in Uttarakhand. African Journal of Biotechnology 13(3), 441–448.

Pryor, B.M., Gilbertson, R.L., 2002a. Relationships and taxonomic status of *Alternaria radicina, A. carotiincultae,* and *A. petroselini* based upon morphological, biochemical, and molecular characteristics. Mycologia 94(1), 49–61.

Pryor, B.M., Michailides, T.J., 2002b. Morphological, pathogenic and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. Phytopathology 92(4), 406–416.

Rangaswami, G., 1958. An agar blocks technique for isolating soil microorganisms with special reference to Pythiaceous fungi. Science and Culture 24, 85.

Riker, A.J., Riker, R.S., 1936. Introduction to research on plant diseases, St. Louis, Chicago, New York and Indianapolis, John's Swift Co., 117.

Roopa, S., Yadahalli, K.B., Kavyashree, M.C., 2016. Morphological and cultural characters of *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato. Biochemical and Cellular Archives 16(1), 125–129.

Rudnicka, M., Kochman, M., 1984. Purification of the juvenile hormone esterase from the haemolymph of the wax moth *Galleria mellonell* (Lepidoptera). Insect Biochemistry 14(2), 189–198.

Sahu, S., Sett, M., Kjellstrom, T., 2013. Heat exposure, cardiovascular stress and work productivity in rice harvesters in India: implications for a climate change future. Industrial health 51(4), 424–431. doi: 10.2486/indhealth.2013–0006

Shahbazi, H., Aminian, H., Sahebani, N., Halterman, D.A., 2010. Biochemical evaluation of resistance responses of potato to different isolates of *Alternaria solani.* Phytopathology 100(5), 454–459.

Sherf, A.F, Mac Nab, A.A., 1986. Vegetable Diseases and Their Control; John Wiley and Sons: New York, NY, USA, Pp 728.

Singh, A., Singh, V., Yadav, S.M., 2014. Cultural, morphological and pathogenic variability of *Alternaria solani* causing early blight in tomato. Plant Pathology Journal 13(3), 167–172.

Tong, Y.H., Liang, J.N., Xu, J.Y., 1994. Study on biology and pathogenicity of *Alternaria solani*. Phytopathology 78, 926–930.

Tuite, J., 1969. *Plant pathological methods*. Burgess Publishing Company, Minneapolis, Minnesota. 2nd edition, 239.

Tymon, L.S., Peever, T.L., Johnson, D.A., 2016. Identification and enumeration of small-spored *Alternaria* species associated with potato in the U.S. Northwest. Plant Disease 100(2), 465–472.

Upadhyay, P., Ganaie, S.H., Singh, N., 2018. Diversity assessment among *Alternaria solani* isolates causing early blight of tomato in India. Proceedings of the National Academy of Sciences, India, Section B: biological sciences 89(3), 987–997.

Walker, J.C., 1952. Diseases of vegetable crops, 1st edn. MacGraw-Hill, New York, 228–246.

Waghunde, R.R., Patel, U.T., Vahunia, B., 2018. Morphological and cultural variability of *Alternaria macrospora* causing leaf blight in cotton. Journal of Pharmacognosy and Phytochemistry 7(3), 3096–3099.

Yadav, S.P., Sonit, K., Rajendra, P., Hina, U., Monika, B., 2016. Identification of morphological, cultural and pathogenic variability of *Alternaria brassicae* causing Alternaria blight of Indian mustard (*Brassica juncea* L.). Indian Phytopathology 69(1), 1–5.

Van der waals, J.E., Korsten, L., Slippers, B., 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. Plant Disease 88, 959–964.

Verma, K.P., Singh, S., Gandhi, S.K., Chaudhary, K., 2007. Variability among *Alternaria solani* isolates associated with early blight of tomato. Indian Phytopathology 60(2), 180–186.