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Physiochemical characteristics of *Alternaria solani* (Ellis and Martin) causal agent of leaf blight of tomato

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Abstract
Tomato one of the leading vegetable crop in India and all over the world. It affects by pathogen *Alternaria solani* in form of early blight of potato which is a potential disease of tomato growing areas. The resistant sources against this phytopathogen were also less thus late blight also found to be most destructive diseases of tomato. In this paper we have discussed the effect of temperature and pH on the growth of *Alternaria solani*. Effect of different temperature on growth of *Alternaria solani* showed that temperature range the highest growth of the *A. solani* isolate i.e. AB2 with 89.4 mm were found in the temperature 25 °C while the lowest growth of fungi were found at the temperature 45 °C i.e. 28.25 mm in AB17. Effect of different pH on growth of *Alternaria solani* showed that The average highest growth of *Alternaria solani* 86.44 mm were observed in the pH of 7 while the lowest fungal growth i.e. 26.52 mm were observed at 10 pH.

Keywords: Pathogen, resistant, growth, disease etc.

Introduction
Tomato (*Lycopersicum esculantum* L.) is most favorite crop for growers for food processing industries. Globally, after potatoes, tomatoes are the vegetable that people eat the most. In the value-adding cycle of processing, tomato has very few other sources. Peru and Mexico are the native home of the tomato. It is possible that the Portuguese brought it to India, even though there are no clear records of when and how that happened. It is a staple food in the Indian cuisine and is used both as raw fruit and as cooked processed foods including soup, ketchup, sauce, pickles, pastes, and powder. As a blood purifier, stomach secretion promoter, and source of nutrients and metabolites (such as folate, potassium, and vitamins A and C), tomato juice and pulp are particularly digestible. About 95% of tomato fruit is water, with the remaining 5% consisting primarily of carbs and fibre. In India tomato is primarily cultivated in Odisha, Andhra Pradesh, Madhya Pradesh, Karnataka, West Bengal, Chhattisgarh, Telangana, Bihar, Gujarat, Rajasthan, and Uttar Pradesh. Tomatoes are mostly grown in Varanasi, Kanpur, Lucknow, Faizabad districts of Uttar Pradesh. With an annual production of 902 thousand tones with 4.4% share in total tomato production of India (Sharma *et al*., 2022) [13].
Propagated through the seed the tomato crop affected with number of fungal, bacterial and viral pathogens. Damping off, septorial leaf spot, bacterial stem and fruit canker, early blight, bacterial leaf spot, bacterial wilt leaf curl and mosaic are some diseases which causes economic losses to crop. Among the diseases of tomato the early blight of tomato caused by *Alternaria solani* is the one of the important disease of tomato. It causes 50 to 86% losses in fruit yield and 20-40% losses in seedling establishment (Pravin *et al*., 2021) [8].
The genus Alternaria is large and consist several economically important plant pathogen including *Alternaria solani*. It is categorised as belonging to the family Pleosporaceae, order Pleosporales, phylum Ascomycota, class Dothideomycetes, subclass Pleosporomycetidiae, genus Alternaria, species solani, and authority Sorauer (Chaerani and Voorrips 2006) [4]. It belongs to the order Hyphales and class Hyphomycetes of the fungus imperfecti (deuteromycotina) family. This family is known to spread a number of diseases to a large variety of horticultural and agronomic plants. Ellis and Martin published the first account of this disease in New Jersey in 1882 (Sherf and MacNab, 1986) [14].
The symptom due to *A. solani* in plants came in each and every stages of plant although the most significant stage of the disease is leaf blight. Dark, tiny, necrotic, coalescing, and concentric lesions that give the leaf surface a target-like appearance are its defining features. Yellow bands are seen around the lesions (Shelf and Macnab, 1986) [14]. As the plant matures, the disease spreads upward, starting on lower, older leaves (Rotem, 1994) [12].

Younger leaves are less prone than older ones. As the disease worsens, there is severe defoliation, which increases respiration rate and decreases photosynthetic rate. The variable nature of pathogen emerge the thrust to evaluate more information on physiology of the pathogen and its growth. The Alternaria blight highly variabile in nature and produces new strains of the pathogen which shows lack of knowledge of the pathogen. The pathogen also found fungicide resistance reports in USA (Pasche & Gudmestad, 2008; Rosenzweig *et al*., 2008a, b; Belcher *et al*., 2010; Fairchild *et al*., 2013) [9, 10, 11, 3, 6]. All previous studies urges more information about the early blight pathogen so it can be managed easily.

Methods and Materials

Isolation, Purification and Maintenance microorganism

Isolation and purification and maintenance of *Alternaria solani* isolates

The sick tomato plants 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 physiological and morphological studies of *Alternaria solani*

Effect of different pH values on the growth of *pathogen* isolates: Different pH of PDA media ranged from acidic to basic were taken for investigate the pathogen behavior in various pH. The pH of media maintained by adding acid and basic solution in to it. For acidic pH HCl were added and for basic media NaoH were added in to media. The pH used in our study was 4, 5, 6, 7, 8, 9 and 10. The 5 mm pure culture disk of Alternaria were put on to the PDA and observation were taken after 15 dyas.

Effect of different temperature on the growth of *pathogen* isolates

For this experiment the PDA media were taken with 5 mm mycelia disk kept on it. After inoculation of the pathogen in to media the petri plates were maintained in different temperature of BOD. The BOD temperature was maintained from 20 °C, 25 °C, 28 °C, 30 °C, 35 °C, 40 °C and 45 °C were kept for observation.

Results

The physiological parameters like temperature and pH having diverse effect on the growth of the microorganisms. In these experimental findings we have also checked the effect of temperature and pH on *Alternaria solani* growth on PDA plates. The experimental findings were described below:

Effect of different physiological parameters on *Alternaria solani* growth

The effect of temperature and pH has been checked for colony growth of the pathogen. The different temperature were 20, 25, 28, 30, 35, 40 and 45 °C and pH *viz*. 4, 5, 6, 7, 8, 9 and 10 were taken. From these different temperature and pH pathogens growth behavior were analysed (Table 4.6 and 4.7).

Effect of different temperature on growth of *Alternaria solani*: The seven wide range of temperature has been taken to evolve the growth pattern of the pathogen. In 20 °C the highest growth was observed in AB20 isolate i.e. 60.3 mm at par with AB8 whereas the lowest mycelia growth was observed in AB17 isolate i.e. 45.3 mm followed by AB14 with 45.6 mm growth. In 25 °C the highest growth of was found in AB2 isolate i.e. 89.4 mm followed by AB20 89.3 mm while the lowest growth in this temperature range was found in AB3 isolate i.e. 80.4 mm followed by AB5 was 85.6 mm. In 28 °C the highest growth of AB3 isolate was 89.1 mm followed by AB5 88.2 mm while the lowest mycelia growth was found with isolate AB12 i.e. 69.3 mm followed by AB15 isolate with 75.7 mm. In 30 °C the highest growth was found in AB3 isolate i.e. 78.3 mm followed by AB6 with 77.3 mm while the lowest fungal growth was observed in AB15 isolate with 39.2 mm followed by AB17 isolate with 64.4 mm. In 35 °C the highest was observed in the isolate AB4 with 66.3 mm followed by AB1 63.2 mm while the lowest growth was observed in AB16 with 45.1 mm followed by AB17 with 46.5 mm growth. In 40 °C the highest growth was observed in AB1 isolate i.e. 53.3 mm followed by AB8 47.2 mm while the lowest growth was observed in AB12 isolate with 35.3 mm followed by AB13 isolate with 36.4 mm growth. In 45 °C the highest growth was observed in AB1 isolate with 44.7 mm followed by AB3 36.1 mm whereas the AB11 isolate showed lowest growth i.e. 29.3 mm followed by AB2 30.5 mm.

Table 1: Effect of temperature range on colony growth of *Alternaria solani*

Sr. N.	Isolates	20 °C	25 °C	28 °C	30 °C	35 °C	40 °C	45 °C
1	AB1	55.3	88.3	86.3	76.3	63.2	51.3	44.7
2	AB2	54.3	89.4	87.1	75.6	62.3	44.2	30.5
3	AB3	56.2	80.4	89.1	78.3	63.1	45.4	36.1
4	AB4	54.4	88.2	87.2	75.6	66.3	46.3	34.3
5	AB5	55.4	85.6	88.2	72.3	52.3	44.3	34.4
6	AB6	46.3	86.5	78.6	77.3	56.4	42.3	35.6
7	AB7	56.2	88.6	79.3	76.2	58.2	42.1	34.2
8	AB8	60.3	86.2	79.9	75.3	58.2	47.2	33.1
9	AB9	55.2	87.3	84.6	76.3	55.2	45.3	34.1
10	AB10	54.3	86.2	76.5	72.3	56.3	42.1	31.2
11	AB11	51.2	88.3	76.5	75.6	54.6	39.6	29.3
12	AB12	55.3	88.4	69.3	75.3	51.2	35.3	34.5
13	AB13	53.1	86.5	77.3	76.8	53.6	36.4	36.1
14	AB14	45.6	87.6	76.4	68.4	54.3	38.7	35.6
15	AB15	48.6	87.2	75.7	39.2	56.3	37.9	34.6
16	AB16	45.6	88.7	78.7	68.7	45.1	39	34.2
17	AB17	45.3	87.7	78.4	64.4	46.5	37	31.3
18	AB18	48.6	88.4	77.8	69.4	46.8	37.6	34.2
19	AB19	50.6	87.6	79.6	66.4	48.9	38.7	35.3
20	AB20	60.3	89.3	78.3	67.3	56.3	44.1	33.1
	Average	52.6	87.3	80.2	71.4	55.3	41.7	34.3

In all the temperature range the highest growth of the A. solani isolate i.e. AB2 with 89.4 mm were found in the temperature 25 °C while the lowest growth of fungi were found at the temperature 45 °C i.e. 28.25 mm in AB17. The average highest growth 87.32 mm were observed in the temperature of 25 °C while the lowest fungal growth were observed at temperature 45 °C (Table 1; Fig. 1).

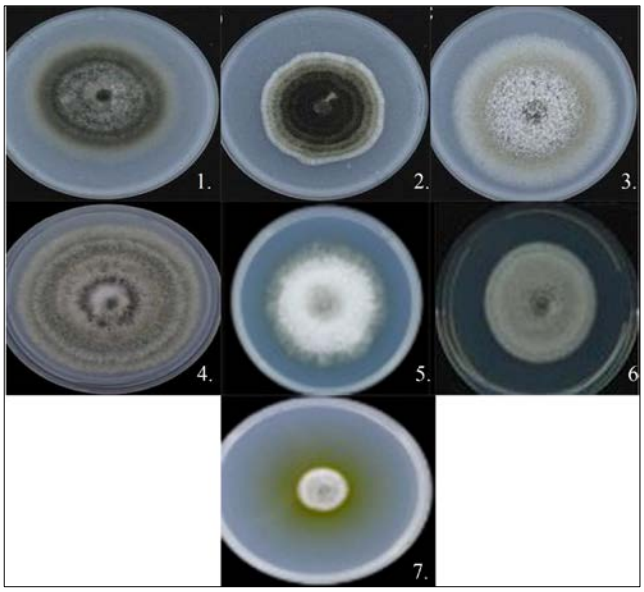

Fig 1: Representative picture for growth of *Alternaria solani* in different pH *viz.* 1) 20 °C 2) 25 °C 3) 28 °C 4) 30 °C 5) 35 °C 6) 40 °C 7) 45 °C

Effect of different pH on growth of *Alternaria solani*

The growth of *Alternaria solani* were checked under the variable pH conditions in fungal growth media. The wide range of pH i.e. 4, 5, 6, 7, 8, 9 and 10 were taken for consideration. The average highest growth of *Alternaria solani* 86.44 mm were observed in the pH of 7 while the lowest fungal growth i.e. 26.52 mm were observed at 10 pH. At the range of 4 pH the highest growth shown by the isolate AB 7 i.e. 28.6 mm while the lowest diameter of the fungal colony were shown by AB11 with 24.3 mm growth followed by AB13 with 24.31 mm growth. In pH range 5 the lowest fungal colony in terms of diameter were observed by AB17 with 34.34 mm followed by AB19 while the highest growth in this pH was observed in the isolate number AB4 with 38.9 mm growth followed by AB9. In pH 6 the lowest growth of fungal colony were observed in the isolate AB9 with 75.64 mm followed by AB8 78.61 mm growth while the highest fungal colony growth were observed in the isolate AB13 with 88.21 followed by AB3 with 86.5 mm growth. At the pH of 7 the highest growth was observed in the AB1 isolate i.e. 88.5 mm followed by AB4 isolate with 88.7 mm while the lowest fungal growth was observed in AB5 isolate i.e. 84.2 mm followed by AB16 84.3 mm. When the pH 8 were maintained in media the lowest growth was observed in AB6 isolate with 60.23 mm growth followed by AB5 isolate with 61.31 mm while the highest growth was observed in AB15 isolate with 66.3 mm growth followed by AB10 isolate with 66.3 mm growth. At the pH of 9 the fungal growth was retarded in comparison to other pH. At pH 9 the lowest growth was observed in AB16 isolate with 43.15 mm followed by AB8 43.2 mm growth while AB6 isolate showed highest growth i.e. 49.1 mm followed by isolate AB1 with 49.1 mm. The growth of different isolates of *Alternaria solani* was also observed at the pH of 10 and it was observed that AB8 isolate showed minimum 24.3 mm growth followed by AB10 isolate with 24.31 mm growth while the AB18 showed highest 28.6 mm growth at this pH followed by isolate AB4 28.6 mm growth (Table 2; Fig. 2).

Table 2: Effect of pH range on colony growth of *Alternaria solani*

Sr. N.	Isolates	4	5	6	7	8	9	10
1	AB1	28.1	38.5	84.6	88.5	65.1	49.1	26.3
2	AB2	28.3	37.5	85.5	87.5	65.3	43.5	28.6
3	AB3	27.5	36.5	86.5	84.6	64.2	48.3	24.4
4	AB4	24.4	38.9	86.4	88.7	62.3	46.8	28.6
5	AB5	28.6	38.4	86.4	84.2	61.3	46.3	27.3
6	AB6	24.4	38.6	84.2	86.2	60.2	49.1	26.3
7	AB7	28.6	37.5	86.2	85.3	64.3	46.2	26.1
8	AB8	27.3	38.2	78.6	87.3	62.3	43.2	24.3
9	AB9	26.3	38.9	75.6	84.5	62.3	46.3	25.4
10	AB10	26.1	37.8	85.6	87.6	66.3	46.2	24.3
11	AB11	24.3	37.8	84.1	86.3	64.3	48.2	24.3
12	AB12	25.4	35.6	84.2	88.1	64.3	46.2	28.1
13	AB13	24.3	38.2	88.2	87.3	65.3	43.5	28.3
14	AB14	24.3	36.4	86.3	86.3	65.3	46.2	27.5
15	AB15	27.6	38.2	86.4	85.6	66.3	44.3	24.4
16	AB16	27.4	37.2	86.3	84.3	64.3	43.2	28.6
17	AB17	27.7	34.3	86.4	86	64.2	44.2	24.4
18	AB18	28.3	36.2	85.6	84.6	62.3	44.3	28.6
19	AB19	28.3	35.2	85.3	87.5	62.3	45.6	27.3
20	AB20	27.3	36.3	84.7	88.3	63.2	46.2	27.3
	Average	26.725	37.31	84.855	86.435	63.77	45.845	26.52

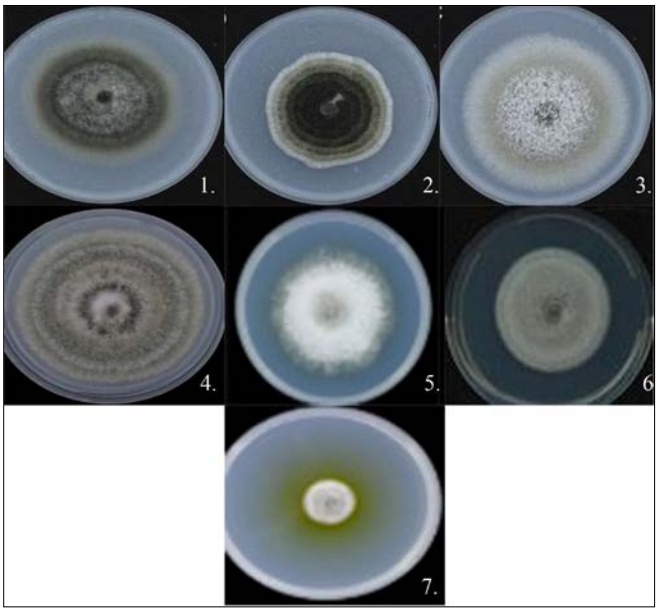

Fig 2: Effect of different pH range on colony growth of *Alternaria solani*

Discussion

The seven wide range of temperature has been taken to evolve the growth pattern of the pathogen. In 20 °C the highest growth was observed in AB20 isolate while the lowest mycelia growth was observed in AB17. In 25 °C the highest growth of was found in AB2 isolate while the lowest growth in this temperature range was found in AB3 isolate. In 28 °C the highest growth of AB3 isolate was while the lowest mycelia growth was found with isolate AB12. In 30 °C the highest growth was found in AB3 isolate while the lowest fungal growth was observed in AB15. In 35 °C the highest was observed in the isolate AB4 while the lowest growth was observed in AB16. In 40 °C the highest growth was observed in AB1 isolate while the lowest growth was observed in AB12. In 45 °C the highest growth was observed in AB1

isolate whereas the AB11 isolate showed lowest growth. In all the temperature range the highest growth of the *A. solani* isolate i.e. AB2 with 89.4 mm were found in the temperature 25 °C while the lowest growth of fungi were found at the temperature 45 °C i.e. 28.25 mm in AB17. The average highest growth 87.32 mm were observed in the temperature of 25 °C while the lowest fungal growth were observed at temperature 45 °C. Somappa *et al*., 2013 [17] studied the effect of temperature on early blight pathogen and it was found that the average mycelia growth was highest at 25 °C which coincide with our study. Alhussaen, 2012 [1] recommended the temperature range of 20-25 °C most suitable for the groth of the Alternaria solani pathogen. Chohan *et al*., 2015 [5] also suggested the 25 °C temperature was best for the growth of the Alternaria pathogen. Mahalaxmi *et al*., 2021 observed that temperature 30 °C was the temperature of highest growth in early blight pathogen.

The average highest growth of *Alternaria solani* 86.44 mm were observed in the pH of 7 while the lowest fungal growth i.e. 26.52 mm were observed at 10 pH. At the range of 4 pH the highest growth shown by the isolate AB 7 while the lowest diameter of the fungal colony were shown by AB11. In pH range 5 the lowest fungal colony in terms of diameter were observed by AB17 highest growth in this pH was observed in the isolate number AB4. In pH 6 the lowest growth of fungal colony were observed in the isolate AB9 while the highest fungal colony growth were observed in the isolate AB13. At the pH of 7 the highest growth was observed in the AB1 isolate while the lowest fungal growth was observed in AB5 isolate. When the pH 8 were maintained in media the lowest growth was observed in AB6 isolate with while the highest growth was observed in AB15 isolate. At the pH of 9 the fungal growth was retarded in comparison to other pH. The growth of different isolates of *Alternaria solani* was also observed at the pH of 10 and it was observed that AB8 isolate showed minimum growth followed by AB10 isolate while the AB18 showed highest growth at this pH. Aruna kumara *et al*., 2015 [2] found the result that the pH range of 6.5-7 was the excellent pH range for the growth of the early blight pathogen which coincides with our study. Sinha and Alam, 2017 drawn the results when studying about the growth characteristics of early blight. In their study they found the pH 7 was the most suitable pH for the growth of the pathogen. Chohan *et al*., 2015 [5] also suggested the 6-7 pH was best for the growth of the Alternaria pathogen. Rao *et al*., 2020 come with conclusion regarding the pH requirement of the early blight pathogen which was between 6-7 pH.

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