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Prevalence, isolation, cultural and morphological characterization of early blight (*Alternaria solani*) of tomato from Uttar Pradesh

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Abstract

Early blight of tomato is one of the most destructive disease and considered to be great threat to tomato production worldwide. The disease causal organism is *Alternaria solani* observed in all district of Uttar Pradesh namely Kanpur, Faizabad, Varanasi and Lucknow. Assessment of disease prevalence is an important step as it helps in designing management strategies which ultimately help in increasing the crop yield. Therefore, a roving survey was conducted to find out the disease severity of tomato early blight in aforesaid locations. The disease was prevalent in all the tomato growing areas during rabi 2018-2019. In survey the maximum incidence of *Alternaria* blight disease was at Prithviganj village of Varanasi in Namdhar-4266 variety. While the lowest incidence of disease was found at Mohanlalganj village of Lucknow in Kashi Vishesh. The isolate from Kanpur (AB1-AB5) showed Light green colony colour, hyphae and septate conidia. The Faizabad isolate AB6 to AB10 showed light green to green colony colour and conidia muriform. The Varanasi isolates AB11-AB15 isolates found dark green colony colour, ovoid to obclavate conidia. The AB16 to AB20 isolates were collected from the region of Lucknow recorded as grey colony colour when colony morphology were analysed with transverse and longitudinal conidia. The collected isolates were checked for colony growth and found >85 mm growth.

Keywords: Prevalence, survey, disease incidence, alternaria, conidia etc.

Introduction

India is one of the largest producers of vegetable crops. Under vegetable crops tomato (*Solanum lycopersicum* L.) covers a large area of vegetables farmers. An estimated 845 thousand hectares of land were under cultivation for tomato production in India for the fiscal year 2021 and contributed 16089.32 thousand tones tomato yield. During the growing seasons, Tomato is severely distributed by the early blight (*Alternaria solani*) Ellis and Martin, which reduces the photosynthesis rate in plants, and makes a yield loss up to 86% and 60% losses and during seedling establishment (Pravin *et al.*, 2021) [1]. Initially appearing as tiny, dark brown to black spots, the disease flecks on leaves later grow to be huge, circular patches with target-like, concentric rings and frequently have chlorotic haloes (Adhikari *et al.*, 2017) [1]. Small, dark brown patches that emerge on the lowest and oldest leaves are the main sign of early blight. If there are several initial lesions, the entire leaf may develop necrosis and chlorosis, and the tissue surrounding the lesions may turn brilliant yellow. The spots grow larger and form concentric rings, giving them a bull's-eye appearance. The amount and quality of tomato fruits are harmed when disease develops on plants, lesions spread widely, and plants begin to defoliate (Agris, 2005) [2]. The conidia are dark muriform, pale golden or olivaceous brown, smooth, typically 150–300 µm long and 15–19 µm thick at their widest point, with 9–11 transverse septa and 1-4 longitudinal or oblique septa; occasionally, they are branching 2.5–5 µm thick and taper gradually (Ellis 1971) [5]. Race claims were made as a result of the morphological and pathogenic diversity of *A. solani* isolates, but this has not yet been verified (Rotem 1966) [15]. The pathogen (*A. solani*) has been reported to exhibit a broad range of diversity in the manifestation of disease symptoms under natural epiphytotic, depending on the tomato cultivars, environmental factors, etc. Researchers have previously described the disease survey, pathogenic, cultural, morphological, and molecular diversity of *A. solani*. In the current study, twenty isolates of *A. solani* obtained from various agroclimatic conditions in Uttar Pradesh were examined for the degree of disease incidence, isolation and cultural variation. From this study pathogenic behavior of *Alternaria solani* will be revealed which ultimately leads towards the path of disease management.

Materials and Methods

Surveying Method

Random survey was conducted from the high tomato growing districts of Uttar Pradesh. The surveyed regions came in to sub-tropical place. In each districts, villages were selected which having the highest incidence of false smut disease. In the selected villages observation was taken with zig-zag marking of one to five 1-m² area in the field. The target observation was number of infected plant and total number of infected plants for percent disease incidence (PDI). The location (Latitude; Longitude) of the survey area taken with the mobile GPS tracker and variety on the farm were write down on envelop and these envelops were used samples collection. This activity helped on taking the same field for evaluation of false smut disease incidence for the next year. Next year the same fields were assessed for early blight of tomato.

Location and sampling of soil samples

In this study, various samples of tomato were collected from major tomato growing districts of Uttar Pradesh. Surveys and sampling were done to select random collection of samples for its occurrence and incidence. The survey was done during the year 2018 and 2019, Rabi period. The same field was choosing for survey on the basis of earlier GPS location in next year. The date of survey was 22/09/2018, 23/09/2018, 06/10/2018, 07/10/2018, 18/10/2018, 03/11/2018 for first survey and for second survey the dates were 26/09/2019, 05/10/2019, 15/10/2019, 22/10/2019 and 06/11/2019 (Table1).

Disease Incidence

Observations were taken in the zig-zag marking of a 1 to 5 m² area made in the farmer's field. Observations were recorded for the PDI as number of infected plant and total number of infected plants, varieties on which disease incidence estimated using formula

$$\text{Percentage disease incidence} = \frac{\text{number infected plants}}{\text{total number of assessed plant}} \times 100$$

Isolation, Purification and Maintenance of *Alternaria solani* isolates

The sick tomato plant part were transported to the lab, where 1 cm sections of the contaminated stem were taken and

washed with tap water for three minutes before being superficially cleaned with 1 percent sodium hypochlorite (NaOCl) for one to two minutes. As a result, they were dried for 5 minutes before being put on PDA-containing petri plates and incubated at 20 to 25 °C. To create pure cultures, mycelial discs with a 4 mm diameter were placed to petri plates containing PDA from the borders of colonies that had developed within 4-5 days. After that, these isolates were put into test tubes containing PDA and kept at +4°C for subsequent examination. Fungal isolates were identified at the species level using morphological keys and spore identification.

Evaluation of cultural and morphological studies of *Alternaria solani*

Growth pattern of pathogen isolates

The pure culture of alternaria isolates were taken for growth pattern parameters like type of growth, sporulation and mycelia growth per day in mm on petriplates observation. Morphological studies were also observed. The basis of observation of morphology of fungi was colour, texture, shape and concentric ring formation. The observation was recorded after 15 day of inoculation. The temperature should be maintained up to 26±1 °C at B.O.D for optimum growth.

Results

Survey and incidence of *Alternaria* blight in collected fields

The prevalence of the early blight disease was observed through the survey of the tomato growing areas of the Uttar Pradesh. Total four states viz. Kanpur, Faizabad, Varanasi, Lucknow and twenty villages were surveyed. The survey has been conducted to know the incidence and status of *Alternaria* disease in tomato growing areas of Uttar Pradesh. In our survey it was found that the maximum incidence of alternaria disease was at Prithviganj village of Varanasi with 35.41 percent disease incidence in Namdhar-4266 variety followed by Bhikaripur village of Varanasi with 31.2 percent disease incidence in Kashi Vishesh variety. While the lowest incidence of disease was found at Mohanlalganj village of Lucknow with 10.2 percent disease incidence in Kashi Vishesh followed by CSSRI, location of Lucknow with 11.2 percent disease incidence in Avinash-3 variety (Table 1; fig. 1).

Table 1: Geographic locations of the *Alternaria solani* infected leafs in tomato and soil samples for processing

Sl. No.	Isolates	Site (Villages)	District/Block, State	Latitude	Longitude
1	AB1	Kalyanpur farm	Kanpur, UP	26.49371	80.29570
2	AB2	Khayoya Khatri	Kanpur, UP	26.53353	80.30904
3	AB3	Prithhvi ganj	Kanpur, UP	26.55881	80.29712
4	AB4	Bilhaur	Kanpur, UP	26.83929	80.06812
5	AB5	Ghatampur	Kanpur, UP	26.14579	80.1555
6	AB6	Farm section	Faizabad, UP	26.5395	81.8299
7	AB7	NDUAT	Faizabad, UP	26.5414	81.8319
8	AB8	Bikapur	Faizabad, UP	26.58952	82.12927
9	AB9	Milkipur	Faizabad, UP	26.590809	81.907387
10	AB10	Ayodhya	Faizabad, UP	26.75412	81.76021
11	AB11	Balipur	Varanasi, UP	25.2245	82.9468
12	AB12	Bhikaripur	Varanasi, UP	25.2673	82.8325
13	AB13	BHU farm	Varanasi, UP	25.2556	82.9950
14	AB14	Narasana	Varanasi, UP	25.2613	82.8295
15	AB15	Farm engineering field	Varanasi, UP	25.2568	82.9915
16	AB16	Mohanlalganj	Lucknow, UP	26.675615	80.981773
17	AB17	Bakshi ka Talab	Lucknow, UP	26.989533	80.923647
18	AB18	Malihabad	Lucknow, UP	26.920589	80.705155
19	AB19	CSSRI	Lucknow, UP	26.8038	80.9236
20	AB20	Allupur	Lucknow, UP	26.879653	80.753689



Fig 1: Survey and collection of *Alternaria solani* samples from random fields

Cultural and morphological variability of collected isolates of *Alternaria blight*

From the survey total twenty isolates of *Alternaria solani* has been recovered and named as AB1, AB2, AB3, AB4, AB5, AB6, AB7, AB8, AB9, AB10, AB11, AB12, AB13, AB14, AB15, AB16, AB17, AB18, AB19 and AB20. The isolate from Kanpur (AB1-AB5) showed Light green colony colour. Hyphae and conidial structure of these isolates varies from septate conidia with chain formation and beakless to Septate oval shape. The AB1, AB2 and AB5 showed concentric zonation while other Kanpur isolates showed almost same sporulation i.e. +++ to ++.

The Faizabad isolate were named as AB6 to AB10. Most of the isolates showed light green to green colony colour. The conidia of collected isolates were recorded muriform type of conidia with septate mycelium. All the isolates of Faizabad showed concentric zonation with high sporulation i.e. +++.

The Varanasi isolates were given named as AB11-AB15. Most of the isolates found dark green colony colour. In Varanasi isolates septate hyphae with ovoid to obclavate conidia were found. The isolates AB11 to AB15 were also showed the concentric zonation as the isolates of Faizabad were shown. Sporulation in Varanasi isolates were came in the range of +++ to ++.

Table 2: Disease incidence and disease severity of *Alternaria blight* of Tomato samples in surveyed fields (Pooled)

District/Block, State	Site (Villages)	Latitude	Longitude	Percent Disease incidence	Variety
Kanpur, UP	Kalyanpur farm	26.49371	80.29570	26.34	Azad-T6
	Khayoya Khatri	26.53353	80.30904	28.33	Azad-T8
	Prithvi ganj	26.55881	80.29712	35.41	Namdhari-4266
	Bilhaur	26.83929	80.06812	30.2	Swarna Sampada
	Ghatampur	26.14579	80.1555	20.22	Namdhari-4266
Faizabad, UP	Farm section	26.5395	81.8299	12.36	Naveen
	NDUAT	26.5414	81.8319	25.34	Avinash-3
	Bikapur	26.58952	82.12927	21.32	Pusa hybrid-4
	Milkipur	26.590809	81.907387	22.42	Namdhar-526
	Ayodhya	26.75412	81.76021	25.61	Pusa Hybrid-2
Varanasi, UP	Balipur	25.2245	82.9468	30.22	Kashi Aman
	Bhikaripur	25.2673	82.8325	31.20	Kashi Vishes
	BHU farm	25.2556	82.9950	20.1	KASHi Amrit
	Narasana	25.2613	82.8295	14.23	Kashi Hemant
	Farm engineering field	25.2568	82.9915	21.3	Kashi Sharad
Lucknow, UP	Mohanlalganj	26.675615	80.981773	10.2	Kashi Vishes
	Bakshi ka Talab	26.989533	80.923647	12.3	KASHi Amrit
	Malihabad	26.920589	80.705155	14.2	Naveen
	CSSRI	26.8038	80.9236	11.2	Avinash-3
	Allupur	26.879653	80.753689	15.3	Pusa hybrid-4
CD@5%				12.64	
S.Em				2.26	
CV				6.480	

The AB16 to AB20 isolates were collected from the region of Lucknow District. The isolates AB16 to AB20 recorded as grey colony colour when colony morphology were analysed. The conidia morphology of these isolates were like transverse

and longitudinal conidia while the zonation in isolates were absent and found no zonation in the isolates. The speculation was lower than other isolates of UP i.e. Kanpur (AB1-AB5), Faizabad (AB6-AB10), and Varanasi (AB11-AB16).

Table 3: Morphological and cultural variability of collected isolates

Isolates	Site (Villages)	District/Block, State	Colony morphology	Hyphae and conidial structure	Zonation	Sporulation	Colony Diameter
AB1	Kalyanpur farm	Kanpur, UP	Light-green compact	Light brown conidia, septate, chain formation, beakless	Concentric zonation	+++	88.56
AB2	Khayoya Khatri	Kanpur, UP	Green	Septate hyphae, germinate in chain formation	Concentric zonation	+++	87.2
AB3	Prithvi ganj	Kanpur, UP	Light green with whitish margin	Gray, septate, oval shaped	Without zonation	++	85.32
AB4	Bilhour	Kanpur, UP	Light green with whitish margin	Septate hyphae, septate chain forming	Without zonation	++	86.31
AB5	Ghatampur	Kanpur, UP	Light green	Septate hyphae, chain forming	Concentric zonation	+++	88.25
Average							87.12
SD							1.34
AB6	Farm section	Faizabad, UP	Light green with white margin	Muriform conidia and septate hyphae	Concentric zonation	++	89.61
AB7	NDUAT	Faizabad, UP	Green	Muriform conidia with beak	Concentric zonation	+++	88.32
AB8	Bikapur	Faizabad, UP	Light Green mixed with white mycelium	Muriform conidia with septate mycelium	Concentric zonation	+++	81.23
AB9	Milkipur	Faizabad, UP	Light green	Muriform conidia with septate hyphae	Concentric zonation	+++	84.25
AB10	Ayodhya	Faizabad, UP	Green	Muriform conidia with beak and septate mycelium	Concentric zonation	+++	88.36
Average							86.35
SD							3.50
AB11	Balipur	Varanasi, UP	White olive brown-green cottony	Septate hyphae with ovoid obclave conidia, transverse and longitudinal septation	Concentric zonation	+++	86.45
AB12	Bhikaripur	Varanasi, UP	Dark green	Conidia brown, transverse septation and septate hyphae	Concentric zonation	+++	85.3
AB13	BHU farm	Varanasi, UP	Dark green and compact	Light gray, ovoid to obclave conidia, transverse and longitudinal septation	Concentric zonation	+++	84.31
AB14	Narasana	Varanasi, UP	Light green whitish growth	Hyphae septate, obclavate conidia, transverse septation	Concentric zonation	++	86.35
AB15	Farm engineering field	Varanasi, UP	Green and at white margin	Hyphae septate, conidia moderate, long, brown septate and chain formation	Concentric zonation	++	88.31
Average							86.14
SD							1.49
AB16	Mohanlalganj	Lucknow, UP	Grey colony colour with white margin	Conidia with transverse and longitudinal septa, beaked	Without zonation	+	85.64
AB17	Bakshi ka Talab	Lucknow, UP	Grey colony colour	Conidia with transverse and longitudinal septa	Without zonation	+	84.31
AB18	Malihabad	Lucknow, UP	Grey colony	Conidia with transverse and longitudinal septa	Without zonation	+	86.34
AB19	CSSRI	Lucknow, UP	Grey colour colony	Conidia with transverse and longitudinal septa	Without zonation	++	81.23
AB20	Allupur	Lucknow, UP	Grey colony colour	Conidia with transverse and longitudinal septa	Without zonation	++	88.78
Average							85.26
SD							2.77

The collected isolates were checked for colony growth. The isolates collected from Kanpur measured 87.12 mm average growth. The isolates of Faizabad was recorded 86.35 mm average growth, Varanasi isolates were showed 86.14 mm

growth. The isolates from Lucknow showed 85.26 mm average growth of the mycelium in the 90 mm petri plates (Table 2; fig. 2).

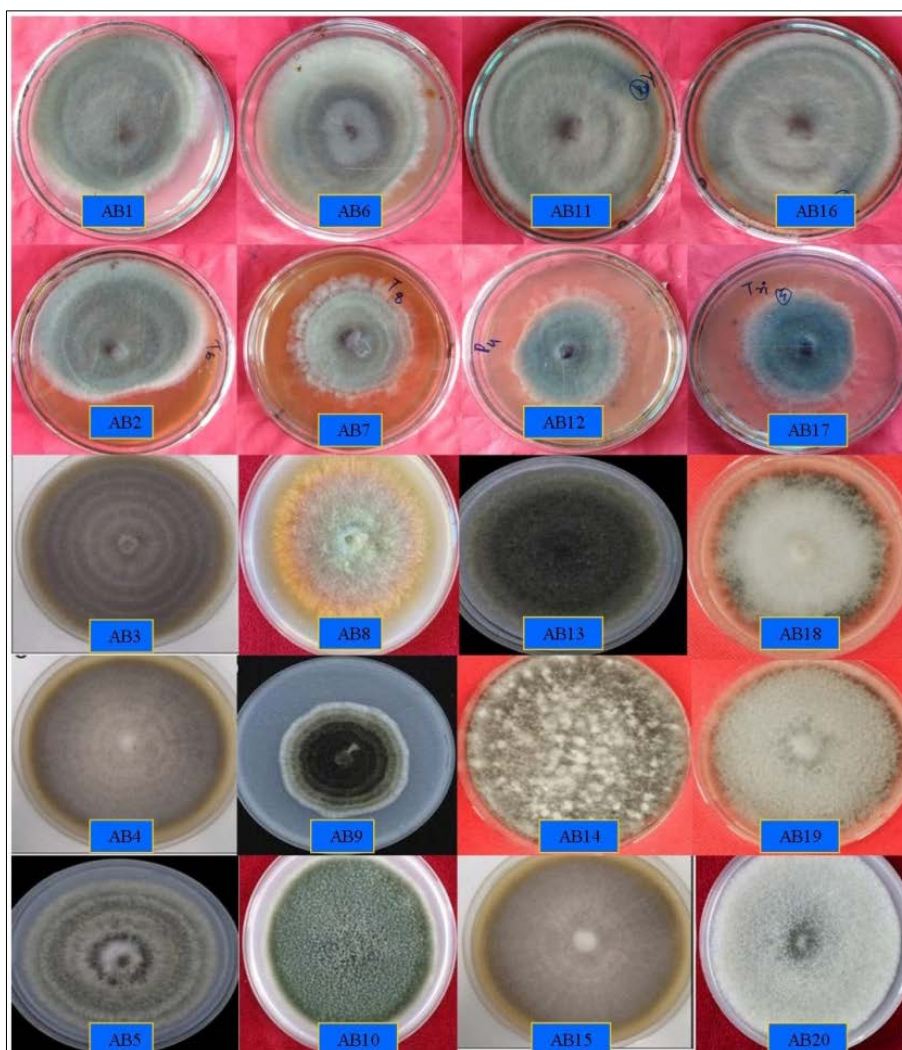


Fig 2: Cultural variability of the collected isolates from survey; AB1-AB5: Kanpur Isolates; AB6-AB10: Faizabad isolates; AB11-AB15: Varanasi Isolates; AB16-AB20: Lucknow Isolates

Discussion

Survey and incidence of Alternaria blight in collected fields

Diseases are the key limiting factor in crop production. The management of such diseases is crucial for sustained crop production and to capture a full yield potential of a particular variety. The survey has been conducted to know the incidence and status of Alternaria disease in tomato growing areas of Uttar Pradesh. In our survey it was found that the maximum incidence of Alternaria blight disease was at Prithviganj village of Varanasi in Namdhar-4266 variety followed by Bhikaripur village of Varanasi in Kashi Vishesh variety. While the lowest incidence of disease was found at Mohanlalganj village of Lucknow in Kashi Vishesh followed by CSSRI, location of Lucknow in Avinash-3 variety. The survey on Alternaria species was by Kalra and Sohi (1984)^[6] in Chandigarh market and they found more than 60% incidence of the disease in the surveyed area. In Nigerian states of Ohio and Logos the survey were also made and found the Alternaria disease as the most prevalent disease in these regions (Chinoko and Nagvi, 1989)^[3]. The survey was made in the states of India viz., Karnataka, Punjab for Alternaria blight disease incidence (Prasad *et al.*, 2002; Deora *et al.*, 2004)^[12, 4]. Kamble *et al.*, 2009^[7] performed roving survey during the 2004-2005 on Alternaria blight disease in Raigarh and Thane districts of Maharashtra and found the

variable proportion of the ranging from 21 to 57% of disease incidence. Another researcher Munde *et al.*, 2013^[10] conducted the survey for the early blight pathogen in four district of M.H. viz., Thane, Raigad, Ratnagiri, and Sindhudurg and found 20% to 51% disease incidence.

Cultural and morphological variability of collected isolates of Alternaria blight

During the survey the diseased samples were also collected and isolated in the pure form were kept for the cultural and morphological identification. From the survey total twenty isolates of *Alternaria solani* has been recovered and named as AB1, AB2, AB3, AB4, AB5, AB6, AB7, AB8, AB9, AB10, AB11, AB12, AB13, AB14, AB15, AB16, AB17, AB18, AB19 and AB20. The isolate from Kanpur (AB1-AB5) showed Light green colony colour, hyphae and conidial structure of these isolates varies from septate conidia with chain formation and beakless to Septate oval shape. The Faizabad isolate were named as AB6 to AB10. Most of the isolates showed light green to green colony colour. The conidia of collected isolates were recorded muriform type of conidia with septate mycelium. The Varanasi isolates were given named as AB11-AB15. Most of the isolates found dark green colony colour. In Varanasi isolates septate hyphae with ovoid to obclavate conidia were found. Isolates showed the concentric zonation as the isolates of Faizabad were shown.

The AB16 to AB20 isolates were collected from the region of Lucknow District. The isolates AB16 to AB20 recorded as grey colony colour when colony morphology were analysed. The conidia morphology of these isolates were like transverse and longitudinal conidia while the zonation in isolates were absent and found no zonation in the isolates. The collected isolates were checked for colony growth. The isolates collected from Kanpur measured 87.12 mm average growth. All the collected isolates showed the colony diameter of >85mm. Kumar *et al.*, 2008^[9] widely studied about the cultural, morphological and molecular variability. They found pigmentation in isolates of *A. solani* on potato dextrose agar medium ranged from yellow, brown, black, brownish, to greenish black. All isolates had radial development on PDA that, in general, fell between 14.9 mm and 32.2 mm. On PDA, the So *Alternaria* isolate had the fastest radial development and the Ka *Alternaria* isolate had the slowest. Conidiogenous hyphal thickness ranged from 1.17 to 9.56 micron, with Va-5 and Ma isolates having the thickest hyphae. The majority of the isolates had uniform mycelial development without concentric zonation, with a round and uneven border. Roopa *et al.*, 2016^[14] have used the morphological and cultural variability for the identification of the of the *Alternaria solani*. They found different conidiophores of the *Alternaria* fungi as formation singly or in group, conidiophores straight or flexuous, colour brown to olivaceous brown and arrangement straight, oblong or ellipsoidal conidia. Rahmatzai *et al.*, 2016^[13] also made experiments on *Alternaria solani* for its morphology, pathogenicity and physiological variability. They found the 7.9 cm mycelial growth on PDA media. The pigmentation, sporulation, and mycelial growth characteristics such colony surface, growth margin, and zonation were substantially variable across *A. solani* isolates. Masurkar *et al.*, 2022 also observed the cultural and morphological variability in *U. virens* pathogen.

Conclusions

The survey of percent disease incidence of *Alternaria solani* pathogen revealed that the disease was most prevalent in Uttar Pradesh state and the cultural and morphological variability of the pathogen were also to enough extent to develop new races of the *Alternaria solani* in the surveyed region. This experimental finding becomes a trigger for the management of the disease in the surveyed area.

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