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STEMPHYLIUM BLIGHT OF GRAM AND ITS CHEMICAL CONTROL

BY

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Stemphylium blight of gram caused by *Stemphylium sarciniforme* was evident as small dark brown spot on leaves, petioles, stem and pods. Leaflets got blighted and were ultimately shed. Modified Richards's medium, pH 5.0, temperature of 25°C were found optimum for growth and sporulation of the pathogen. Sporulation was excellent in darkness and in presence of glucose, maltose and starch but better growth was obtained on glucose, fructose, mannose and sucrose. Glutamic acid, asparagine and arginine were better nitrogen sources. Addition of boron and zinc stimulated growth of the fungus which appeared partially deficient for inositol and ascorbic acid.

Thiride or Bavistin+Thiram sprays (0.1%) gave complete protection to gram plant against stemphylium blight. Captan was also effective while Ronilan, Bavistin and Fytolan did not gave adequate protection.

INTRODUCTION

Leaf spot of gram caused by *Stemphylium sarciniforme* was first reported in India by Das and Sengupta (1961) from West Bengal. Subsequently, *S. sarciniforme* has been reported to cause gram blight from Bihar by Prasad *et al.* (1969) and from Uttar Pradesh by Chauhan and Singh (1973). Kaiser (1972) observed it along with *Ascohyta rabiei* and *Sclerotinia sclerotiorum* to assume epiphytotic from in southestern Iran. During 1978-79 crop season, this disease in association with botrytis blight completely destroyed about 20,000 hactares of gram crop in Bihar (Laha and Grewal, 1983). Keeping in view, the severe incidence of the disease, the present study was undertaken to work out the physiological characteristics and mode of perpetuation of the pathogen and suitable chemical control measures.

MATERIALS AND METHODS

Naturally infected diseased plants were collected from different localities of West Bengal and Bihar. The pathogen was isolated from the diseased plant in

each case to get a number of isolates. Among the isolates a virulent isolate was selected for the study. Modified Richard's liquid medium (Sucrose 5.0 g, KNO_3 2.0 g, KH_3PO_4 5.0 g, Mg SO_4, $7H_2O$ 2.5 g, $FeCl_3$ 0.02 g, distilled water to make upto 1000 ml) was used for physiological studies. To study the effect of carbon source and amino acid, sucrose of the said medium was replaced with suitable carbohydrate and nitrogen compound by amino acid respectively. The pathogen was grown on PDA slants and exposed to different combination of light and darkness to see the effect of light on growth and sporulation.

The medium was made free from trace elements present in it as contaminants by adding 5 g of Al_2O_3/litre as suggested by Donald *et al.* (1952). It was then autoclaved at 10 p.s.i. pressure for 20 minutes. On removal from the autoclave the hot contents were swirled to wet the inside of the flask. Later the medium was filtered through Corning G-5 sintered glass filter. Then the desired quantities of Zn, Fe, Mn, and Cu as sulphates, B as boric acid and Mo as amonium molybdate were added (in concentrations given in Table 3) singly as well as in different combinations to aliquots of the filtered medium. The different treatments were dispensed (20 ml) in 100 ml flasks and steam sterilized for one hour for three consecutive days Inoculum used was obtained by growing the fungus on trace element-free medium for three generations.

The basal medium was rendered vitamin free by following the method described by Mathur *et al.* (1950). The medium was boiled with activated charcoal added at the rate of 5 g/litre. Charcoal was allowed to settle down and the medium was filtered through Corning G-5 sintered glass filter to remove traces of activated charcoal present in the medium. The filtrate was dispensed (20 ml) in 100 ml flasks and steam sterilized for one hour for three consecutive days. Thiamine hydrochloride, biotin, riboflavin, inositol, nicotinic acid, ascorbic acid, pyrodoxine hydrochloride and calcium pantothenate were dissolved singly as well as in various combinations in double glass distilled water and sterilized by passing through acid treated Corning G-5 sintered glass filter. They were then aseptically added to sterilized vitamin-free medium so as to give desired concentrations. Inoculum for these studies was obtained by growing the fungus on vitamin-free medium for three generations.

In spray trial eight fungicides namely Ronilon (50% vincozonil), Bavistin + Thiram (25% methyl-2-benzimidazole carbamate + 50% tetramethyl thiuram disulphide), Bavistin (50% methyl-2-benzimidazole carbamate), Captan (75% N-trichloro-methyl thio 4-cyclohexane-1,2, dicarboximide), Thiride (75% tetramethyl thiuram disulphide), Dithane M45 (78% manganous ethylene bisdithiocarbamate + 2% Zn ion), Dithane Z78 (75% Zinc ethylene bisdithiocarbamate), Fytolan (88% copper oxychloride) were used. Four replications of fortyfive days old plants in pots were sprayed individually with 0.05, 0.1 and 0.2 percent suspensions of the

above fungicides. One day after protective spray the pots were inoculated with spore suspension of *S. sarciniforme*. The inoculated plants were then kept in humidity chamber for 36 hours to provide optimum condition for disease development and subsequently maintained at relatively high humidity conditions. The disease incidence was recorded on the basis of 1-9 scale 1=no infection ; 3=few scattered lesions on leaflets seen after careful infection ; 5=stem infection rare, foliage infection upto 25% ; 7=stem infection common, foliage infection 25-50%, lesions larger in size with ashy borders ; 9=stem infection very common and damaging, foliage infection more than 50%, extensive drying of leaflets with defoliation).

RESULTS AND DISCUSSIONS

The disease appeared as light brown lesions on leaves, petioles, stem and pod within 3-4 days of inoculation. Later, these lesions turned dark brown. Contrary to the observations of Das and Sengupta (1961), the leaf spots observed in the present study were small, irregular and dark brown. Later, leaflets became yellow, got blighted and were shed. Similar observations have been reported by Kaiser (1972) from Iran. In the present study stem lesions were dark brown, elongated and sunken and were similar to those described by Prasad *et al* (1969).

Out of the five synthetic media, namely Richards, Mayer, Armstrong, Czapeak-Dox, Asthana and Hakker used, modified Richards's medium was found to be best for growth and sporulation of *S. sarciniforme*. Similar results with Richards's solution were reported by Young and Bennett (1922).

The present isolate of *S sarciniforme* could grow at 10-35°C with optimum growth at 25°C. The growth of the fungus at 20° and 30°C was significantly less than that at 25 C, but sporulation was equally well at these temperatures. It was also found that pH 5.0 was optimum for growth and a pH range of 4.0 to 6.0 was for sporulation.

Total darkness supported excellent sporulation of *S. sarciniforme*, while continuous light supported good mycelial growth and poor sporulation. In the presence of continuous light ten days old growth on PDA was greyish black in colour with profuse aerial mycelium, Sporulation was moderate with light and darkness combinations of 16 : 8h and 12 : 12h, while 8 : 16h combination sporulation was good.

Among the carbon sources (Table 1), glucose, fructose, mannose and sucrose supported good growth, while starch and mannitol were reletively poor sources of carbon. Cellulose did not support any growth. Curren (1986) also reported glucose and sucrose as good and cellulose as poor sources of carbon for growth of

S. radicinum. Sporulation of *S. sarciniforme*, was exeellent on glucose, maltose and starch, good on xylose, fructose and sucrose and poor on cellulose and mannitol.

Table 1. *Growth and sporulation of* S. sarciniforme *on various carbon sources*

Carbon source	Mycelial dry weight (mg)	Sporulation
Xylose	28.7	***
Glucose	36.6	****
Fructose	35.5	***
Mannose	34.7	*
Sucrose	34.6	***
Maltose	33.8	****
Raffinose	30.1	**
Starch	20.9	****
Cellulose	4.0	—
Mannitol	15.2	—
Control (no carbon)	2.8	—
C.D. at 5%	1.33	

— absent, *poor, **fair, ***good, ****excellent.

The test pathogen made excellent growth on glutamic acid, asparagine and arginine as nitrogen sources in place of potassium nitrate (Table 2). Similar results with *S. radicinum* were obtained by Curren (1968) but Breiman and Barash (1976) reported that ammonium nitrogen added to the medium containing aspargine inhibited the growth of *S. botryosum*. Cysteine inhibited growth and sporulation.

Table 2. *Growth and sporulation of* S. sarciniforme *on some amino acids*

Amino acid	Mycelial dry weight (mg)	Sporulation
Arginine	27 9	**
Glutamic acid	27.4	**
Asparagine	27.3	**
Isoleucine	24.8	**
Phenylalanine	23.0	*
Alanine	22 0	*
Cysteine	8.7	*
Control (no nitrogen)	3.0	*
Control (KNO_3)	22.5	**
C.D. at 5%	2.24	

— absent, * poor, ** fair, *** good, **** excellent.

Data presented in the Table 3 showed that 5 ppm of B and Zn, added individually, increased the growth of *S. sarciniforme*. Contrary to this, Young and Bennet

(1972) as well as Sengupta and Das (1964) reported Zn to be inhibitory for growth of *S. sarciniforme*. Addition of Mn or Cu in traces reduced the growth of the fungus. Sengupta and Das (1964) also reported similar results with Cu. They further reported that B, Mo and Fe were stimulatory to growth of *S. sarciniforme*, which is in accordance with the observations of the present study. Sporulation was stimulated by Cu and supressed by B.

Data presented in Table 4 showed that growth of *S. sarciniforme* was significantly improved when 0.5 ppm of inositol or ascorbic acid was added to the vitamin

Table 3. *Effect of trace elements on growth and sporulation of* S. sarciniforme

Trace elements	Addition of trace element			Omission of trace element	
	Concentration (ppm)	Mycelial dry weight (mg)	Sporulation	Mycelial dry weight (mg)	Sporulation
B	5	40.8	*	32.2	***
Zn	5	40.4	*	34.1	**
Mo	5	36.3	**	36.0	**
Fe	10	33.8	**	34.9	**
Mn	1	23.8	**	40.6	**
Cu	1	22.4	***	42.1	*
All trace elements added				37.2	**
No trace element added		29.3	*	30.2	*
C.D. at 5%		1.35		1.94	

— absent, *poor, **fair, ***good.

Table 4. *Effect of vitamins on growth and sporulation of* S. sarciniforme

Vitamins	Vitamin added			Vitamin omitted	
	Concentration (ppm)	Mycelial dry weight (mg)	Sporulation	Mycelial dry weight (mg)	Sporulation
Inositol	0.5	39.7	++	30.1	++
Ascorbic acid	0.5	36.7	++	29.3	++
Calcium pantothenate	0.5	35.1	+++	33.3	++
Nicotinic acid	0.5	34.6	++	35.4	++
Riboflavin	0.5	34.4	+	33.4	++
Biotin	0.005	34.2	++	32.3	++
Pyridoxine hydrochloride	0.5	33.7	++	33.7	++
Thiamine hydrochloride	0.5	30.1	+++	36.3	+
All vitamins added				33.8	++
No vitamin added		34.5	++	34.2	++
C.D. at 5%		1.52		1.37	

— absent, +poor, ++fair, +++good.

free basal medium and their ommision from the minimal medium reduced growth. B-complex group of vitamins had no stimulatory effect on growth and thiamine actually depressed it. Contrary to these observations, Sengupta and Das (1964) reported that B complex group of vitamins, particularly thiamine, riboflavin stimulated the growth. However, thiamine and calcium pantothenate, in the present siudy, were found to improve sporulation. All other vitamins tested had no effect on sporulation.

The was found to remain viable for one year in infected materials stored at room conditions. This indicates that bits of infected tissues, mixed with the seeds may serve as source of infection.

Results of control trial (Table 5) showed that protective sprays with thiride as well as bavistin+thiram (0.1%) gave complete protection to gram plants against infection by *S. sarciniforme*. Captan (0.1% and 0.2%) was also highly effective in this respect. The results were in accordance with the reports of *in vitro* sensitivity of *S. sarciniforme*, isolated from gram against thiram, captan and ceresan wet (Chauhan and Singh, 1973). They further recorded that benlate, bavistin, difolatan and dithane M45 were least effective. In the present trial also ronilan, bavistan and fytolan gave inadequate protection against stemphylium blight, while dithane M45 and dithane Z78 gave intermediate protection.

Table 5. *Relative efficacy of different fungicidal spray against aerial i fection of* S. sarciniforme

Fungicides	Per cent concentration used/Disease incidence 0.2	0.1	0 05
Thiride	0	0	3.8
Bavistin (25%)+thiram	0	0	4.6
Captan	1.1	2.7	5.3
Dithane M45	4.3	7.5	15.1
Dithane Z78	5.7	14.8	41.3
Ronilan	16.5	24.1	45.8
Bavistin	18.2	25.7	43.5
Fytolan	28.9	51.4	65.1
Control		74.8	

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