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Research Article

EFFECT OF TEMPERATURE ON *Alternaria alternata* CAUSING BROWN SPOT OF POTATO

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Abstract- A total of thirty isolates of *A.alternata* were evaluated for their characteristics at four different temperatures *viz.,* 15, 20, 25and 30°C on potato dextrose agar medium (PDA).The data recorded on colony diameter and rate of growth after incubation at various temperature was found significantly different. Results revealed that most of the isolate had maximum growth at 30°C followed by 25°C. Minimum growth for most of the isolates was recorded in 15°C followed by 20°C. Rate of growth at different temperatures for most of the isolates of *A.alternata* was also found significantly maximum at 30°C followed by 25°C except few isolates like Pindra, Chirrayyakot, Jamalpur (JJ), Keshavpur, Bhikharipur, Raibarely and Nalanda where growth rate at 25°C was higher than 30°C.

Key words- *Alternaria alternata*, Brown spot, Potato, Temperature, Uttar Pradesh

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Introduction

The genus *Alternaria* contains a diverse and ubiquitous population of fungi, including aggressive and opportunistic plant pathogens. Of the two best known and economically important members of the genus one is *A. solani*, the causal agent of early blight on potato, the most infamous one and the other is *Alternaria alternata* (Fr.) Keissler which was better known for its saprophytic nature. However, over the time scale, it has bagged pathogenic status by its virtue of producing toxins in crops like Tomato, Pepper, Mandarins, Bean, Tobacco, Durum Wheat, Mango, Pistachio, Pears, Citrus, Almond and Persimmon etc [1, 2]. Quite recently *A.alternata* has been reported to cause brown necrotic lesions on potato foliage and black pit disease on tubers from Israel [3], Brazil [4], North America [5], South Africa [6], Pakistan [7] and several other parts of the world. Losses due to the disease have typically been estimated to around 20 percent. However, there have been instances of losses amounting to 70–80 per cent, when the disease was left uncontrolled [8]. The occurrence of this disease was recently observed in several parts of Eastern Uttar Pradesh. In order to understand the physiological variability of the pathogen a study was conducted to know the effect of different temperatures on the growth characteristics.

Materials and Methods

Present investigations on *Alternaria alternata* causing brown spot of potato were carried out 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, Raebarelly, Kanpur and Dumraon & Nalanda of Bihar. Description of place of collection of isolates and code assigned to them is legibly mentioned in [Table-1].

Isolation and maintenance of isolates: Leaf sample with typical brown spot symptoms were excised from the collected potato samples and cleaned under tap water to remove dust particle. The excised tissue was cut into small bits and sterilized in 0.1% sodium hypochlorite for 30 seconds under aseptic condition. These bits were than washed thrice in sterile 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. After observing growth of fungus sub-culturing was done in another Petri dish these purified cultures were then maintained for further studies in slants by sub-culturing and will be preserved under refrigerated condition (at 5°C).

Identification of the pathogen: The pathogen was identified on the basis of its cultural & morphological characteristics as well as pathogenicity test. 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 [6, 9-13]

Evaluation method: Growth of different isolates of *A. alternata* was studied in four different temperatures *viz.,* 15, 20, 25and 30°C on potato dextrose agar medium (PDA). Petri plates were poured with about fifteen to twenty millilitres sterilized molten medium aseptically. Each isolate was replicated twice under each temperature to be studied. After solidification of the medium each plate was centrally inoculated with 10 mm mycelial disc from the margin of 8 days old culture (maintained at 26± 1°C on PDA) with the help of a sterilized cork borer. The colony diameter was measured eight days after inoculation. Also, the rate of growth of each isolate was assessed by measuring radial mycelial growth of each isolate daily.

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 = Growth rate (mm hr^{-1}), S = Colony diameter (mm), T = 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.Em±) was computed in each case. Coefficient of variance per cent was also worked out for all the characters.

Result and Discussion

The identity of the fungi causing brown spot disease was confirmed as *A.alternata* on the basis of its cultural and morphological features. The recorded characteristic of all the thirty isolates employed in the current study was in accordance with the literature [9-11].

Radial growth of *A. alternata* isolates at different temperatures: a statistically significant variability of radial growth at different temperatures was noticed [Table-1]. All the isolates achieved a maximum growth of 90mm at temperature of 30°C after eight days post inoculation making it most preferred temperature for the growth of *A. alt*ernata. Bhikharipur (90.00mm) was found to grow maximum when cultures were incubated at 25°C. Followed by isolate Jansa (86.00mm) and Nalanda (85.00mm) which were found at par with each other. Least radial growth at 25°C was observed in isolate Leva (69.50mm), Mohommadabad (69.50mm), Kanpur-M (68.50mm) and Makdumpur (61.50mm). The maximum radial growth at 20°C was recorded in Bhikharipur (61.5mm) which was statistically at par with Jamalpur, Keshavpur, Jafrabad, Kandhiya, Jansa and BHU isolates. At 20°C, significantly maximum radial growth was shown by Alternaria alternata isolate Jansa (60.00mm) followed by Bhikharipur (61.50mm), Jamalpur (61.00mm), Jafrabad (60.50mm), Keshavpur (60.00mm), Kandhiya (60.00mm) Jansa (60.00mm), BHU (59.00mm), Rajpur-1 (58.00mm), Nalanda (58.00mm) and were found at par each other. Least growth was observed in isolates Kanpur-M (46.50mm), Raibarely47.50mm), Ugapur (48.50 mm), Raipur (48.50mm) and Chunar (50.00mm). At 15°C, significantly maximum radial growth was observed in Chirrayyakot (31.00 mm) and was found statistically at par with Chunar (28.50mm), Kandhiya (28.00mm) and Jamalpur (27.50mm). Isolates having minimum radial growth were Rajpur-2 (14.00mm) Kanpur-M (14.00mm) which were found at par with Raipur (15.00mm).

Rate of growth of isolates of *Alternaria alternata* on different temperature: Rate of radial growth at temperature 15°C was found significantly maximum in isolateJansa (2.75mm) statistically at par with Chirrayyakot (2.92mm)**,** Chunar (2.83mm), Kandhiya (2.75mm), Jamalpur (2.67mm), Pindra (2.5mm), BHU (2.42mm), Jamalpur (2.33mm), Vindhyanchal (2.33mm), Rajepur (2.25mm), Mohobbatpur (2.25mm), Bhikharipur (2.25mm), Rajpur-1 (2.17mm) and Bharpura-2 (2.17mm).At temperature 20°CJansa (7.33mm) had maximum rate of radial growth followed by BHU (6.75 mm), Sidhona (6.67mm), Bhikharipur (6.58 mm), Bharpura-2 (6.5 mm), Pindra (6.5 mm), Jafrabad (6.5mm), Jamalpur (6.42mm), Rastamau (6.42mm), Leva (6.33mm), Vindhyanchal (6.25mm) and Rajepur (6.17mm) which were found statistically at par with each other. Isolates among minimum growth are Ugapur (5.08mm) followed by Raipur (5.17mm), Rajpur-2 (5.25mm), Raibarely (5.42mm), Mohobbatpur (5.42mm), Chunar (5.50mm), Dumraon (5.58mm), Kanpur-M (5.67mm) and Chirrayyakot (5.67mm) which were found at par minimum. At 25°C isolate Bhikharipur (10.33mm) had maximum rate of growth and was found at par with Jansa (10.17mm), Pindra (9.75mm), Jamalpur (9.67mm) and Raibarely (9.67mm). isolates having least rate of growth were Makdumpur (5.92mm) followed by Kanpur-M (7.25mm), Chunar (7.50mm), Rajpur-2 (7.67mm), Mohommadabad (7.75mm), Jafrabad (7.83mm), Sidhona (7.92mm) and Mohobbatpur (7.92mm), which were found at par each other. When cultures were incubated at 30°C Jansa (10.67mm) had significantly maximum rate of growth followed by isolate Rajpur-1 (9.92mm), Rajpur-2 (9.92mm), Kandhiya (9.83mm), Rajepur (9.75mm) and Jafrabad (9.75 mm). Lowest rate of growth was observed in Chunar (9.00mm). Raipur, Keshavpur, Bhikharipur, Bharpura-2 and Rastamau had 9.08 mm rate of growth [Table-2].

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

S. N.	Place	District	Isolate code	Morphological identity of pathogenic isolates
1.	Jansa	Varanasi	JV	*Alternaria alternata*
2.	BHU		BV	*Alternaria alternata*
3.	Pindra		PV	*Alternaria alternata*
4.	Leva	Chandauli	LC	*Alternaria alternata*
5.	Raipur	Ghazipur	RG	*Alternaria alternata*
6.	Makdumpur		MG	*Alternaria alternata*
7.	Mohommadabad	Mau	MMu	*Alternaria alternata*
8.	Chirrayyakot		CMu	*Alternaria alternata*
9.	Rajepur	Azamgarh	RA	*Alternaria alternata*
10.	Mohobbatpur		MA	*Alternaria alternata*
11.	Jamalpur	Jaunpur	JJ	*Alternaria alternata*
12.	Keshavpur		KJ	*Alternaria alternata*
13.	Jafrabad		JFJ	*Alternaria alternata*
14.	Kandhiya	Bhadoi	KB	*Alternaria alternata*
15.	Bhikharipur		BB	*Alternaria alternata*
16.	Ugapur		UB	*Alternaria alternata*
17.	Vindhyanchal	Mirzapur	VM	*Alternaria alternata*
18.	Rajpur-1		RM-1	*Alternaria alternata*
19.	Rajpur-2		RM-2	*Alternaria alternata*
20.	Bharpura-1		BM-1	*Alternaria alternata*
21.	Bharpura-2		BM-2	*Alternaria alternata*
22.	Chunar		CM	*Alternaria alternata*
23.	Jamalpur		JM	*Alternaria alternata*
24.	Raibareli	Raibarely	RR	*Alternaria alternata*
25.	Rastamau	Amethi	RMA	*Alternaria alternata*
26.	Sidhona	Faizabad	SF	*Alternaria alternata*
27.	Kanpur-M	Kanpur	KK-1	*Alternaria alternata*
28.	Kanpur-C		KK-2	*Alternaria alternata*
29.	Nalanda	Nalanda	NNB	*Alternaria alternata*
30.	Dumraon	Buxar	DBB	*Alternaria alternata*

Table-2 *Variability in Growth of thirty isolates of Alternaria alternata at different temperatures*

S. No.	Isolate	Temperature (°C)			
		15°C	20°C	25°C	30°C
1.	Jansa	26.50	60.00	86.00	90.00
2.	BHU	27.00	59.00	75.00	90.00
3.	Pindra	26.00	54.00	81.00	90.00
4.	Leva	21.00	50.50	69.50	90.00
5.	Raipur	15.00	48.50	75.00	90.00
6.	Makdumpur	18.50	51.00	61.50	90.00
7.	Mohommadabad	16.00	53.00	69.50	90.00
8.	Chirrayyakot	31.00	54.00	77.00	90.00
9.	Rajepur	23.50	57.00	75.00	90.00
10.	Mohobbatpur	23.50	51.00	72.50	90.00
11.	Jamalpur	24.00	56.50	82.50	90.00
12.	Keshavpur	24.00	60.00	82.50	90.00
13.	Jafrabad	17.00	60.50	71.00	90.00
14.	Kandhiya	28.00	60.00	80.00	90.00
15.	Bhikharipur	25.00	61.50	90.00	90.00
16.	Ugapur	20.00	48.50	70.00	90.00
17.	Vindhyanchal	24.00	57.50	80.50	90.00
18.	Rajpur-1	23.00	58.00	80.00	90.00
19.	Rajpur-2	14.00	51.50	72.50	90.00
20.	Bharpura-1	19.00	53.50	75.00	90.00
21.	Bharpura-2	24.00	56.50	77.50	90.00
22.	Chunar	28.50	50.00	74.50	90.00
23.	Jamalpur	27.50	61.00	80.00	90.00
24.	Raibarely	22.00	47.50	80.50	90.00
25.	Rastamau	19.00	57.00	74.50	90.00
26.	Sidhona	18.00	57.50	70.00	90.00
27.	Kanpur-M	14.00	46.50	68.50	90.00
28.	Kanpur-C	17.50	56.00	80.00	90.00
29.	Nalanda	17.50	58.00	85.00	90.00
30.	Dumraon	16.00	51.00	70.00	90.00
		Isolate (I)	Temperature (T)	I × T	
SEm (±)		1.07	0.39	2.15	
CD (0.01)		3.98	1.45	7.96	
CV		5.01			

Table-3 *Variability in Growth rate of thirty isolates of Alternaria alternata at different temperatures*

S. No.	Isolate	15°C	20°C	25°C	30°C
1.	Jansa	2.75	7.33	10.17	10.67
2.	BHU	2.42	6.75	8.83	9.33
3.	Pindra	2.50	6.50	9.75	9.67
4.	Leva	1.83	6.33	8.25	9.58
5.	Raipur	0.83	5.17	8.67	9.08
6.	Makdumpur	1.42	5.83	5.92	9.50
7.	Mohommadabad	1.00	5.92	7.75	9.17
8.	Chirrayyakot	2.92	5.67	9.25	9.17
9.	Rajepur	2.25	6.17	8.42	9.75
10.	Mohobbatpur	2.25	5.42	7.92	9.17
11.	Jamalpur	2.33	5.83	9.67	9.33
12.	Keshavpur	2.00	6.00	9.58	9.08
13.	Jafrabad	1.17	6.50	7.83	9.75
14.	Kandhiya	2.75	6.08	9.42	9.83
15.	Bhikharipur	2.25	6.58	10.33	9.08
16.	Ugapur	1.67	5.08	8.33	9.17
17.	Vindhyanchal	2.33	6.25	9.33	9.75
18.	Rajpur-1	2.17	6.00	9.00	9.92
19.	Rajpur-2	0.67	5.25	7.67	9.92
20.	Bharpura-1	1.50	5.83	8.00	9.25
21.	Bharpura-2	2.17	6.58	8.75	9.08
22.	Chunar	2.83	5.50	7.50	9.00
23.	Jamalpur	2.67	6.42	9.17	9.75
24.	Raibarely	1.83	5.42	9.67	9.25
25.	Rastamau	1.50	6.42	9.00	9.08
26.	Sidhona	1.33	6.67	7.92	9.50
27.	Kanpur-M	0.67	5.67	7.25	9.25
28.	Kanpur-C	1.25	6.00	8.75	9.25
29.	Nalanda	1.25	5.92	9.33	9.17
30.	Dumraon	1.00	5.58	8.58	9.50

	Isolate (I)	Temperature (T)	I × T
SEm (±)	0.18	0.39	2.15
CD (0.01)	0.68	1.45	7.96
CV	8.00		

Results presented above are corroborating with study conducted by Maheshwari, et al., (2000) [14] who reported optimum temperature for the growth of the fungus *Alternaria alternata* was 28°C. Study carried by Hubballi, et al., (2010) [15] on effect of temperature 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 temperature range of 25 - 30°C. Kantwa, et al., (2015) [16] observed maximum mycelial growth and sporulation on potato dextrose agar at 25°C temperature and 100 per cent relative humidity. According to study of Rout, et al., (2015) [17] *Alternaria alternata* grows best at a temperature range of 25-30°C.The temperature 25°C and pH 7.0 was found optimum for the growth and sporulation of *Alternaria alternata* [18]. Mishra and Thawani, (2016) concluded by their study that the best growth and sporulation of the fungus *Alternaria alternata* was observed at 27˚C [19]. Tiwari, et al., (2016) also reported that *Alternata alternata* prefers 30ºC for maximum growth and sporulation followed by 25ºC and 20ºC [20].

Conclusion

The present study can be concluded that, *A. alternata* causing brown spot can be grown at 25 - 30°C for the maximum radial growth. The variability in growth rate indicates the ability of the pathogen to adjust to various temperatures.

Application of research: The results of study indicate that the pathogen can survrive in a wide range of temperature and hence the results of this study can be used further by the researchers for characterisation of pathogen and to design efficient management strategy.

Research Category: Plant Pathology

Abbreviations:
PDA: Potato Dextrose Agar

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