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STUDIES ON THE PHYSIOLOGY OF GROUNDNUT BLIGHT PATHOGEN

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ABSTRACT

Phoma microspora Balasubrm. *et.* Narayan. causes a blight disease of groundnut. The paper reports on requirements of the fungal pathogen for its growth and sporulation *in vitro*. Lactose supported maximum mycelial growth of the pathogen, while galactose was associated with maximum conidial production. Among the nitrogen sources tested, ammonium oxalate and ammonium chloride favoured maximum growth and sporulation respectively. Growth and conidial production were optimum at 25°C and at pH 7.

KEY WORDS : Groundnut blight, Mycelial growth, Sporulation, Physiology.

Phoma microspora Balasubrm. *et.* Narayan. causes a blight disease of groundnut (*Archis hypogaea* Linn.) (Balasubramanian and Narayanasamy, 1980). This disease is known to cause considerable loss in some groundnut growing areas of Tamil Nadu. This paper reports on the findings on requirements of the pathogen for its growth and sporulation *in vitro*.

MATERIALS AND METHODS

The blight pathogen, *P. microspora* was isolated by tissue transplant method and purified by monoconidial isolation. The fungus was grown on potato dextrose agar and mycelial discs of 5 mm dia were used for inoculation. After an incubation period of 10 days at 25°C, mycelial dry weights were determined. Drying and weighing were repeated until two consecutive weights tallied. The conidial production under various treatments was determined by macerating the pycnidia along with the mycelial growth using 50 ml of sterile water and centrifuging the whole contents at 2000 rpm for 10 min to bring the conidia in suspension. The number of conidia per ml was assessed in different treatments using a hemocytometer.

Effect of carbon sources on growth and sporulation : The fungus was grown in Czapek's broth and also in amended media in which sucrose was replaced by various carbon sources on equal weight basis. Medium without any carbon source served as control. With a view to finding out the differential utilization of various nitrogen sources as reflected in mycelial weight and sporulation, sodium nitrate in Czapek's broth was substituted with various other inorganic and organic nitrogen sources. A suitable control was also maintained. The test-fungus was grown in Czapek's broth and incubated at different temperatures and the dry mycelial weights were recorded. The pathogenic fungus was grown in Czapek's broth adjusted to different pH levels and the dry mycelial weights were recorded.

RESULTS AND DISCUSSION

Effect of different carbon sources on growth and sporulation of *P. microspora* :

Lactose supported maximum mycelia growth followed by sucrose, while dextrose

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favoured the conidial production to a maximum extent followed by fructose, maltose and galactose. Galactose favoured conidial production remarkably with minimal effect on mycelial growth, while sucrose caused an increase in mycelial growth and it had no significant effect on sporulation (Table 1).

Table 1. Effect of carbon sources on mycelial growth and sporulation in *Phoma microspora*

Treatment	Mean* dry mycelial weight(mg)	Mean* number of conidia (10^6/ml)
Dextrose	560.00	15.50
Sucrose	635.00	10.00
Maltose	588.00	14.75
Starch	575.00	13.00
Lactose	705.00	12.25
Xylose	602.00	11.75
Galactose	544.00	14.75
Fructose	562.00	15.25
Control	107.00	0.25
CD (P = 0.05)	27.18	2.25

* Mean of four replicates

Reports of Misra and Mahmood (1960) and Tandon and Chandra (1962) indicate that lactose was the preferred carbon source for several species of *Colletotrichum*. An ally of lactose, melibiose was utilized by *Phyllosticta caricapapayae* (Tandan and Bilgrami, 1957). Sucrose was also utilized by many fungi like *Gercospora musae* (Kolandaisamy, 1964), *Colletotrichum papayae* (Ghosh *et al.*, 1965), *Cercospora sorghi* Ell and Everh. (Srinivas Rao, 1960) and *Helminthosporium oryzae* Breda de Haan (Misra and Mukherjee, 1962). The present study clearly indicates that the carbon sources which favoured the mycelial growth did not induce sporulation efficiently and those that supported the conidial production had no significant effect on mycelial growth.

Effect of different nitrogen sources on growth and sporulation of ***P. microspora***

Ammonium oxalate exerted maximum favorable influence on growth. Similar effects on growth were also observed due to the presence of ammonium orthophosphate, ammonium nitrate and ammonium acetate. Ammonium chloride favoured maximum sporulation and calcium nitrate and potassium nitrate also were equally efficient in accelerating sporulation. The effect on the conidial production was the least due to sodium nitrite. Among the organic sources of nitrogen, beef extract induced maximum mycelial growth and the highest sporulation was induced by casein. The organic nitrogen sources were less effective in increasing the mycelial growth and conidial production when compared to inorganic sources of N (Table 2).

Table 2. Effect of various nitrogen sources on growth and sporulation of *Phoma microspore.*

Treatment	Mean* dry mycelial weight (mg)	Mean* number of conidia (10^6/ml)
A. Inorganic nitrogen sources		
Ammonium nitrate	696.25	10.00
Ammonium sulphate	611.00	8.25
Ammonium chloride	470.00	15.75
Ammonium oxalate	721.25	8.50
Potassium nitrate	417.25	14.75
Sodium nitrate	643.50	7.50
Sodium nitrate	654.50	6.50
Ammonium acetate	686.25	6.75
Ammonium orthophosphate	705.50	6.75
Calcium nitrate	596.25	15.50

Treatment	Mean* dry mycelial weight (mg)	Mean* number of conidia (10^6/ml)
Control	104.00	0.50
CD (P = 0.05)	27.07	1.20
B. Organic nitrogen sources		
Beef extract	498.00	4.50
Casein	385.50	10.25
Urea	360.50	4.50
Asparagine	375.25	4.25
Peptone	372.25	8.25
Control	110.25	0.25
CD (P = 0.05)	24.32	1.86

* Mean of four replicates

The present finding is in line with the report of Sathiabalan Samuel (1969) who found that maximum growth of *Alternaria sesami* was induced by ammonium oxalate and ammonium acetate. Rane and Patel (1956) found ammonium nitrate the best source for the growth of *Alternaria macrospora* and Ahmed (1960) found the same to support the maximum mycelial growth of *A. palandui*. Ammonium nitrate was found to influence the mycelial growth of *Cercospora medicagenis* (Goyal and Patel, 1968) and *Helminthosporium gramineum* (Chandwani and Munjal, 1963) favourably.

Effect of different temperatures of mycelial growth and sporulation of *P. microspora*

The optimum temperature for the growth and sporulation of the fungus was found to be 25°C and the growth was suppressed at temperatures above 30°C and below 20°C (Table 3). Miller (1953) reported that temperature ranges of 25-32°C and 25-30°C were optimum for *Cercospora arachidicola* and *Cercosporidium personatum* respectively. The temperature requirements of the *Leptosphaerulina trifolii* and *L. crassiasca* were between the temperature ranges of 20-25°C and 25-30°C respectively (Graham and Luttrell, 1961). It has been reported that 25°C was optimum for the growth of several other fungi like *Alternaria tenuis* (Ashour and Kadi, 1960). *A. citri, A. solani* (Hasija, 1970) and *Phoma exigua* var. *exigua* (Paulson and Shoenewiss, 1971). Macek (1969) reported that a temperature range of 25-30°C favoured better growth of *P. glomerate*.

Table 3. Comparison of growth and sporulation in *Phoma microspora* and different temperatures.

Treatment	Mean* dry mycelial weight (mg)	Mean* number of conidia (10^6/ml)
0°C	144.00	0.25
5°C	165.25	0.50
10°C	252.00	2.50
15°C	311.50	6.00
20°C	407.50	8.00
25°C	645.25	15.00
30°C	620.50	13.00
35°C	487.25	9.50
CD (P = 0.05)	22.42	1.12

* Mean of four replicates

Effect of different pH levels on the growth and sporulation of *P. microspora*

The data (Table 4) reveal that mycelial growth and sporulation were maximum at pH 7. An increase in acidity or alkalinity was equally harmful to physiological processes. Spiers (1976) found that *Phoma exigua*, the

cause of leaf blotch of *Populus* required an initial pH of 7. Misra and Mukherjee (1962) reported pH between 6.0 and 7.0 to be the optium for the growth of *Helminthosporium oryzae*. Similar results were also obtained with *H. gramineum* (Chandwani and Munjal, 1963).

Table 4. Effect of different pH levels on mycelial growth and sporulation in *Phoma microspora*

Treatment	Mean* dry mycelial weight (mg)	Mean* number of conidia (10^6/ml)
pH 2	173.50	2.00
pH 3	194.50	4.00
pH 4	211.50	5.00
pH 5	256.25	5.25
pH 6	401.00	9.75
pH 7	665.00	15.25
pH 8	623.00	12.00
pH 9	406.00	5.50
pH 10	228.25	1.13
CD (P = 0.05)	23.45	1.78

* Mean of four replicates

The requirements of *P. microspora* are similar to that of well known pathogens and the pathogen causing the blight disease could be quite destructive causing ultimate death of the plants under favorable conditions.

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