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MORPHOLOGICAL AND CULTURAL CHARACTERS OF *ALTERNARIA SOLANI* (ELLIS AND MARTIN) JONES AND GROUT CAUSING EARLY BLIGHT OF TOMATO

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ABSTRACT : Identification of the pathogen on the basis of morphology and cultural characters which helps us to know the nutrition and conditions that favours the pathogen. The *Alternaria* species obtained on cultural media showed conidiophores of the fungus, formed singly or in groups. Morphologically, pathogen produces straight or flexuous and brown to olivaceous brown conidiophores on which solitary straight or slightly flexuous, oblong or ellipsoidal conidia were produced. Determination of optimum growth period is essential to study the physiology of fungi. Maximum dry mycelial weight in the present study was obtained on 9th day of inoculation on potato dextrose broth (249.27mg), which is indicative of the optimum growth of the fungus. Maximum growth of the fungus was observed in PDA (89 mm). In the present study on the influence of temperature, *A. solani* showed maximum dry mycelial weight of 251.42 mg and growth of 88.7 mm at 25°C.

Key words : Tomato, *Alternaria solani,* early blight.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) regarded as poor man's apple, is one of the widely grown and most popular vegetable crop, in India and throughout the world. Among the vegetables, tomato ranks second, next only to potato, in world acreage and ranks first among the processing crops. Many factors operate in successful cultivation as well as marketing of quality tomato, of which diseases play an important role. Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease incurring loss both at pre and post harvest stages in tomato growing tracks of India, so also in Karnataka. The pathogen *Alternaria solani* belongs to class Deuteromycetes, order Moniliales, family Dematiacea, genus *Alternaria* and species *Solani*. The causal organism is air borne and soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). Looking in to this economically important disease, the attempt has been made to identify pathogen on the basis of morphology and cultural characters which helps us to know the nutrition and conditions that favours the pathogen.

MATERIALS AND METHODS

Identification of the fungus was carried out based on the morphological characters of the isolated fungus.

Studies on the growth phase of the pathogen

The flasks inoculated with fungus were incubated at 27±1°C. A set of three flasks were harvested starting from first day up to 14th day after inoculation. The culture was filtered through Whatman No.1 filter paper. Before filtering the filter papers were dried to a constant weight by drying in hot air oven at 60°C. The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed immediately on an analytical balance. The difference between final and initial weight of filter disc was taken as the weight of the mycelial mat. The data were analyzed statistically and maximum growth period was determined. Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper.

Growth characters of *Alternaria solani* on different solid media

The cultural characters for the pathogen were studied on the following solid media *viz*.

1. PDA - Potato Dextrose Agar
2. Richard's Agar
3. Czapek's Dox Agar
4. Sabouraud's dextrose Agar
5. Corn meal Agar
6. Oat meal Agar
7. Fungal agar

8. Host leaf extract Agar
9. V8 Juice Agar
10. Potato Carrot Agar

I. Potato Dextrose Agar (PDA)

In most of the experimental studies the Potato dextrose agar (PDA) was used. The composition of PDA is as follows

Potato (peeled)	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000.00ml (to make up)

Two hundred grams of peeled and cleaned potato was made into small pieces. Later these pieces were boiled in distilled water and then extract was collected by filtering through muslin cloth. Dextrose 20.0 g and agar 20.0 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water. Known quantity of such medium was dispensed into a number of conical flasks and plugged with non-absorbent cotton and finally wrapped with brown paper. The flask containing dispensed medium were sterilized at 1.1 kg/cm^2 pressure for 20 minutes.

II. Richards' agar

Sucrose ($C_{12}H_{22}O_{11}$)	20.00 g
Potassium nitrate (KNO_3)	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	5.00 g
Magnesium sulphate ($MgSO_4$. $7H_2O$)	2.50 g
Ferric chloride ($FeCl_3$. $6H_2O$)	0.02 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients except Potassium dihydrogen phosphate was dissolved separately in 450 ml of distilled water. Agar melted in 500 ml distilled water was mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml water and mixed together to get final volume of 1000 ml and sterilized.

III. Czapek's (Dox) agar

Sucrose ($C_6H_{12}O_6$)	30.00 g
Sodium nitrate ($NaNO_3$)	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Magnesium sulphate ($MgSO_4$. $2H_2O$)	0.50 g
Potassium chloride (KCl)	0.50 g
Ferric chloride ($FeCl_3$. $6H_2O$)	0.01 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

Agar-agar was melted in 500 ml distilled water. Ingredients were dissolved in remaining 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml and sterilized.

IV. Sabouraud's dextrose agar

Dextrose ($C_6H_{12}O_6$)	40.00 g
Peptone	10.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to one litre and sterilized as described earlier.

V. Corn meal agar

Corn meal	60 g
Agar	15 g
Distilled water	1000 ml

Sixty grams of powdered corn meal was placed in clean muslin bag and tied. The bag was steamed in 500 ml of distilled water in a beaker for one hour. Agar was melted separately in 450 ml of distilled water. The boiled corn meal was then strained into the melted agar and the volume was made to 1000ml and then sterilized.

VI. Oatmeal agar

Oat flakes	30.00 g
Agar agar	20.00 g
Distilled water	1000.00 ml (to make up)

First oat flakes were boiled in 500 ml distilled water for thirty minutes and filtered through muslin cloth. Agar agar was melted in 500 ml of water separately and then both the solutions were mixed thoroughly and the volume was made up to one liter and sterilized.

VII. Fungal agar

Papaic digest of soyabean meal	10g
Dextrose	10g
Agar	15g

Suspended 35g of fungal agar in 1000ml distilled water. Heat to boiling the media to dissolve completely. Sterilize by autoclaving at 15lbs pressure (121ºC) for 15 minutes.

VIII. Host leaf extract agar

Healthy tomato leaves	200 g
Agar	15 g
Distilled water	1000 ml

Tomato leaves were boiled in 500 ml of water for 30 minutes. Extracts was collected by filtering through muslin cloth. The agar was melted in 500 ml of water. Both the solutions were mixed and the volume was made to 1000 ml and sterilized.

IX. V-8 Agar

L- Aspargine	10g
V-8 Juice (100ml)	8.30g
Yeast extract	2g
Calcium carbonate	2g
Glucose	2g
Agar	20g
Distill water	1000 ml
Suspend	44.3g

Dissolve 44.3g in 1000ml distilled water. Sterilize by autoclaving at 15lbs pressure (121ºC) for 15 minutes. If slight precipitate appears after sterilization shakes well to suspend the precipitate evenly before dispensing

X. Potato carrot agar

Grated potato	20 g
Grated carrot	20 g
Agar agar	20 g

Distilled water 1000 ml (volume to make up)

Boil grated vegetables for 1 hr. in the tap water. Strain through fine sieve and add agar. Boil over water bath till agar dissolves, sterilize at 15 p.s.i. for 20 min.

Twenty ml of each medium listed above was poured into 90 mm diameter Petri plates. After solidification, five mm discs of the *A. solani* were selected from actively growing culture using a cork borer and a single disc placed at the center of Petridish. Each set of experiment replicated three and they were incubated at 27 ± 1°C for 9 days. The colony diameter in the culture plates and cultural character such as colony diameter, colour, type of margin, aerial growth sporulation were also recorded. The sporulation was graded as follows.

Sl. No.	Score	Grade	Description (conidia/microscopicfiled [100 X])
1.	++++	Excellent	> 75
2.	+++	Good	50-75
3.	++	Fair	25-50
4.	+	Poor	1-25
5.	-	No sporulation	—

Effect of temperature on the growth of *A. solani*

Effect of temperature on the growth of fungus was tested at 15, 20, 25, 30, 35 and 40°C. Thirty ml of potato dextrose broth (PDB) was added into each of 100 ml conical flask and sterilized at 1.1 kg/cm^2 pressure for 20 minutes at 121°C. After sterilization these flasks were allowed to cool and then inoculated with 5 mm disc from 9 days old culture and incubated at 27 ± 1° C for nine days. For each temperature level four replications were maintained. After incubation period the culture was filtered through Whatman No. 42 filter paper of 9 cm diameter. Dry mycelial weight was recorded for each flask as described earlier.

RESULTS AND DISCUSSION

The description of the fungus isolated is as follows. The conidiophores were formed singly or in groups, straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 150-300 ìm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks were flexuous, pale and sometimes branched. The description of this fungus agreed with the description given for *Alternaria solani* by Common Wealth Mycological institute, Kew, Surrey, England (Ellis, 1971). Thus, the pathogen causing early blight of tomato has been identified as *Alternaria solani* (Ellis and Martin) Jones and Grout.

Studies on the growth phase of the pathogen

Growth and sporulation of *Alternaria solani* on different solid media

Fungi secure food and energy from the substrate upon which they live in nature. Not all media are not equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow. So, different media were tried to study the variation in growth and cultural characteristics of *A. solani* the results have been presented in Table 2. Among ten solid media maximum growth was observed on PDA (89 mm), followed by Richards agar (79.50 mm) after 9 days of inoculation which may be attributed to complex nature of natural media supporting good fungal growth. The results are on par with the results obtained in an experiment conducted by Gemawat and Ghosh (1980), where as among seven solid media tested for growth of *A. solani* PDA showed good growth with excellent sporulation after 10 days of incubation. Bonde (1929), Neergaard (1945), Mazzonetto *et al* (1996) and Arunkumar (2006) observed potato dextrose agar as the best medium for *Alternaria* spp. Morphological variations such as colony colour, margin of colony and substrate colour were noticed on *A. solani*. Majority of isolates produced brown and grey pigmentation in the culture media. The results are in

Table 1 : Growth phase studies of *Alternaria solani* in Potato Dextrose Broth.

Intervals (days)	Dry mycelial weight (mg)
1	57.62
2	92.48
3	109.33
4	155.10
5	184.77
6	213.80
7	234.83
8	244.00
9	249.27
10	236.23
11	227.78
12	221.70
13	220.47
14	205.80
S.Em.±	0.96
C.D. at 1%	2.65

Cultural studies

Studies on the growth phase of the pathogen

The fungus was grown on potato dextrose broth in 100 ml conical flasks containing 30 ml of the medium in order to study the growth pattern of *Alternaria solani.* The mycelial growth from inoculated flasks was harvested everyday starting from first day up to 14th day. Maximum dry mycelial weight in the present study was obtained on 9th day of inoculation on potato dextrose broth (249.27 mg), which is indicative of the optimum growth of the fungus. Afterwards the growth declined gradually with the increment in number of days of incubation (Table 1). This may be possibly due to autolysis of the fungus and exhaustion of nutrients in the medium as opined by Lilly and Barnett (1951) who also pointed out that the growth of the fungus as in other organisms follow a definite pattern which depend on species, environmental and nutritional conditions. The present studies are also in conformity with Kulkarni (1998) and also Arunkumar (2006) who found that the maximum growth of fungus was attained on 7th day of incubation.

Table 2 : Cultural and morphological characters of *Alternaria solani* on different solid media.

Different Media	Mycelium				Sporulation
	Radial growth (mm)	Colour	Type of growth	Topography	
Oatmeal agar	72.8	Dull white	Flat growth circular	Merged	+
Corn meal agar	23.2	Dark brown	Sparse, irregular	Merged	+++
Fungal agar	24.2	Grey at centre and white at margin	Smooth, irregular	Submerged	-
Czapek's (Dox) agar	44.0	White	Smooth, irregular	Submerged	-
Potato dextrose agar	89.0	Grey	Smooth, irregular	Aerial	+++
Potato carrot agar	33.0	Dark grey	Smooth, irregular	Merged	++++
Sabouraud's dextrose agar	55.2	Grey	Rough, irregular	Submerged	+
Richards's agar	79.5	Dull white	Smooth, irregular	Aerial	+
V-8 Juice agar	39.5	Grey	Smooth, regular	Merged	++
Host leaf extract agar	73.0	white	Smooth, regular	Aerial	++
S.Em. ±	0.14				
C.D. at 1%	0.39				

Sporulation:	Conidia/microscopic field (100x)
++++	> 75
+++	50 – 75
++	25 – 50
+	1 – 25
-	No conidia

confirmation with Bonde (1929), Henning and Alexander (1959) and Kaul and Saxena (1988) they also observed differences in cultural characters like growth rate, type of growth, colony colour, colour of the substrate and sporulation on different media.

Cultural characteristics of *Alternaria solani* on different solid media

The radial growth, colony characters and sporulation of the fungi were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungus are presented in Table 2. Maximum radial growth of *A. solani* was recorded on potato dextrose agar (89.00 mm), which

Table 3 : Effect of temperature on growth of *Alternaria solani* on PDB and PDA.

Temperature (°C)	PDB	PDA	
	Dry mycelial weight (mg)	Radial growth (mm)	Sporulation
15	55.44	0.00	-
20	163.25	43.9	++
25	251.42	88.7	++++
30	227.66	52.5	+++
35	89.82	12.8	+
40	57.81	0.00	-
S.Em. ±	1.19	0.01	
C.D. at 1%	3.42	0.03	

Speculation: Conidia/microscopic field (100x)
++++ > 75
+++ 50 – 75
++ 25 – 50
+ 1 – 25
- No conidia

was found to be significantly superior to all other media followed by Richards's agar (79.50 mm), Host leaf extract agar (73.00mm), Oat meal agar (72.8mm), Sabouraud's dextrose agar (55.2mm), Czapek (Dox) agar (44.00 mm), V-8 juice agar (39.50), Potato carrot agar (33.0 mm). The least radial growth was recorded in fungal agar (24.2) and corn meal agar (23.2 mm). Mycelium colour varied from white to black. The growth varied from flat, raised fluffy to sparse. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation. Excellent sporulation was recorded on potato carrot agar and good sporulation in potato dextrose agar and cornmeal agar. No sporulation was recorded in fungal agar and Czapek's (Dox) agar. Several workers notably Bonde (1929), Henning and Alexander (1959) and Kaul and Saxena (1988) also observed differences in cultural characters like growth rate, type of growth, colony colour, colour of the substrate and sporulation on different media.

Effect of temperature on growth of *Alternaria solani* on solid and liquid media

A. solani was grown on potato dextrose broth and potato dextrose agar at six temperature levels *viz.,* 15, 20, 25, 30, 35 and 40°C to know the optimum temperature required for maximum mycelial growth and sporulation. Growth of *A. solani* on PDA showed gradual increase as temperature increased from 15 to 30° C and later declined with further increase in temperature. The maximum mycelial growth was recorded at 25° C (88.7 mm), which was significantly superior to all other temperatures tested, followed by 30° C (52.5 mm), 20°C (43.9 mm) and no growth was found in 15°C and 40°C. The growth differences at all the temperatures were statistically significant from each other. The sporulation was excellent at 25°C, good at 30°C and moderate at 20° C. Poor sporulation was recorded at 35° C and no sporulation at 15°C and 40°C. The maximum growth on potato dextrose broth showed at 25°C which found to be significantly superior to other temperature levels by recording the maximum dry mycelial weight (251.42 mg) followed by 30° C (227.66mg). The least dry mycelial weight was recorded at 15° C (55.44 mg). Further, dry mycelial weight was at 35° C (89.82 mg) and at 40°C (57.81 mg) (Table 3). Kaul and Saxena (1988) also reported the temperature of 25°C being good for the growth of *A. solani*. Choudhary (1944) observed maximum growth of *A. carthami* at 30°C and 25°C. Bonde (1929) and Arunkumar (2006) reported that growth rate of *A. solani* ranged from 15-40°C and they observed maximum growth of the fungus at 30°C.

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