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Effect of Culture Media, *p*H and Temperature on Growth Behaviour of *Alternaria brassicae* and *Drechslera graminea*

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The optimum growth and sporulation of *A. brassicae* was observed at 22°C at *p*H 5.5 whereas that of *D. graminea* was observed at 24°C at *p*H 6.0.

Key Words: *A. brassicae*, **Culture media**, *D. graminea*, **Growth**, *p*H, **Temperature**

Introduction

Fungi are sensitive to nutritional and environmental factors and their growth and sporulation are therefore greatly influenced by the composition of the nutrient media, *p*H and temperature. The present work was, therefore, undertaken to study the effect of these factors on the growth of *A. brassicae* and *D. graminea*.

Materials and Methods

A. brassicae and *D. graminea* were isolated from the infected leaves of mustard and barley respectively. After performing their pathogenicity test their cultures were maintained on potato-dextrose agar (PDA) medium at 25±1°C. In all the experiments 7-day-old sub-cultures were uesd.

To study the effect of different media and temperature, the *p*H of the semi-solid as well as liquid media was adjusted to 5.5 for *A. brassicae* and 6.0 for *D. graminea*. For investigating effect of different *p*H, the media were adjusted to *p*H 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8 and 9. For estimating dry mycelial weight, conical flasks (of 150 ml capacity) were used in which 25 ml of the liquid medium (PDA) was poured. Autoclaving was done at 15 lb pressure/in.^{2} for 20 min and cooled to 35°C. These fungi were also grown on semi-solid medium in Petri dishes.

The semi-solid as well as liquid media were inoculated with 5 mm agar disks of hyphal tips taken from 5 days old culture. Five replicates were used for each treatment. All the experiments were carried out at 25± 1°C except those meant for the study of effect of temperature. For this purpose the

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temperatures maintained were 15°C, 20°C, 22°C, 24°C, 26°C, 28°C, 30°C and 35°C. The radial growth of the colony in Petri dishes was measured after 3, 6, 9 and 12 days of incubation for *A. brassicae* and after 2, 4, 6 and 8 days for *D. graminea*. In both the cases the average growth rate/day was calculated and expressed in mm. Dry mycelial-weight was estimated after 14 days of incubation for both the fungi.

Grades of sporulation on different media were recorded on the basis of the number of spores in a field observed under the low power of microscope. The occurrence of 1–7 spores in a field was graded as poor, 8–15 as good and above 15 as excellent. To prepare a homogeneous spore suspension, fungal blocks of equal size (5 mm in diam.) were cut from different areas of the colony, eight such blocks were added to the conical flasks containing 40 ml of sterilized distilled water.

Results and Discussion

Effect of media

A. brassicae and *D. graminea* showed good growth on various natural media (table 1)

Table 1 *Effect of culture media on growth behaviour of* A. brassicae *and* D. graminea

Media	Average growth rate/day in mm		Sporulation		Average dry mycelial wt. in mg after 15 days		*p*H of the filtrate	
	Ab	Dg	Ab	Dg	Ab	Dg	Ab	Dg
Alfalfa seed decoction	2.8	1.2	P	—	175.5	80.0	5.9	6.4
Asthana and Hawker	2.2	2.3	G	G	99.8	133.3	6.1	7.2
Corn meal agar	4.6	11.9	P	—	195.7	134.5	6.3	7.3
Czapek's agar	2.9	3.4	G	E	230.5	168.5	6.9	8.8
Dextrose-asparagine-phosphate	1.5	2.3	G	E	201.3	190.0	6.1	5.8
Glucose-asparagine	4.6	4.9	G	G	291.8	109.0	6.5	7.4
Glucose peptone agar	2.5	3.9	P	P	126.7	104.0	5.8	7.5
Gram meal agar	4.5	11.5	P	—	195.3	164.0	5.8	7.7
Host leaf decoction	5.1	10.0	P	—	229.9	122.0	7.6	8.4
Host seed decoction	4.1	9.4	P	—	222.8	97.5	6.4	6.3
Houston agar	4.7	1.9	P	G	139.3	215.3	5.7	6.4
Lima bean agar	4.0	9.3	P	—	166.5	108.7	8.0	8.5
Malt extract agar	2.9	4.2	G	G	178.3	123.3	5.9	6.2
Martin's agar	1.1	3.9	E	G	150.5	115.7	6.1	7.7
Oat meal agar	6.1	9.9	G	P	221.6	250.0	6.3	8.1
Potato-dextrose agar	3.0	6.7	E	G	235.7	184.3	5.9	7.4
Rice meal agar	4.9	10.3	—	P	295.8	198.5	5.8	6.4
Richard's agar	4.1	1.3	G	E	326.7	139.0	7.1	6.3
Thind and Mandhar	1.5	1.1	G	E	143.7	75.3	5.8	6.3
Wheat meal agar	5.1	11.7	G	—	326.7	267.7	7.1	7.7

Colony diameter and dry mycelial weight are based on the record of average of 5 replicates
Ab=*Alternaria brassicae*, Dg=*Drechslera graminea*; *E*, Excellent, *G*, Good; *P*, Poor; —, No sporulation

but oat meal agar proved best for *A. brassicae* and corn meal agar for *D. graminea*. Amongst the semisynthetic and synthetic media the glucose-asparagine medium supported best growth of *A. brassicae* which is in accordance with the observation of Prasada et al. (1970).

Houston agar medium supported the least growth. Sporulation was favoured by Martin's and PDA media. Maximum dry weight of the mycelium was observed in Richard's and wheat meal media and minimum dry weight in Asthana and Hawker's medium.

PDA medium was best for supporting the growth of *D. graminea*. Sporulation was favoured by Czapek's, dextrose asparagine phosphate, Richard's and Thind and Mandhar media. Dry weight of the mycelium of *D. graminea* was maximum in wheat meal and lowest in Thind and Mandhar medium. In general, natural and semisynthetic media were more favourable for growth of the test fungi than the synthetic ones.

Effect of pH

pH between 4.5 and 6.0 supported good growth of *A. brassicae*, the maximum occurring at pH 5.5 (table 2) which is in conformity with the findings of Prasada et al. (1970). pH had marked effect on sporulation which was nil at pH below 5 and above 7. Good growth of *D. graminea* occurred between pH 5 to 6.5 with optimum at pH 6.0 (table 2) which is in accordance with the observation of Chandwani and Munjal (1963).

It is evident from the present results that both the pathogens changed the pH of the medium by the end of the incubation period. It was noticed that whenever the initial pH was low, it drifted towards neutrality or alkaline range, whereas in highly alkaline media this was reverse. Since fungi differ in their metabolic activity and rate of growth, the pH changes brought about in the culture medium also differ. The pH changes caused by the growth of the same fungus will depend upon the composition of the medium used. The changes in

Table 2 *Effect of* pH *on growth response of* A. brassicae *and* D. graminea

pH adjusted	pH after autoclaving		pH of the filtrate	Average growth rate/day in mm		Average dry mycelial wt. in mg after 15 days	
	Ab	Dg		Ab	Dg	Ab	Dg
3.0	3.2	4.2	4.5	2.4	4.6	76.5	148.2
4.0	4.3	5.9	6.0	2.9	6.1	130.4	182.3
4.5	4.7	6.9	6.2	3.2	7.2	240.7	190.5
5.0	5.1	7.0	6.5	3.4	8.7	280.5	201.4
5.5	5.5	6.8	6.5	4.1	9.9	288.4	215.0
6.0	6.2	7.1	7.9	3.8	10.5	268.2	220.9
6.5	6.7	8.0	8.0	3.2	9.3	158.9	180.5
7.0	7.0	7.1	7.9	2.4	5.5	152.2	160.8
8.0	7.8	6.9	7.8	1.4	4.3	108.5	129.0
9.0	8.9	7.4	7.5	1.3	3.6	86.5	98.8

Ab=*Alternaria brassicae*; Dg=*Drechslera graminea*

*p*H are due to changes in the relative amounts of acids and bases formed or withdrawn and to the ionization constants of these compounds.

Table 3 *Effect of temperature on growth response of* A. brassicae *and* D. graminea

Temperature in °C	Average growth rate/day in mm		Average dry mycelial wt. in mg after 15 days	
	Ab	Dg	Ab	Dg
15	1.3	1.9	98.5	126.5
20	3.9	5.8	221.4	138.4
22	4.6	7.7	289.9	161.3
24	3.6	8.3	252.3	225.5
26	1.9	7.2	208.5	211.9
28	1.6	5.5	178.3	180.5
30	0.8	4.7	20.4	119.3
35	—	1.6	—	85.3

Ab=*Alternaria brassicae*; Dg=*Drechslera graminea*

Effect of Temperature

Good growth and sporulation of *A. brassicae* was observed at 22°C (table 3) which is in accordance with the findings of Neergard (1945), and Taber et al. (1968). *D. graminea* grows and sporulates over a wide range of temperature i.e. 15°C to 35°C with optimum at 24°C. The optimum temperature for *D. graminea* was reported to be 25°C by Johnson (1925), Shands (1934) and Chandwani and Munjal (1963).

Statistical analysis reveals that media, *p*H, temperature and incubation period have significant (P=0.01) effect on variation in radial growth of *A. brassicae* and *D. graminea*

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