Physical Factors in Relation to Conidial Germination in *Alternaria porri*, the Incitant of Purple Blotch of Onion

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R.B.L. 굽타 · V.N. 파닥 · O.P. 베르마 : 양파검은무늬병균의 포자발아에 미치는 물리적 요인

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ABSTRACT Influence of temperature, relative humidity, spore washing and spore drying on conidial germination of *Alternaria porri*(Ell.) Cif. was studied. Maximum conidial germination occurred at 100% relative humidity prevailing for 6 hours or more at 25°C. Conidial germination decreased with increase in number of spore washings. Drying of conidia for more than half an hour caused significant decrease in germination. In all the experiments, conidial germinatio increased with increase in incubation period.

KEY WORDS *Alternaria porri*, Conidial germination, Physical factors, Incubation period.

INTRODUCTION

Purple blotch incited by *Alternaria porri* (Ell.) Cif. is a serious disease of onion in several countries(Ajrekar, 1922; Durrell, 1929; Skiles, 1953; Horin and Palti, 1960; Pandotra, 1964; Boelma and Ehlers, 1967). Except a few preliminary investigations on conidial germination(Nolla, 1927; Bock, 1964; Khare and Nema, 1982), the aspect has not been studied systematically. The present paper reports effects of temperature, relative humidity, spore washing and spore drying in relation to incubation period which is usually ignored in mycophysiological studies.

MATERIAL AND METHODS

Conidia of the most aggressive isolate AP-5 of *Alternaria porri*(Gupta, 1983) formed 20 days after inoculation on leaves of onion cv. 'Nasik Red' were employed in the studies. Spores were suspended in sterile water and shaken on a mechanical shaker for 10 minutes. The suspension was filtered through double folds of muslin and conidial concentration was adjusted to 0.95×10^3 conidia per ml. A glass slide having a drop of spore suspension was inverted and placed on two glass rods positioned in a humid chamber. A humid chamber was prepared by lining bottom and cover of a Petri dish with double layers of moist blotter papers. The moist chambers were then incubated at desired temperature for 5 incubation periods viz., 1, 3, 6, 12 and 24 hours.

Effect of temperature: Slides having drops of spore suspension were exposed to 10, 18, 22, 25, 28, 30, 35 and 40°C for different periods of incubation.

Effect of RH: Different levels of RH viz., 40, 50, 60, 70, 80, 90 and 100 percent were produced by mixing different proportions of sterilized distilled water and concentrated sulphuric acid(Buxton and Mellanby, 1934). Ten *ml* of the required mixture was placed in each Petri dish in which a slide containing air dried spores was kept in an inverted position. The Petri dishes were sealed with alkathene tape and incubated at 25±1°C for diff-

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Table 1. Effect of temperature on germination of conidia of *A. porri* at different incubation periods.

Temperature(°C)	Per cent conidial germination after incubations periods(hours) of					Mean
	1	3	6	12	24	
10	9.43(18.50)	48.30(44.03)	63.60(52.91)	75.33(60.24)	76.03(60.71)	54.54(47.34)
18	24.17(29.44)	57.07(49.06)	96.57(79.52)	97.50(81.10)	97.60(81.28)	74.60(64.08)
22	35.03(36.29)	93.07(74.82)	97.13(80.39)	97.40(80.88)	97.60(81.28)	84.04(70.73)
25	39.40(38.83)	96.80(79.84)	97.60(81.35)	97.73(81.55)	97.93(81.97)	85.89(72.72)
28	31.87(34.36)	78.63(62.48)	96.63(79.70)	93.23(80.66)	97.50(81.17)	80.37(67.68)
30	33.13(35.13)	75.27(60.19)	94.43(76.37)	95.20(77.38)	95.43(77.69)	78.69(63.35)
35	28.50(32.26)	66.53(54.66)	78.63(62.47)	78.87(62.63)	79.37(62.99)	66.38(55.00)
40	6.77(15.00)	16.53(23.98)	20.97(27.25)	21.67(27.73)	21.90(27.89)	17.57(24.37)
Mean	30.02	56.13	67.49	69.02	69.37	
L.S.D.(5%)	for Temperature		1.25			
	for Incubation		0.98			
	for Temperature×Incubation		2.79			

Figures in parentheses are angular values.

erent incubation periods.

Effect of spore washing: Ten m*l*. of spore suspension poured into each of the 4 centrifuge tubes were centrifuged at 2,000 r.p.m. for 10 minutes. The supernatants were poured off. The sediment of one tube(S_1) was processed for incubation. For this, the sediment was suspended in 0.5m*l* of sterile water, a drop of the suspension was placed on a glass slide and incubated for germination. The sediments in rest 3 tubes were suspended in sterile water(10m*l* per tube) and centrifuged again at 2,000 r.p.m. for 10 minutes. The supernatants were poured off. The sediment (S_2) of one tube was processed for incubation as described above. The sediments in rest two tubes were centrifuged again. Likewise, the process was repeated upto 4 cycles to get the spores washed once, twice, thrice and four times. The spores after different washings and the unwashed spores(control) were incubated at 25±1°C for different incubation periods.

Effect of spore drying: Drops of spore suspension placed on glass slides were air-dried at room temperature(20~32°C) for different durations viz. 0.5, 1, 3, 6, 9, 12, 18, 24 and 36 hours. After the drying, a drop of sterile water was placed on the dried spores and the slides were incubated at 25±1°C for different durations. The undried spores served as control.

Germination was recorded by examining 8 slides constituting four replicating four replications in a treatment. A drop of lactophenol was added to the drop of spore suspension after each incubation period. Five microscopic fields were randomly selected in each drop to record percent germination at 100X.

RESULTS

Effect of temperature: Although conidia germinated in a wide range of temperature (10~40°C), maximum germination occurred at 25°C(Table 1). A sharp decline in germination was recorded at 40°C. Increasing incubation period upto 12 hours resulted in increased germination. Beyond 12 hours of incubation, no significant increase in germination was observed. Interactions between temperature and incubation period were significant. At 25°C, 96.8 percent germination occur-

Table 2. Effect of relative humidity on germination of conidia of *A. porri* incubated at 25±1°C for different periods.

RH(%)	Per cent conidial germination after incubation periods(hours) of					Mean
	1	3	6	12	24	
40	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
50	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
60	0.00(0.00)	0.00(0.00)	0.00(0.00)	15.97(23.55)	16.30(23.81)	6.45(9.47)
70	0.00(0.00)	0.00(0.00)	28.97(32.56)	39.50(38.94)	40.33(39.43)	21.76(22.18)
80	0.00(0.00)	21.13(27.63)	35.47(36.55)	50.23(45.13)	51.70(45.97)	31.70(31.00)
90	26.83(31.19)	55.30(48.04)	66.30(54.52)	94.73(77.14)	97.70(81.43)	68.17(56.47)
100	37.30(37.64)	72.03(58.08)	96.17(79.08)	97.70(81.52)	98.13(82.28)	80.26(67.72)
Mean	9.83	17.64	28.96	38.04	38.99	
L.S.D.(5%)	for RH		4.51			
	for Incubation		3.81			
	for RH×Incubation		10.10			

Figures in parentheses are angular values.

Table 3. Effect of washing on germination of conidia of *A. porri* incubated at 25±1°C for different periods.

Washing No.	Percent conidial germination after incubation periods (hours) of					Mean
	1	3	6	12	24	
0	37.50(37.74)	77.60(61.78)	96.06(78.56)	97.00(80.18)	97.50(80.98)	81.13(67.85)
1	31.93(34.40)	54.96(47.83)	81.76(64.73(	95.50(77.76)	96.20(78.85)	72.07(60.72)
2	30.60(33.56)	50.90(45.51)	76.60(61.08)	93.73(75.74)	95.40(77.76)	69.44(58.73)
3	33.50(35.38)	45.60(42.51)	73.80(59.25)	93.36(75.27)	94.43(76.74)	68.13(57.83)
4	27.03(31.32)	35.16(36.33)	51.90(46.11)	63.00(52.54)	71.13(57.52)	49.64(44.77)
Mean	34.48	46.79	61.95	72.30	74.37	
L.S.D.(5%)	for Washings		1.36			
	for Incubation		1.36			
	for Washing×Incubation		3.05			

Figures in parentheses are angular values.

red within 3 hours, whereas longer incubation period was required to get statistically same germination percentage at 18, 22, 28 and 30°C.

Effect of RH: The conidia failed to germinate at RH less than 60 percent (Table 2). Maximum germination occurred at 100 percent RH. Increasing incubation periods upto 12 hours resulted in higher germination percentage. Interactions between RH and incubation period were significant.

Effect of spore washing: Spore washing caused reduction in spore germination though this reduction was nonsignificant in spores washed twice and thrice. Spores washed 4 times had only 49.64 percent germination as against 81.13 percent in unwashed spores. Increase in incubation period caused significant increase in percent germination. Interactions between number of washing and incubation period were significant(Table 3).

Effect of spore drying: The germination was significantly reduced when spores were dried for one hour or more. Only 4.61 percent germination was recorded in spores dried for 36 hours as against 80.36 percent in undried spores(Table 4). Increase in incubation period

Table 4. Effect of drying on germination of conidia of *A. porri* incubated at 25±1°C for different periods.

Period of drying (hours)	Percent conidial germination after incubation periods(hours) of					Mean
	1	3	6	12	24	
0	35.83(36.77)	78.60(62.46)	94.93(77.19)	96.13(78.86)	96.33(79.12)	80.36(66.88)
0.5	37.26(37.62)	77.40(61.61)	94.20(77.11)	96.50(79.36)	96.73(79.69)	80.41(66.88)
1	29.33(32.79)	67.86(56.16)	88.83(70.51)	96.43(79.16)	96.73(79.69)	75.83(63.65)
3	24.80(29.86)	57.96(49.58)	76.00(60.68)	82.53(64.46)	83.66(66.18)	64.99(54.15)
6	24.26(29.51)	44.90(42.07)	66.63(54.72)	80.70(63.95)	81.50(64.53)	59.59(50.96)
9	0.00(0.00)	0.00(0.00)	32.56(34.79)	45.66(42.51)	48.10(43.91)	25.26(24.24)
12	0.00(0.00)	0.00(0.00)	20.33(26.79)	40.93(39.77)	49.60(44.77)	22.17(22.27)
18	0.00(0.00)	0.00(0.00)	0.00(0.00)	25.56(30.36)	30.93(33.79)	11.26(12.83)
24	0.00(0.00)	0.00(0.00)	0.00(0.00)	11.30(19.63)	15.60(23.39)	5.38(8.60)
36	0.00(0.00)	0.00(0.00)	0.00(0.00)	9.13(17.57)	13.93(21.89)	4.61(7.89)
Mean	16.66	27.19	40.08	51.56	53.69	

L.S.D.(5%)	for Drying	1.34
	for Incubation	0.71
	for Drying×Incubation	3.01

Figures in parentheses are angular values.

caused significant increase in germination of conidia dried for different durations. Interactions between duration of drying and incubation period were significant. Spores dried for 9 and 12 hours could not germinate upto 3 hours of incubation and those dried for more than 12 hours failed to germinate upto 6 hours of incubation.

DISCUSSION

Spore germination was found dependent upon temperature and there was a sharp decline in spore germination at 40°C. Inability of the fungus to germinate well as higher temperatures may partially account for low incidence of the disease in seasons having high temperatures during the crop growing period. The duration of incubation period influenced the spore germination at different temperatures. Increase in incubation period at less favourable temperatures annulled the adverse effect of the less favourable temperatures.

The conidia failed to germinate at RH below 60 percent. Maximum germination occurred at 100 percent RH. The role of incubation period upto 12 hours proved significant at different RH levels. The germination percentage at 6 hours of incubation at 80 percent RH were at par with the germination percentage of one hour at 100 percent RH. These observations may have epidemiological bearings. It is likely that longer durations of low RH (not below 60 percent) may also favour the disease epidemic. The results obtained in the present studies are in consonance of those obtained by various workers (Bock, 1964; Boelma and Ehlers, 1967; Khare and Nema, 1982) in related studies.

Reduced germinability of washed conidia might be due to the removal of germination stimulants or leaching out of nutrients from conidia during the process of washing. However, it appears that adverse effect of washing could be negated by longer incubatin period. Reduction in conidial germination due to drying partly explains the poor secondary spread of the disease under dry conitions.

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