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Morphological and cultural studies of Septoria vignicola

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Pycnidial formation starts six days after inoculation. These are globose, dark and range between 59.28-109.20 µm in diameter. The pycnidial wall is composed of 2-3 layers of brown cells. The pycnidiospores ooze 8 days after inoculation. They are filiform, straight to curved, hyaline, septate with 2-4 septa, ends pointed or round and 21.84-68.64 × 1.25-2.15 µm. Mycelial growth was most profuse on Czapek Dox's agar medium, whereas maximum sporulation took place on Coon's, Sabauraud's and Potato dextrose agar media. The fungus could grow over a wide range of temperature from 12-36°C (optimum: 24 ± 1°C) and showed no growth at 40°C or above. Pycnidial formation occurred at 16-28°C with a maximum at 24 ± 1°C. Out of seven carbon sources tested, sorbose was the best for growth and sporulation of *Septoria vignicola* whereas maltose was a poor source for sporulation. The fungus responded very well to the supply of carbon in culture media as no growth of the fungus could be observed when the carbon supply was with held. Aspartic acid supported optimum growth among all the organic sources tried, whereas among inorganic sources potassium nitrate was the best, least growth observed on ammonium nitrate.

INTRODUCTION

Leaf spot disease of Cowpea (*Vigna sinensis* L. Savi. ex Hassk.) caused by *Septoria vignicola* is a serious disease in Karnataka (R a w a l 1977) and Maharashtra (R a o 1963) states. During favourable weather conditions (June – November) it causes severe defoliation, thereby causing the failure of the crop. Studies have been made on the epidemiology and control of the disease. In the present studies emphasis was laid on the morphology of the fungus.

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MATERIALS AND METHODS

The fungus used in these studies was isolated from the diseased specimens collected form Hessaraghatta (Bangalore) through single spore culture. Potted plants were inoculated with spore suspensions prepared in sterilized distilled water for morphological studies, thin hand sections were out from the infected spots on the inoculated leaves and mounted in lactophenol cotton blue strain.

The different solid media namely, Cowpea Meal Agar, Czapek-Dox's Agar, Potato Dextrose Agar, Richard's Agar, Dextrose Asperagine Agar, Brown's Agar, Malt Extract Agar, Sabouraud's Agar, Standard Agar and Coon's Agar were tried for cultural studies. These were prepared as given by M c L e a n and C o o k (1965). All the media were autoclaved at 15 lb pressure for 20 minutes and the pH was adjusted to 6.5 with N sodium hydroxide or N hydrochloric acid. The modified Coon's medium (without agar) was used to study the effect of different temperatures and sources of carbon and nitrogen. Temperatures ranging from 8 - 40°C were tried for study. The carbon sources were added to the above medium at the rate of 7.75 g of carbon per litre, whereas nitrogen sources at the level of 277 mg of nitrogen per litre. Glucose was used as a source of carbon when effect of different nitrogen sources were studied. Similarly potassium nitrate was used as a source of nitrogen when different carbon sources were studied. Four flasks (100 ml) were used in each treatment containing 25 ml of the medium. These were then inoculated aseptically with uniform spore suspension containing 20-25 spores/ml. The initial pH was adjusted to 6.5 after autoclaving at 15 lb pressure per Sq. inch for 20 minutes.

RESULTS

The mycelium of the fungus isolated from the diseased tissue was of two types: hyaline and thin walled, brown and thick walled. The hyaline mycelium was characteristic of the young and actively growing hyphae which were dominant during the early stages of the fungus infection. The sporogenous tissue within the pycnidium was also composed of similar mycelium. The mycelium was septate. The hyphal walls were thin and hyaline. The brown hyphae were characteristics of the older mycelium and were associated with the formation of pycnidial walls. It was frequently branched ans septate.

Pycnidial formation was not definitely positioned in the host tissue. Pycnidia were formed both in the pallisade tissue and spongy parenchyma of the leaves. These were first observed 6 days after the inoculations but continued to appear for 10 days. During rainy weather the pycnidia were erumpent, these were globose and ranged between 29.28-109.20 μm in diameter. The pycnidial wall was composed of 2 - 3 layers of brown cells.

Table 1

Growth of Septoria vignicola on different solid media

Sl. No.	Medium	Colony diameter /mm/		Incubation period /days/	Extent of pycnidial formation	Extent of spore exudation	Growth characteristics
		Max.	Min.				
1	Cowpea meal agar	31.00	25.00	4	++	++	Colony black with light margins, poor mycelial growth.
2	Czapek dextrose agar	31.33	24.00	5	+++	+++	Colony chocolate with margins black, good raised mycelial growth, spore ooze present
3	Potato Dextrose agar	30.00	25.00	3	+++	++++	Colony black with light margins, good raised mycelial growth, spore ooze present
4	Richard's agar	23.00	16.25	3	++++	+++	Colony chocolate with black margins, medium mycelial growth, spore ooze present
5	Dextrose asparagine agar	29.00	25.00	3	+++	+++	Colony chocolate with light margins, medium mycelial growth, spore ooze present
6	Brown's agar	16.50	13.25	3	+++	+++	Colony chocolate with dark margins, poor raised mycelial growth, spore ooze present
7	Malt extract agar	25.50	21.50	3	++	++	Colony chocolate, poor mycelial growth, sparse pycnidial formation
8	Sabouraud's agar	30.00	22.50	3	++++	++++	Colony chocolate with dark margins, good raised zonate mycelial growth, spore ooze present
9	Standard agar	29.00	25.00	3	++++	++++	Colony chocolate with dark margins, good white mycelial growth
10	Coon's agar	30.50	26.50	3	++++	++++	Colony chocolate, good raised white mycelial growth, spore ooze present

\+ Poor ++ Fair +++ Moderate ++++ Excellent Max. Maximum Min. Minimum

The pycnidiospores started coming out of the pycnidia 8 days after inoculation. These were filliform, straight to curved, hyaline and septate having 2 - 4 septa, with pointed and rounded ends and were 21.84-68.64 × 1.25-2.15 µm in size.

The morphology of the fungus was studied on Potato dextrose agar medium. The fungus produced scanty mycelial growth on this medium at 24 ± 1°C. The hyphae were thin hyaline, septate, cottony white and irregularly branched. Pycnidial formation initiated after 4 days in the form of black dots. Both serial as well as submerged pycnidia were observed. The spores started coming out in the form of ooze from the pycnidia in 5 - 6 days time. The pycnidia measured 37.44-109.22 µm in diameter, whereas the spores were 20 - 35 × 1.87 - 2.5 µm in size. The culture lost sporulation after 10 months of storage with subculturing at monthly intervals.

The growth of the fungus in terms of colony diameter, pycnidial formation and spore exudation was best on Sabouraud's, Coon's and potato dextrose agar medium (Table 1), while Czapek-Dox's agar medium gave moderate sporulation. Malt extract and cowpea meal agar media showed very poor pycnidial formation and sporulation.

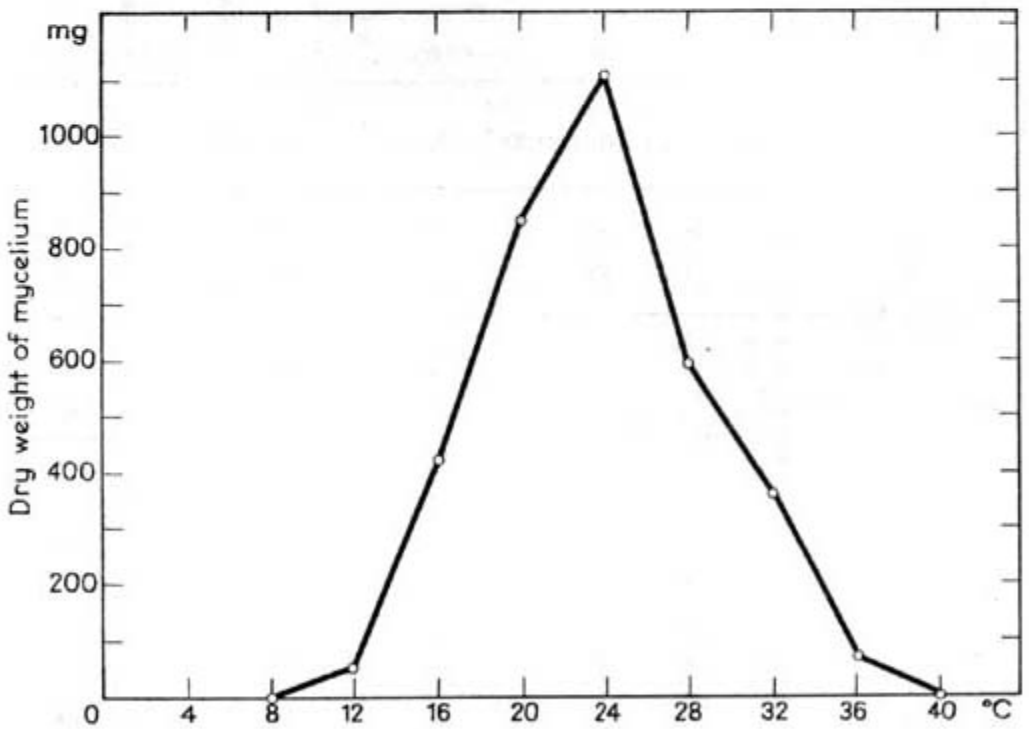

Fig. 1. Effect of different temperature on the growth of *Septoria vignicola*

The data (Fig. 1) show that the fungus could grow over a wide range of temperature i.e. 12 - 36°C. The minimum, maximum and optimum temperatures for growth of the fungus were 12,36 and 24°C, respectively. It was observed that at

low temperatures the fungus showed a tendency to grow on the walls of the flask, whereas it was not so at high temperatures. The mycelial growth was good at high temperatures. Maximum sporulation was observed at 24°C. There was not much difference in sporulation at 12 or 36°C.

The data (Fig. 2) indicated that sorbose was the best source of carbon followed by sorbitol, galactose, sucrose and lactose as the next best sources in order of effects on fungal growth. Maltose was the poorest source of carbon utilized by the fungus as indicated by sporulation. In the absence of a carbon courd the fungus did not grow at all.

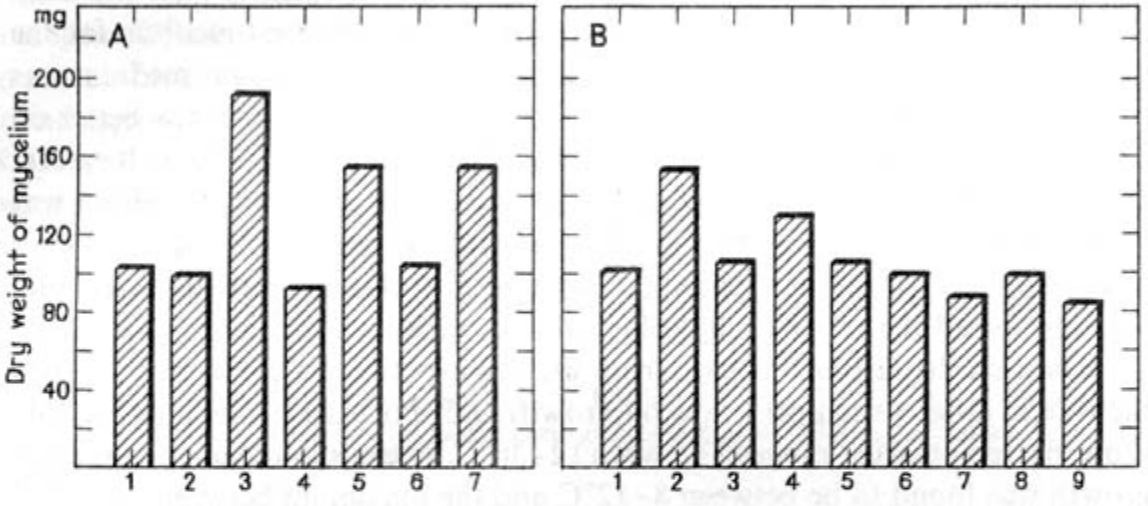

Fig. 2. Effect of various carbon and nitrogen sources on growth of *Septoria vignicola*

A – carbon source, *1* – lactose, *2* – glucose, *3* – sorbose, *4* – maltose, *5* – galactose, *6* – sucrose, *7* – sorbitol; *B* – nitrogen source, *1* – L-threonine, *2* – aspartic acid, *3* – L-arginine, *4* – L-serin, *5* – potassium nitrate, *6* – sodium nitrate, *7* – ammonium nitrate, *8* – urea, *9* – control

The fungus could grow on all the nitrogen sources tested which included both organic and inorganic compunds. Among the organic sources, asparatic acid was the best source, followed by serine. Among the various inorganic nitrogen compounds, potassium nitrate was the best source. Ammonium nitrate was the poorest source of nitrogen utilised by the fungus. Growth of the fungus was also observed on the basal medium.

DISCUSSION

Detailed morphological and pathogenicity studies conducted on the material received from various localities of South India on different cowpea varieties revealed that *Septoria vignicola* was the only species prevalent in all these localities. The same species was first reported by R a o (1963) from Poona. It differs from *Septoria glycines* and *Septoria vignae* in its morphology. It was similar to the one reported by R a o (1963) having pycnidia of the size of 63-

113.4 µm with 2-4 septate sporae measuring 21-67.2 × 1.2-2.1 µm as against 50-70 µm with 3-5 septate spore of the size of 25-35 × 1 µm in *S. vignae* (Saccardo 1913) and 44-100 µm with 1-4 septate spores of the size 21-52.5 × 1.4-2.1 µm in *S. glycines* (Saccardo 1931), (Nikolaeva, Afferova 1971). Since the measurements of S. Kozopolanski are not available in the literature hence it was not possible to compare that species with *S. vignicola.*

All the media tried supported the growth of the fungus – pycnidial formation and spore ooze was comparatively better on Coon's agar followed by potato dextrose agar, Sabouraud's agar and standard agar media. Sohi and Sokhi (1973) reported potato dextrose agar to be the best medium for the growth of *Septoria lycopersici.* In their studies Czapek-Dox's agar medium was found to be the poorest. In the present findings mycelial growth was better on Czapek-Dox's agar medium but it did not support sporulation. The malt extract agar medium supported growth with poor sporulation. Brown's medium was found to be poor for mycelial growth as well as sporulation. Since Coon's medium gave goot mycelial growth along with sporulation, it was used as basal medium for all the in vitro physiological and nutritional studies.

Based on the results obtained from various experiments conducted to study the role of different temperatures on growth and sporulation, the fungus could grow in a temperature range of between 12-36°C. The minimum temperature for growth was found to be between 8-12°C and the maximum between 36-40°C. The optimum temperature for growth and sporulation ranged between 20-28°C. The fungus failed to sporulate at 28°C and above. Taking all aspects into consideration (mycelial growth, sporulation and spore germination) the optimum temperature was found to be 24 ± 1°C. Such temperature optima have been observed for many fungal pathogens. Sohi and Sokhi (1973) noticed optimum growth and sporulation at 25°C in *Septoria lycopersici.* The optimum temperature for *Septoria apii* is reported to be 22-24°C (Walker 1957). Richards (1951) found that the cultures of *Septoria nodorum* below 18°C or above 24°C failed to sporulate. The temperature optima for various plant pathogenic fungi have been collected by Togashi (1949). He found two categories: one having the temperature optima 20-30°C and other between 26-30°C. The present fungus falls in the first category.

The utilization of carbon compounds by different fungi depends upon their enzymatic make up. The present fungus showed differentiated responses to the presence of various carbon sources tried. Out of all the carbon sources tried sorbose was utilized most effeciently as a source of carbon. Maltose was found to be a poor source for sporulation. Basu and Nandi (1974) while studying the effect of different carbon sources on the growth and sporulation of *Sphaceloma curcumae* found sorbose to be the best source and maltose a poor

source of carbon. Richards (1951) found galactose to be the most favourable source of sporulation of *Septoria nodorum*. Sohi and Sokhi (1973) reported glucose to be a good source of carbon in their studies on *S. lycopersici*. The present fungus also showed good response to galactose and glucose as sources of carbon.

The investigated fungus utilized various amino acids more efficiently than inorganic nitrogen. Of the various aminoacids *Septoria vignicola* could utilize aspartic acid more efficiently. It proved comparatively a better source of nitrogen for growth and sporulation than others tried. Lilly (1963) stated than an individual amino acids or a mixture of amino acids may by good source of nitrogen. Glycine, asparagine, glutamic acid and aspartic acid support good growth of fungi in general (Cochrane 1958). Sohi and Sokhi (1973) found asparagine to be the best source of nitrogen for growth and sporulation of *S. lycopersici*. Richards (1951) reported good sporulation in *S. nodorum* when glycine was used as a source of nitrogen. Out of the inorganic nitrogen compounds tested, potassium nitrate proved most favourable for growth and sporulation. Similar results were also obtained by Mix (1933) for growth and sporulation of *Phyllosticta solitaria*.

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