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Isolation, pathogenicity and suitability of different culture media on growth and sporulation of *Alternaria solani* (ELL. & MART.) Inciting early blight disease of tomato

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Abstract

Early blight is the most common and destructive disease of tomato plants caused by the fungus *Alternaria solani*. Six culture media were used in this study, namely PDA, Glucose Peptone Yeast Agar, Malt Extract Agar, Tomato Leaf Extract, Oatmeal Agar and Dox Czapek agar extract and evaluated the suitability of the culture medium for fungal growth. It has been noted that PDA and Tomato Leaf Extract Agar promotes pathogen development through the formation of radial growth of 9.0 cm and mainly 8.5 cm within 10 DAI. Oatmeal agar also appeared to be the best medium after PDA and Tomato Leaf Extract Agar followed by Czapek's Dox Agar. The least supported medium was glucose peptone agar with radial growth of fungi of only 4.00 cm. The colony ranged in color from white, cream and olive green to black. When sporulated, oat agar and potato dextrose agar showed excellent sporulation. While on Tomato Leaf Extract Agar and Czapek Dox Agar it was bad.

Keywords: Early blight, PDA, tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the most important vegetable crop in India. It is a widely used vegetable because of its high nutrition and nutritional values. However, India faces a serious problem of diseases that severely affect tomato productivity. Among the leaf diseases of tomatoes, one of the major diseases is the early blight disease caused by *Alternaria solani*. The first symptoms of the early blight disease caused by *Alternaria solani* in tomatoes appeared as small brown water-soaked lesions on the older leaf. Older leaves become infected first and then move upwards. The spots were oval or square in shape, ranging from 1 to 5 mm in diameter. A narrow chlorotic zone was also found around the site. Later stage points were enlarged and concentric rings formed in the middle. In advance, the color of the spots was changed from brown to dark brown and eventually adjacent spots merged to form large irregular spots. A severe attack of the disease causes the leaves to dry out and defoliate. When the plants were old, symptoms appeared on stems and petioles as brown to dark brown, elongated, target plate-type spots. The *Alternaria solani* colony was observed to be grayish-black in color with a circular edge and rough surface and to have a dark colored mycelium. They produce short, simple, upright conidiophores that carry single or branched chains of conidia. The conidiophores were formed individually and in groups, they were straight or pliable, and brown to olive-brown in color. The conidia were individually observed to be straight and slightly flexible, elongated or muriform or ellipsoid, and tapered to beak, pale or olive brown, length 150-300µm and 15-20µm thick in the broadest part with 8-10 transverse and 0-4 Longitudinal septa. The beaks of the conidia were pliable, pale, and sometimes branched.

2. Materials and Methods**2.1 Examination of diseased samples****2.2 Visual observations**

Visual observations of disease symptoms were recorded in the field in order to learn about the development of the disease in the plant population *in vivo*.

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2.3 Disease sample

The leaf samples showing typical symptoms were taken from Agriculture Farm, BHU and collected in polythene bags and sealed. They were brought to laboratory for isolation of pathogen.

2.4 Microscopic examination

These samples were then rinsed with tap water to remove foreign matter. Temporary abutments were made from diseased specimens in lactophenol cotton blue and examined under a compound microscope for the presence of microorganisms, if any.

2.5 Isolation of causal organism

Small pieces of the desired size were cut from infected samples, making sure that each piece contained half of the infected and half healthy. These pieces were then disinfected with 0.1% mercury chloride (HgCl_2) for 1 minute, followed by three washes in sterile distilled water to remove traces of mercury chloride. These pieces were then placed in sterilized pads to dry. The suitably dried pieces were aseptically transferred to sterile Petri dishes containing solidified sterilized PDA medium. The plates were incubated in a BOD incubator at 24 ± 1 °C until the fungal mycelium completely covered the surface of the medium. The obtained fungal growth was then transferred to tilted PDAs and stored as a stock culture for further study.

2.6 Pathogenicity test of the isolated organism

To confirm the identification of an early blight disease and its causative agent, a pathogenicity test was performed in an open container. 30-day-old tomato seedlings were transplanted into pots. The pathogen from a 10-day culture grown on a PDA was added to 100 ml of sterile distilled water and shaken appropriately to release the conidia. The suspension was filtered using gauze to remove PDA. A conidia suspension was prepared in 3×10^6 ml⁻¹ of pathogen spores. The spore suspension was sprayed on the tomato plants 20 days after transplanting the seedlings and the inoculated tomato plants were sprayed with sterile water for 2 days to maintain optimal humidity. Symptoms were observed in plants inoculated with *A. solani* and compared with the initial symptoms of early disease. The pathogen was re-isolated from artificially inoculated tomato leaves and the morphological and cultural properties were compared with those of the original pathogen.

2.7 Effect of culture media on *A. solani*

The medium was prepared with the specified composition. The initial pH of each medium was adjusted to 6.5 before autoclaving. The medium was prepared with the specified composition and poured into an Erlenmeyer flask. The bottles were sealed with non-absorbent cotton swabs and autoclaved at 15 pounds. Psi for 20 minutes. The petri dishes were sterilized in a hot air drying oven at 160 °C for 1 hour. The sterilized Petri dishes were filled with 20 ml of molten medium and allowed to solidify. A disc of the examined fungus with a diameter of 5 mm was cut out with a burnt cork tip and inoculated in the center of the Petri dishes. The seeded plates were then incubated for 7 days at room temperature (27 ± 20 °C.). All multimedia compositions used come from the Ainsworth and Bisby's Dictionary, as noted below in Table 1.

20 ml of each medium was poured into 90 mm Petri dishes. The 5 mm disc of the fungal culture was cut from the pure

culture margin of the 7-day-old *Alternaria solani*, which had been grown on a PDA using a sterilized cork drill. A culture disk was placed upside down in the center of each Petri dish containing different culture media; H. Potato dextrose agar medium (PDA), tomato leaf extract medium, malt extract medium agar, glucose peptone yeast medium, oat meal medium and Czapek Dox Agar medium and 7-10 days at 25 ± 2 °C Incubated. The subsequent rate of mycelium growth was recorded after incubation. Three replicates were maintained for each medium.

Table 1: Media used and their composition

1)	Potato Dextrose Agar Medium (PDA)	
i)	Potato	:200g
ii)	Dextrose	:20g
iii)	Agar	:20g
iv)	Distilled water	:1000ml
2)	Tomato Leaf Extract Agar Medium (TLEA)	
i)	Healthy tomato leaves (Green)	:200g
ii)	Agar	:20g
iii)	Distilled water	:1000ml
3)	Malt Extract Agar Medium (MEA)	
i)	Malt extract	:20g
ii)	Glucose	:5g
iii)	Peptone	:1g
iv)	Agar	:20g
v)	Distilled Water	:1000ml
4)	Glucose Peptone Yeast Agar Medium (GPYA)	
i)	Glucose	:40g
ii)	Peptone	:5g
iii)	Yeast extract	:5g
iv)	Agar	:15g
v)	Distilled Water	:1000ml
5)	Oat Meal Agar Medium (OMA)	
i)	Rolled oats	:40g
ii)	Agar	:20g
iii)	Distilled water	:1000ml
6)	Czapek's Dox Agar Medium (CDA)	
i)	Sodium nitrate (NaNO_3)	:2g
ii)	Potassium dihydrogen phosphate (K_2HPO_4)	:1g
iii)	Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:0.5g
iv)	Ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	:0.01g
v)	Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	:20g
vi)	Agar	:20g
vii)	Distilled water	:1000 ml

2.8 Statistical analysis

The data obtained were statistically analysed by the methods suggested by Gomez and Gomez (1986). The standard error and critical difference were worked out and the results obtained were compared statistically.

3. Results and Discussion

3.1 Examination of diseased samples

3.1.1 Visual observation

An early blight tomato disease (*Solanum lycopersicum* L.) caused by *Alternaria solani* (Ell. & Mart.) Has been detected in moderate to severe on a farm at the Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh. The disease initially appeared as small, round, dark yellow spots on the lower leaves. These spots later expanded into circular areas with concentric rings and may have been surrounded by a yellow halo. Subsequently, these spots enlarged and formed lesions ranging from gray to black with a diameter of 0.5 to 1 cm. As the disease progressed, the lesions were accompanied by a pattern of the target plate due to the formation of many

concentric rings with wavy edges. The centers were covered in masses of black soot spores, which later fell off, forming holes.

3.1.2 Microscopic examination

Temporary mounts were made from affected cotton samples with lactophenol blue. Microscopic examination revealed the presence of fungal structures such as mycelium and conidia. Conidia were riveted to a long beak with transverse (10-11) and longitudinal (2-3) septa. The conidia are slightly narrowed in the direction of the transverse septa. The conidia were light brown to gray in color and measured $103.0-141.0 \times 11.63-16.85 \mu\text{m}$. The length of the beak is $42.35-71.57 \mu\text{m}$.

3.2 Isolation and proving pathogenicity

The pathogen was successfully isolated on potato-dextrose agar medium from diseased tissue showing well-developed lesions along with a healthy portion transported to the laboratory from naturally infected tomato plants. The seeded plates were incubated in a BOD incubator for 5-7 days at $25 \pm 20^\circ\text{C}$. The fungus culture obtained by isolating it from the diseased tissue was transferred to the PDA in Petri dishes and replicated in the laboratory.

3.3 Purification of fungal culture

After seven days of incubation, the test fungus produced greenish-gray to black, loose, crumbly cotton growths on potato-dextrose agar medium. The pure culture phases were sealed with paraffin and stored in the refrigerator for later use in the laboratory.

3.4 Suitability of different culture media

Six different culture media were used in this study, namely Potato Dextrose Agar (PDA) Agar, Yeast Peptone Glucose Agar, Malt Extract Agar, Tomato Leaf Extract Agar, Oatmeal Agar Medium extraction of Czapek's Dox agar is evaluated for the suitability of the culture medium for the growth of fungi. The final growth that develops on different types of culture media is shown in Table 1. Potato Dextrose Agar (PDA) Agar and Tomato Leaf Extract Agar were found to contribute significantly to the development of pathogens through the formation of outgrowths. Radial 9.0cm and 8.5cm within 10 DAI. Oat meal Agar was also the best medium after PDA and Tomato Leaf Extract, followed by Czapek's Dox Agar. The least supported medium was agar medium with glucose-peptone-yeast agar with radial growth of fungi of only 4.00 cm which was showed in the table 2 and fig 1.

Table 2: Suitability of different culture media

S. No.	Culture Media	Radial growth (cm) at different interval				
		2 DAI	4 DAI	6 DAI	8 DAI	10 DAI
1.	Potato Dextrose Agar Medium	2.30	4.93	6.70	8.00	9.00
2.	Tomato Leaf Extract Agar Medium	2.00	4.70	6.33	7.50	8.50
3.	Malt Extract Agar Medium	1.63	3.00	4.60	5.63	6.30
4.	Glucose Peptone Yeast Agar Medium	1.30	2.53	2.90	3.53	4.00
5.	Oat Meal Agar Medium	2.10	4.70	6.41	7.70	8.00
6.	Czapek's Dox Agar Medium	1.70	3.23	4.63	6.27	7.00
	S.Em \pm	0.099	0.085	0.163	0.119	0.121
	CD at 5%	0.307	0.264	0.507	0.369	0.377

The results obtained in this study are supported by the find of several employees. Prasanna *et al.* (2018) tested the growth of

fungi in 11 different solid culture media and studied their maximum growth. They showed that the diameter of the fungal colonies was highest on potato dextrose agar medium (9cm), followed by oat meal agar medium (7.6 cm) and Richard's agar medium (5.1 cm), they reported. While PDA is a suitable culture medium for the growth and sporulation of *Alternaria* spp.

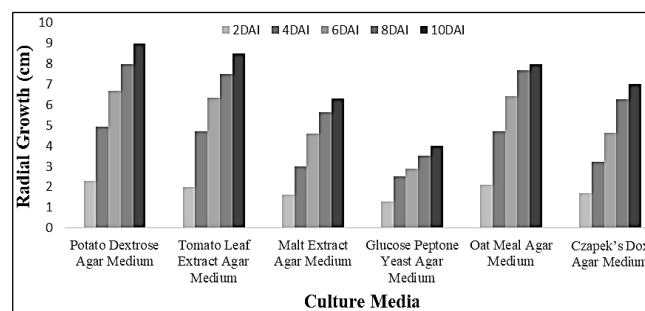


Fig 1: Suitability of different culture media for growth of *Alternaria solani*

Valvi *et al.* (2019) investigated eight different culture media for the growth of *Alternaria brassicae*, including PDA medium (9 cm), which proved to be more suitable and promoted maximum radial mycelium growth, followed by medium with host extract (8.7 cm) and oat meal agar (7.1 cm).

4. Conclusion

In conclusion, on the basis of the results of present study it can be concluded that early blight of tomato caused by *Alternaria solani* (Ell. & Mart.) is an important disease of tomato in varanasi region. Among the various biotic factors responsible for low production and productivity of tomato, *Alternaria solani* is one of the constraints.

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