

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.704.102>

Variability in *Alternaria alternata* Causing Potato Brown Spot under Different Hydrogen Ion Concentration

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ABSTRACT

Keywords

Alternaria alternata, Brown spot, pH, Potato, Uttar Pradesh

Article Info

Accepted:

10 March 2018

Available Online:

10 April 2018

A total of thirty isolates of *A. alternata* were collected from eastern Uttar Pradesh and parts of Bihar, India. All thirty isolates were evaluated for their growth and rate of growth at three different H⁺ ion concentrations (pH) viz. four, six and eight in PDA medium. Radial growth for all of the isolates of *A. alternata* was also found significantly maximum at pH 6 followed by pH 8 and pH 4. Also, it was noted that none of the isolate had statistically similar growth in different pH range. At pH 4, range of radial growth for thirty isolates ranged between 28.50 to 38.00mm, at pH 6, 90.00mm to 66.00mm whereas for pH 8 growth of *A. alternata* isolates ranged between 49.50 and 58.00mm. Likewise, rate of growth for all of the isolates of *A. alternata* was also found significantly maximum at pH 6 followed by pH 8 and pH 4. None but one of the isolate had statistically similar rate of growth in different pH range *i.e.* Mohobbatpur had 4.67mm and 4.58mm rate of growth at pH 8 and 4 respectively which were found statistically at par each other.

Introduction

The genus *Alternaria* was established in 1817 with *Alternaria alternata* (originally *Alternaria tenuis*) as the type-isolate (Thomma, 2003). The genus *Alternaria* is an important pathogen on standing crop in field and it poses a serious threat after harvest as well. By virtue of mycotoxin producing capability, it is also seen as a contaminant in food items. Droby *et al.*, (1984) for the first time reported pathogenicity of *Alternaria alternata* in potato. This report was followed by several other reports from all over the world (Boiteux and Reifschneider, 1994; Thomma, 2003; Van der Waals *et al.*, 2011

and Shahid *et al.*, 2018). Potato is one of the major crops of India and Uttar Pradesh in particular, which stands first in potato production within India (URL 1).

The potato crop is known to be affected by several diseases among them, early blight caused by *Alternaria solani* and late blight caused by *Phytophthora infestans* cause huge economic losses (Singh and Rana, 2014).

Recently, potato crop in eastern Uttar Pradesh has been seen with new symptoms similar to brown spot disease reported from other parts of the globe. The involvement of the *A. alternata* with the disease was confirmed (data

unpublished). Present study was conducted to understand the variability in the pathogenic isolates.

Materials and Methods

Present investigations on *Alternaria alternata* causing brown spot of potato were carried out in at Department of Mycology and Plant Pathology, Institute of Agriculture Sciences, BHU- Varanasi, Uttar Pradesh.

Collection of samples

Samples were collected from potato growing districts of Eastern Uttar Pradesh viz., Sultanpur, Faizabad, Amethi, Jaunpur, Azamgarh, Mau, Ghazipur, Varanasi, Bhadoi, Mirzapur Chandauli, Raebarely, Kanpur along with Dumraon and Nalanda areas of Bihar. A code was assigned for ease of handling to each isolate. Details of place of collection of isolates and code assigned are given in Table 1.

Isolation and maintenance of isolates

Leaf sample with typical spot symptom were taken from the collected samples and washed under tap water to remove dust particle. Under aseptic condition of laminar air flow small bits of sample portion showing symptom were cut with the help of sterilized blade and surface sterilized in 0.1% sodium hypochlorite for 30 seconds. These bits were then washed thrice in sterilised distilled water for 15 seconds and placed in sterilized blotting paper to remove excess moisture. These bits were placed aseptically in Petri dish containing solidified Potato Dextrose Agar (PDA) medium. These inoculated plates were placed in BOD incubator for growth of the organism at 26±2°C. On the basis of morphology of conidia and conidial chains the pathogen was identified as *A. alternata* and purified by single spore isolation method.

Identification of the pathogen

The pathogen was identified on the basis of its pathogenicity test, cultural and morphological characteristics. Slides from cultured mycelia were prepared on lacto phenol and observed under compound microscope.

Identification of the pathogen was done on the basis of cultural and morphological grounds as described by various authors (Gilman, 1975; Subramanian, 1971; Barnett and Bany, 1972; Ellis, 1971; Droby *et al.*, 1984; Ellis and Gibson, 1975 and Van der Waals, 2011).

Evaluation of effect of different pH

A total three pH level *i.e.* 4, 6 and 8 were selected to study the variability among different isolates in different H⁺ ion concentrates. The pH of potato dextrose medium was adjusted to three different levels mentioned above with the help of digital pH meter using NaOH (0.1 N) and HCl (0.1N) solution. The pH was also recorded by using pH strips before autoclaving for keeping the pH constant. The sterilized media of different pH levels were poured in the sterilized Petri plates in about 20 ml quantities and allowed to solidify. After solidification of the medium each plate was centrally inoculated with 10mm mycelial disc taken from the actively growing tips of eight days old culture with the help of a sterilized cork borer. Each treatment was replicated twice. Inoculated Petri plates were incubated at 26+ 1°C. The observations on radial mycelial growth or colony diameter (mm) on each pH level were recorded at 48 hrs interval and continued till eight days after inoculation.

The growth rate of the fungus on each medium was calculated as follows.

$$GR = \frac{S_{x+1} - S_x}{T_{x+1} - T_x}$$

Where, $G R = \text{Growth rate (mm hr}^{-1}\text{)}$, $S = \text{Colony diameter (mm)}$, $T = \text{Time (days)}$.

Statistical analysis

The data recorded during course of investigations were subjected to statistical analysis using STPR software. The significance of treatment difference was tested by F- test on the basis of null hypothesis. The appropriate standard error ($S.E.m \pm$) was computed in each case. Coefficient of variance per cent was also worked out for all the characters

Results and Discussion

Based on the morphological and cultural characteristics all the thirty fungal isolates were identified as *A. alternata* on the basis of its cultural and morphological structures observed under compound microscope. Identity was confirmed by literature (Gilman, 1975; Subramanian, 1971; Barnett and Bany, 1972). Data pertaining radial growth and rate of growth of the test organism in five different pH viz., 4, 5 and 6 is presented in table 2 and 3 respectively.

Radial growth *A. alternata* isolates at different H^+ ion concentration (pH)

At pH four no isolate grew more than 38.00mm at eight days post incubation (DPI). Significantly maximum growth was observed in isolate Mohobbatpur (38.00mm) which was found statistically at par with Keshavpur (37.50mm), Raipur (37.00mm), Jafrabad (37.00mm), Ugapur (36.50mm) and Dumraon (36.50mm). Minimum radial growth was observed in isolate Chunar (28.50mm) followed by Jansa (29.50mm), Kandhiya, Chirrayyakot, Makdumpur, Pindra each with 31.00mm radial growth. At pH six, a total of five isolates viz., Pindra, Jamalpur (JJ), Bhikharipur, Raibarely, Kanpur-C, were found

to grow 90.00mm radial growth. Chunar (88.50mm) was found statistically at par maximum. Minimum radial growth at pH six was found in isolate Sidhona (66.00mm) followed by Mohammadabad (67.50mm), Kanpur-M (68.50mm) and Raipur (68.50mm) found at par each other. Similarly, at pH eight, radial growth of *A. alternata* isolates varied from 58.00mm to 49.50mm. significantly maximum radial growth was found in Keshavpur (58.00mm) followed Vindhyanchal (57.00mm), Chirrayyakot (56.50mm), Raibarely (56.50mm), BHU (56.00mm), Mohammadabad (56.00mm), Pindra (55.50mm), Ugapur (55.50mm), Sidhona (55.50mm), Kanpur-M (55.50mm), Nalanda (55.50mm) and Bharpura-2 (55.00mm), were found at par each other. Minimum radial growth was present in isolate Kandhiya (49.50mm) which was found at par Bharpura-1 (50.00mm), Mohobbatpur (51.00), Rajepur (51.50mm) and Kanpur-C (51.00mm).

Rate of growth *A. alternata* isolates at different H^+ ion concentration (pH)

At pH four Keshavpur (4.92mm) was observed to have maximum rate of growth and was found at par with Raipur (4.75mm), Rajepur (4.67mm), Mohobbatpur (4.58mm), Jamalpur (4.50mm), Mohammadabad (4.42mm) and Vindhyanchal (4.42mm).

Minimum rate of radial growth was observed in isolate Bhikharipur (3.08mm) followed by Pindra (3.5mm), Chunar (3.5mm), Rajpur-1 (3.67mm), Rajpur-2 (3.83mm), Kandhiya (3.92mm), Raibarely (3.92mm), Dumraon (3.92) and Jafrabad (4.00mm) which were found statistically at par each other. At pH six significantly maximum rate of growth was observed in Raibarely (12.92mm) followed by Rastamau (11.08mm), Vindhyanchal (10.92mm), Nalanda (10.92mm) and Kanpur-C (10.75mm) which were found at par each other.

Table.1 Isolates of *A. alternata* their place of collection and isolate codes

S. No.	Place	District	Isolate code	Morphological identity of pathogenic isolates
1.	Jansa	Varanasi	JV	<i>Alternaria alternata</i>
2.	BHU	Varanasi	BV	<i>Alternaria alternata</i>
3.	Pindra	Varanasi	PV	<i>Alternaria alternata</i>
4.	Leva	Chandauli	LC	<i>Alternaria alternata</i>
5.	Raipur	Ghazipur	RG	<i>Alternaria alternata</i>
6.	Makdumpur	Ghazipur	MG	<i>Alternaria alternata</i>
7.	Mohammadabad	Mau	MMu	<i>Alternaria alternata</i>
8.	Chirrayyakot	Mau	CMu	<i>Alternaria alternata</i>
9.	Rajepur	Azamgarh	RA	<i>Alternaria alternata</i>
10.	Mohobbatpur	Azamgarh	MA	<i>Alternaria alternata</i>
11.	Jamalpur	Jaunpur	JJ	<i>Alternaria alternata</i>
12.	Keshavpur	Jaunpur	KJ	<i>Alternaria alternata</i>
13.	Jafrabad	Jaunpur	JFJ	<i>Alternaria alternata</i>
14.	Kandhiya	Bhadoi	KB	<i>Alternaria alternata</i>
15.	Bhikharipur	Bhadoi	BB	<i>Alternaria alternata</i>
16.	Ugapur	Bhadoi	UB	<i>Alternaria alternata</i>
17.	Vindhyanchal	Mirzapur	VM	<i>Alternaria alternata</i>
18.	Rajpur-1	Mirzapur	RM-1	<i>Alternaria alternata</i>
19.	Rajpur-2	Mirzapur	RM-2	<i>Alternaria alternata</i>
20.	Bharpura-1	Mirzapur	BM-1	<i>Alternaria alternata</i>
21.	Bharpura-2	Mirzapur	BM-2	<i>Alternaria alternata</i>
22.	Chunar	Mirzapur	CM	<i>Alternaria alternata</i>
23.	Jamalpur	Mirzapur	JM	<i>Alternaria alternata</i>
24.	Raibareli	Raibarely	RR	<i>Alternaria alternata</i>
25.	Rastamau	Amethi	RMA	<i>Alternaria alternata</i>
26.	Sidhona	Faizabad	SF	<i>Alternaria alternata</i>
27.	Kanpur-M	Kanpur	KK-1	<i>Alternaria alternata</i>
28.	Kanpur-C	Kanpur	KK-2	<i>Alternaria alternata</i>
29.	Nalanda	Nalanda	NNB	<i>Alternaria alternata</i>
30.	Dumraon	Buxar	DBB	<i>Alternaria alternata</i>

Table.2 Variability in growth of thirty isolates of *Alternaria alternata* at different H⁺ ion concentration (pH)

S. No.	Isolate	pH		
		4	6	8
1.	Jansa	29.50	86.00	52.50
2.	BHU	33.50	80.50	56.00
3.	Pindra	31.00	90.00	55.50
4.	Leva	33.50	81.50	52.00
5.	Raipur	37.00	68.50	53.00
6.	Makdumpur	31.00	70.00	54.50
7.	Mohammadabad	32.50	67.50	56.00
8.	Chirrayyakot	31.00	80.50	56.50
9.	Rajepur	34.00	79.00	51.50
10.	Mohobbatpur	38.00	80.00	51.00
11.	Jamalpur	34.50	90.00	54.00
12.	Keshavpur	37.50	82.50	58.00
13.	Jafrabad	37.00	81.00	54.50
14.	Kandhiya	31.00	78.50	49.50
15.	Bhikharipur	32.00	90.00	53.50
16.	Ugapur	36.50	70.00	55.50
17.	Vindhyanchal	33.50	80.50	57.00
18.	Rajpur-1	33.50	82.50	53.00
19.	Rajpur-2	33.00	83.50	52.00
20.	Bharpura-1	33.50	76.00	50.00
21.	Bharpura-2	33.50	79.50	55.00
22.	Chunar	28.50	88.50	53.00
23.	Jamalpur	33.50	83.50	53.00
24.	Raibarely	32.50	90.00	56.50
25.	Rastamau	31.50	80.00	53.50
26.	Sidhona	32.50	66.00	55.50
27.	Kanpur-M	34.00	68.50	55.50
28.	Kanpur-C	32.50	90.00	51.50
29.	Nalanda	34.50	85.00	55.50
30.	Dumraon	36.50	69.00	53.00
		Isolate (I)	pH (p)	I × p
SEm (±)		0.92	0.29	1.60
CD (0.01)		3.45	1.09	5.98
CV		4.08		

Table.3 Variability in rate of growth of thirty isolates of *Alternaria alternata* at different H⁺ ion concentration (pH)

S. No.	Isolate	pH		
		4	6	8
1.	Jansa	3.58	11.25	5.00
2.	BHU	4.17	9.50	5.83
3.	Pindra	3.50	10.25	5.50
4.	Leva	4.17	9.83	4.75
5.	Raipur	4.75	8.08	5.75
6.	Makdumpur	4.08	8.58	5.58
7.	Mohammadabad	4.42	8.00	5.25
8.	Chirrayyakot	4.08	10.00	6.00
9.	Rajepur	4.67	9.25	4.92
10.	Mohobbatpur	4.58	9.25	4.67
11.	Jamalpur	4.25	8.50	5.17
12.	Keshavpur	4.92	10.17	6.25
13.	Jafrabad	4.00	10.17	5.17
14.	Kandhiya	3.92	7.08	4.33
15.	Bhikharipur	3.08	9.25	5.83
16.	Ugapur	4.33	8.17	6.83
17.	Vindhyanchal	4.42	10.92	5.25
18.	Rajpur-1	3.67	9.92	4.67
19.	Rajpur-2	3.83	10.58	5.50
20.	Bharpura-1	4.33	10.00	4.75
21.	Bharpura-2	4.33	9.42	5.00
22.	Chunar	3.50	10.50	4.58
23.	Jamalpur	4.50	10.00	5.08
24.	Raibarely	3.92	12.92	6.08
25.	Rastamau	4.25	11.08	5.00
26.	Sidhona	4.25	7.75	5.00
27.	Kanpur-M	4.17	7.42	5.50
28.	Kanpur-C	4.17	10.75	5.00
29.	Nalanda	4.17	10.92	5.08
30.	Dumraon	3.92	7.67	4.67
		Isolate (I)	pH (p)	I × p
SEm (±)		0.17	0.56	0.311
CD (0.01)		0.50	0.15	0.87
CV		5.66		

Least rate of growth was observed in isolate Kandhiya (7.08mm) which was found at par Kanpur-M (7.42mm) and Dumraon (7.67mm). At pH eight, Ugapur (6.83mm) was found to have maximum rate of growth followed by Keshavpur (6.25mm), Raibarely (6.08mm), Chirrayyakot (6.00mm), BHU (5.83mm), Bhikharipur (5.83mm) and Raipur (5.75mm) which were found at par each other. Minimum rate of growth was observed in isolate Kandhiya (4.33mm) followed by Chunar (4.58mm), Mohobbatpur (4.67mm), Rajpur-1 (4.67mm), Dumraon (4.67mm), Leva (4.75mm) and Bharpura-1 (4.75mm).

Results presented above are corroborate with study conducted by Maheshwari *et al.*, 2001 who reported optimum pH for the growth of the fungus *Alternaria alternata* was pH 6.5 respectively. Study carried by Hubballi *et al.*, (2010) on effect of hydrogen ion concentration on mycelial growth of *Alternaria alternata* causing leaf blight of Noni is also corroborant with the present study.

Results of their experiment indicated that the growth of *Alternaria alternata* was maximum in pH range of 6.00- 6.50. Kantwa *et al.*, 2015 observed maximum mycelial growth and sporulation on potato dextrose agar at pH 6.5. The pH 7.0 was found optimum for the growth and sporulation of *Alternaria alternata* (Zarger *et al.*, 2015).

Mishra and Thawani (2016) concluded by their study that the best growth and sporulation of the fungus *Alternaria alternata* was observed at slightly acidic and neutral pH range. Relationship of pH to the mycelial growth of *Alternaria alternata* was determined at different pH levels viz. 4.0 to 8.0 at 25 ± 1 °C for 7 days. Of all the eight pH levels, pH 6.5 was found to be ideal and produced the maximum dry mycelial weight (Choudhary *et al.*, 2017). Results are also in

accordance with earlier reports of Gawai and Mangnalikar (2018) who reported that the pH 6.5 is optimum for the growth *Alternaria alternata*.

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How to cite this article:

Shrishti Lingwal and Asha Sinha. 2018. Variability in *Alternaria alternata* Causing Potato Brown Spot under Different Hydrogen Ion Concentration. *Int.J.Curr.Microbiol.App.Sci*. 7(04): 961-968. doi: <https://doi.org/10.20546/ijcmas.2018.704.102>