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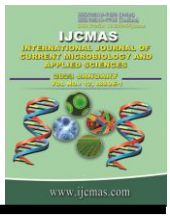

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In vitro Physiological and Morphological Characterization of *Alternaria solani* in Chhattisgarh, India

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A B S T R A C T

Early blight of tomato is found to be one of the most devastating diseases of tomato, hence a roving survey was conducted in 3 districts of Chhattisgarh and maximum incidence was observed in Rampur village of Durg district and the disease incidence was least in khichri village of Raigarh. Further characterization of the causal agent, *Alternaria solani* revealed that PDA was the best media for the mycelial growth which was followed by OMA and HEA while the growth was best at pH 7.5 and minimal at 4.5 pH. Potassium nitrate and glucose were found to be the best nitrogen and carbon source for growth. 7 different colours were evaluated to find its effect on *A. solani* and it was found that blue light gave maximum mycelial growth while minimum growth was obtained in orange colour. The colony characteristics under various treatments also varied accordingly.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops of both tropics and subtropics of the world, belongs to the family Solanaceae. It is herbaceous annual plant with bisexual flowers. Tomato is the world's largest vegetable crop and known as protective food both because of its special nutritive value as well as also for its wide spread production.

Tomato is being extensively grown as an annual plant all over the world. Tomato as a crop also gains its importance due to its value addition and production of various value-added products such as sauce, ketchup, soup, puree, etc. The yield of tomatoes is highly affected by various disease infestations, *Alternaria* blight is one of the main diseases which cause a drastic reduction in yield and quality of fruits produced. The disease is characterized by light or dark spots on older leaves which further expands with characteristics of target board appearance or bull eye appearance.

During survey it was found that variability existed between the various strains of *A. solani* thus the study was focused on its physiological and morphological characterization so that proper control measures could be adopted on the basis of the various character shown by *Alternaria.*

Materials and Methods

Survey on severity of early blight of tomato in different districts of Chhattisgarh

To determine the severity of the tomato early blight disease, a comprehensive roaming survey was carried out in the Chhattisgarh districts of Durg and Raigarh during the Rabi 2021–2022 season. Random localities where the tomatoes were cultivated were chosen in each district. The table2.1 provides details.

Percent Disease Incidence (PDI) was worked out by using formula given by (Yengkhom *et al.,* 2016).

$$\text{Percent disease Incidence (PDI)} = \frac{\text{Number of Plants infected by a particular disease}}{\text{Total number of Plants observed}} \times 100$$

Effect of solid culture media on *A. solani*

The experiment was carried out to determine the impact of various cultural media on the cultural traits of *A. solani* isolates. A 90 mm glass Petri plate was poured with 20 ml of each type of medium. A five-mm disc of fungus was taken from the margin with the help of a sterilised cork borer of *A. solani* culture that had been growing on PDA for seven days. In the middle of each petri plate, a culture disc was placed inverted. At 25±2^{0}C, the petri plates were incubated. For every media, three replications were kept (each replication having three Petri plates). The investigation used the following solid media. The following provides information about cultural media's composition and preparation process:

Potato Dextrose Agar Medium (PDA)

Tomato Leaf Extract Agar Medium (TLEA)

Malt extract Agar Medium (MEA)

Oat meal Agar Medium (OMA)

Asthana and Hawker's Medium (AH)

Czapek's Dox Agar Medium (CDA)

Sabouraud's Agar Medium (SA)

Richard's Agar Medium (RA)

Effect of pH on *Alternaria solani*

To ascertain how pH levels affects the growth properties of *Alternaria solani* isolate, from village Linjir on potato dextrose agar medium, tests were conducted at pH values of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. Before sterilisation, the pH of the medium was adjusted using a digital pH metre and the addition of 0.1N HCL and 0.1N NaOH. Different pH-level sterilised medium were poured to the sterilised Petri plates in quantities of around 20 ml, and they were left to solidify. With the use of a sterilised cork borer, 5mm culture discs were cut from the border of a 7-day-old pure culture of *Alternaria solani* isolate. In the middle of each petri plate, one culture disc was placed inverted. These petri plates were kept at 25±2^{0}C. in the BOD incubator. For each pH level, the three replications were maintained (Ramjegathesh and Ebenzer, 2012).

Effect of Nitrogen sources on *Alternaria solani*

To ascertain if nitrogen source influences *Alternaria solani*'s growth properties, the effects of nitrogen sources were assessed. The nitrogen requirement of the fungus was studied in Czapek's Dox medium. Based on their molecular weights, the amount of nitrogen compounds added was calculated to supply the same amount of nitrogen as the potassium nitrate contained in the basal medium. Ammonium sulphate, potassium, sodium, urea, magnesium, and glycine were the nitrogen sources used in the study. The sterilised melted warm medium was poured in the sterilised petri plates and allowed to solidify. As a control, Czapek's Dox medium devoid of a nitrogen source was used. The centre of the petri plate was positioned with the 5 mm disc culture, which had been developing actively for 7 days. At

25±2^{0}C, the plates were incubated. The mycelial growth's diameter was measured. There were kept three replications (Gholve *et al.,* 2015).

Effect of Carbon sources on *Alternaria solani*

Alternaria solani growth parameters were determined by studying the fungus carbon requirement in Czapek's Dox medium. The amount of carbon compounds added was calculated using their molecular weight. Glucose, fructose, lactose, mannitol, maltose, sucrose, and starch were the carbon sources employed in the study. In the sterilised petri dish, the sterilised melting warm medium was poured and allowed to solidify. The petri dish was filled with an actively growing 7-day-old culture of 5 mm discs. The plates were incubated at 25±2^{0}C. Three replications were kept (Ramjegathesh and Ebenzer, 2012).

Effect of different colours of light on *Alternaria solani*

To ascertain sporulation and mycelium development patterns as a result of light absorption by *Alternaria solani*, the colours of light that fungi require were tested. Red, green, blue, yellow, and white were the different light colours used in the study. 90 mm petri plates were filled with 20 ml of PDA.

A five mm disc of *A. solani*, grown for seven days, was collected and placed in the middle of Petri plates. The plates were set at BOD at 25±2^{0}C and coated with coloured polythene. There were kept three replications.

Results and Discussion

Survey on severity of early blight of tomato in different districts of Chhattisgarh

In order to determine the prevalence of the tomato blight disease, a thorough roving survey was conducted in the months of October, November, and December in the Chhattisgarh districts of Durg, Raigarh, and Raipur. The results in Table 3.1, which shows the percent disease incidence (PDI). The highest percent disease incidence was recorded (85.29%) at village Rampur followed by 56.67 % at village Basni belongs to dist. Durg. In Raigarh district highest PDI was 51.67 % at Linjir village followed by 30.5% at Khichri village. In Raipur district the highest PDI was recorded 38.17 % at Horticulture far IGKV Raipur followed by Mujgahan 15.83 % and lowest PDI was 11.67% at Tekari village.

Similar findings were reported by Sahu *et al.*, (2013), who reported Abhanpur block had the highest disease severity (51.31%), followed by Aarang block (49.35%), and Dharsiwa block had the lowest severity (44.24%). Similar findings were reported by Pachori and Sharma (2016); Munde *et al.,* (2013).

Effect of solid culture media on *A. solani* isolates

An experiment was conducted to know the effect of different media on variability of *A. solani* and the observation were recoded as mycelial growth came 90 mm.

The data depict from the table 3.2 that highest growth of *A. solani* was 90 mm in 10 days in Potato dextrose agar medium followed by Oat meal agar (87.71 mm), both being statistically at par with each other.

Host extract agar (83.75 mm), Malt extract agar (82.38) Czapek's Dox agar (80.84 mm), Asthana hawker's agar (80.77 mm) Richard's agar (80.73 mm) and Sabouraud's agar (80.71 mm) were second group of media, being statistically at par with each other. Some findings were supporting the above result.

Koley and Mahapatra (2015) reported that *A. solani* on different solid media showed that PDA medium developed highest mycelial diameter growth, followed by oat meal agar medium. Some other similar findings were reported by Munde *et al.*, (2013).

Effect of pH on *Alternaria solani*

An experiment was conducted to know the effect of different pH on variability of *A. solani*. The observation were recorded when mycelial growth came 90 mm. The data depict from the table 3.3 that highest growth of *Alternaria solani* was 90 mm in 10 days in pH 7.5 followed by pH 7.0 (79.21 mm), pH 6.5 (73.14mm), pH 6.0 (64.85mm), were differ significantly with each other whereas pH 5.5 (60.88 mm) and pH 5 (58.19mm), were statistically at par with each other. The least growth was 53.44 mm in pH 4.5 which was significant over other tested pH.

The present findings were in accordance with the Alhussaen (2012); Hubbali *et al.,* (2010). Some other findings were reported by Khatri *et al.*, (2014); Chohan *et al.*, (2015) and Ramjegathesh and Ebenzer, (2012).

Effect of Nitrogen sources on *Alternaria solani*

An experiment was conducted to know the effect of different nitrogen on variability of *A. solani*. The observation were recorded when mycelial growth came 90 mm. The laboratory temperature was 39.4 ^{0}C at the time of experiment.

The data illustrated in table.3 that highest growth of *Alternaria solani* was 90 mm in 20 days in KNO_3 followed by $NaNO_3$ (77.46 mm), glycine (77.64 mm), $Mg(NO_3)_2$ (70.30 mm) and urea (70.03 mm), were being statistically at par with each other. The least colony diameter of 25.68 mm was obtained in $(NH_4)_2SO_4$.

The present finding is in accordance with the reports of Ramjegathesh and Ebenzer (2012) reported that Potassium nitrate showed maximum growth followed by Sodium nitrate. Some other findings were reported by Mahadevaswamy *et al.,* (2018); Gholve *et al.,* (2015); Taware *et al.,* (2014); Khatri *et al.,* (2014).

Effect of Carbon sources on *Alternaria solani*

An experiment was conducted to know the effect of variability on *A. solani* and the observation were recoded as mycelial growth came 90 mm. The laboratory temperature was 39.4 ^{0}C at the time of experiment. The data depict in table 3.5 that highest growth of *Alternaria solani* was 90 mm in 20 days. It was found that maximum radial growth was in glucose (84.05 mm) which was followed by lactose (77.44 mm) and starch (77.23 mm) both being statistically at par with each other. Sucrose (69.30 mm), Maltose (69.15 mm), fructose (75.07) and mannitol (74.53 mm) were statistically at par with each other.

Similar kinds of result were reported by Khatri *et al.,* (2014). They revealed that glucose supported maximum and maltose supported minimum dry mycelial weight. Mahadevaswamy *et al.,* (2018); Gholve *et al.,* (2015); Taware *et al.,* (2014) reported highest radial mycelial growth on glucose.

Table.1 Details of districts and village selected for survey

S No.	District	Village
1.	Durg	Basni
2.	Durg	Rampur
3.	Raigarh	Linjir
4.	Raigarh	Khichri
5.	Raipur	Mujgahan
6.	Raipur	Tekari
7.	Raipur	Horticulture Farm

Table.2 Severity of early blight disease on tomato in different districts of Chhattisgarh during *Rabi* 2021-22

S. No.	Village	Coordinates	Variety	Area (ha)	PDI(%)
A. Durg					
1.	Basni	21.421816 N, 81.351401 E	Saaho	0.58	56.67
2.	Rampur	21.259372 N,81.494354 E	Abhilash	0.30	85.29
B. Raigarh					
1.	Linjir	21.541799 N, 83.25084 E	Karishma	0.45	51.67
2.	Khichri	21.541208 N, 83.268626 E	Rubee Dawn	0.60	30.5
C. Raipur					
1.	Mujgahan	21.145857 N, 81.667287 E	Saaho	1.5	15.83
2.	Tekari	21.112107 N, 81.628537 E	Saaho	0.55	11.67
3.	Horticulture Farm	21.232831 N, 81.712033 E	Pusa Ruby	0.048	38.17

Table.3 Effect of solid culture media on *A. solani* isolates

Treatment	Mean colony diameter (mm)	Colony colour	Substrate colour	Mycelium texture	Sporulation*
OMA	87.71	Dark Grey	Brown	Velvet	+++
HEA	83.75	Dark Grey	Dark black	Velvet	++++
SA	80.71	Grey	Blackish brownish	Velvet	+
PDA	90.00	Dark Grey	Dark grey	Velvet	++++
CDA	80.84	Grey	Blackish	Mat	++
MEA	82.38	Grey	Dark black	Velvet	+
RA	80.73	Light grey	Whitish	Mat	++
AHA	80.77	Whitish	Whitish	Velvet	+
CV	2.71				
SE(m)±	1.30				
CD at 1%	3.94				

*0= - (nil), 1-10=+(poor), 11-20= ++(moderate), 21-30= +++(good), 31-40= ++++(excellent)

Table.4 Effect of pH on of *A. solani*

Treatment (pH)	Mean colony diameter (mm)	Colony colour	Substrate colour	Mycelium Texture	Sporulation*
4.5	53.44	Light brownish grey	Brownish	Mat	++
5.0	58.19	Light grey	Blackish	Mat	++
5.5	60.88	Grey	Blackish	Mat	+
6.0	64.85	Grey	Whitish	Mat	++
6.5	73.14	Grey	Blackish	Velvet	+++
7.0	79.21	Grey	Whitish black	Velvet	+++
7.5	87.39	Grey	Blackish	Velvet	++++
CV	4.78				
SE(m)±	1.88				
CD at 1%	5.76				

*0= - (nil), 1-10=+(poor), 11-20= ++(moderate), 21-30= +++(good), 31-40= ++++(excellent)

Table.5 Effect of Nitrogen sources on *Alternaria solani*

Treatment	Mean colony diameter(mm)	Colony colour	Substrate colour	Mycelium Texture	Sporulation*
$(NH_4)_2SO_4$	25.68	Whitish	Dark brownish	Velvet	+++
Mg $(NO_3)_2$	70.30	Grey	Dark blackish	Mat	+
Urea	70.03	Grey	Blackish	Velvet	++
KNO_3	78.61	Dark grey	Dark blackish	Velvet	+++
Glycine	77.64	Grey	Brownish	Velvet	+++
$NaNO_3$	77.46	Dark Grey	Blackish brownish	Velvet	++++
CV	10.23				
SE(m)±	3.94				
CD at 1%	12.26				

*0= - (nil), 1-10=+(poor), 11-20= ++(moderate), 21-30= +++(good), 31-40= ++++(excellent)

Table.6 Effect of Carbon sources on *Alternaria solani*

Treatment	Mean colony diameter (mm)	Colony colour	Substrate colour	Mycelium Texture	Sporulation*
Sucrose	69.30	Very dark	Blackish	Velvet	++
Glucose	84.05	Dark Grey	Blackish	Velvet	+++
Starch	77.23	Grey	White Blackish	Velvet	+
Maltose	69.15	Light grey	Blackish	Mat	+
Fructose	75.07	Grey	Blackish	Velvet	+
Lactose	77.44	Grey	Blackish	Velvet	+++
Mannitol	74.53	Dark grey	Blackish	Mat	++
CV	5.85				
SE(m)±	2.54				
CD at 1%	7.79				

*0= - (nil), 1-10=+(poor), 11-20= ++(moderate), 21-30= +++(good), 31-40= ++++(excellent)

Table.7 Effect of different colours of light on *Alternaria solani*

Treatment	Wavelength (nm)	Mean colony diameter (mm)	Colony colour	Substrate colour	Mycelium Texture	Sporulation*
Red	620-750	83.44	Grey	Dark black	Velvet	++++
Green	495-570	82.21	Grey	Dark black	Velvet	+
Blue	450-495	85.05	Grey	Dark black	Velvet	++
Yellow	570-590	83.52	Grey	Dark black	Velvet	++
White	400-780	82.70	Grey	Dark black	Velvet	++++
Orange	590-620	81.10	Grey	Dark black	Velvet	+
Violet	380-450	82.02	Grey	Dark black	Velvet	++
CV %		2.86				
SE(m)±		1.37				
CD at 1%		N/A				

*0= - (nil), 1-10=+(poor), 11-20= ++(moderate), 21-30= +++(good), 31-40= ++++(excellent)

Effect of different colours of light on *Alternaria solani*

An experiment was conducted to know the effect of different light on variability *A. solani* and the observation were recoded as mycelial growth came 90 mm. The data depict in table 3.6 that highest growth of *Alternaria solani* was 90 mm in 18 days. The colony diameter was found maximum in blue colour (85.05 mm) which was followed by yellow (83.52 mm), red (83.44 mm), white (82.70 mm), green (82.21 mm) and violet (82.02). Minimum colony diameter was found in orange (81.10 mm) colour. All treatments were statistically at par with each other.

The results obtained from this investigation were supported by findings of Igbalajobi *et al.*, (2019) they observed that different colours of light shows difference in sporulation of *Alternaria alternata.*

From the present investigations, it was observed that Rampur village of Durg district had maximum percent disease incidence of early blight of tomato. Potato Dextrose agar medium and pH 7.5 was found as most supportive media and pH respectively for the growth of *A. solani.* KNO3 was found as best nitrogen source while glucose was found to be the best carbon source for the growth of *A. solani* and the growth was found best in blue colour light (450-495 nm).

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