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Biochemical Studies on *Myrothecium Roridum* Tode. Ex. Fries Causing Leaf Spot of Soyabean

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ABSTRACT

Myrothecium leaf spot of soyabean caused by Myrothecium roridum Tode.ex. Fries is an important disease occurring in almost all the major soyabean growing areas of India causing about 30 per cent yield loss. The culture filtrate M. roridum resulted in maximum per cent loss of viscosity in CMC (88.57 %) and PG (90.67 %) at PH 7.0. Culture filtrate in attached leaf of soyabean caused necrosis and browning appeared after 6 days of inoculation and death of the plant occurred after 15 days.. Out of three different methods of inoculation, leaf scratch method was found most suitable for disease development in detached method as symptoms of uniform browning appeared after 3 days of inoculation and 100 per cent leaf area turned blackish after 11 days. Germination of soyabean seeds after inoculation by standard blotter method resulted in 85.00 per cent total mortality in treated seed, where as 90 per cent mortality was found in blotter treated and 100 per cent in seed and blotter treated method.

KEYWORDS : Myrothecium roridum, soyabean, biochemical studies and toxic effect

Soyabean (Glycine max (L.) Merrill) is one of the most important oil seed crops of India covering about 9 million ha yearly. Out of different diseases, leaf spot of soyabean caused by Myrothecium roridum Tode ex. Fries was described as an important and serious disease entailing into colossal losses to this important oleiferous crop in Madhya Pradesh by Shrivastava and Khan (1994). It caused an epidemic in Madhya Pradesh, India during 1994-95 (Singh and Shrivastava, 1994). Initial symptoms of Myrothecium roridum appears as small round or oval, brown spots developed with dark brown margin on leaves in the infected plant. Under favourable conditions (high humidity) creamy layer of erumpent fungus appeared and then oval to irregular shaped sporodochia formed on the infected area of leaf spot. In absence of adequate studies on pathological characteristics of this organism, studies on its enzymatic activity were considered important for development of proper management practices against this fungus.

Myrothecium. spp. is capable of producing a range of cellulolytic enzymes (Moreira et al., 2005). The phytopathogenic strain of M. roridum is capable of producing cellulases and xylanase enzyme in submerged cultures (Okunowo et al., 2010). M. roridum also produces toxins in culture medium (Murakami et al., 1998 and Khisal et al., 2002).

Materials and Methods

The present experiment was conducted in the Department of Plant Pathology, I G K V, Raipur during the period 2009-2011.

Biochemical studies

Studies on activities of Cellulase (Cx) and Polygalacturonase (PG) were carried out. Cellulase activity was determined by measuring the reduction in viscosity of 0.5 percent Carboxy Methyl Cellulose (CMC) solution by Ostwald's viscometer at time intervals of 0, 60, 120, 180, 240, 300, 360, 420 & 480 minutes. The reaction mixture consisted of 5 ml of 0.5% Carboxy Methyl Cellulose solution, 2 ml of sodium citrate buffer (at PH level of 4.0, 7.0, & 9.2) and 2 ml of enzymes preparation (Broth fungus culture filtrate).

Polygalacturonase activity was determined by measuring loss in viscosity of 1.2 percent pectin solution. Viscometric measurements were made with Ostwald's viscometer at different time interval i.e. 0, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720,780, 840, 900, 960 & 1020 minutes. The reaction mixture consisted of 5 ml of 1.2 % pectin solutions, 2 ml of sodium citrate buffer (at PH level of 4.0, 7.0, & 9.2) and 2 ml of enzymes preparation (Broth fungus culture filtrate).

In both the cases the enzyme to be prepared is heated to 1000C for 10 minutes served as control. 15 days old culture filtrate solution was used in the experiment.

Toxic effect of culture filtrate

Toxic effects of culture filtrate were carried out on leaf (Attached method and Detached method) and on seed.

Results and Discussion

Influence of culture filtrate of Myrothecium roridum on the activity of Carboxymethyl cellulase (CMC) at different PH levels

Table 1 clearly shows that cellulolytic activity differed with the change of PH levels from 4.0 to 9.2 & time interval from 0 to 480 minutes. Viscosity loss was very high (88.57 %) at PH 7.0 as compared to 85.54 and 77.16 per cent at PH 4.0 and 9.2 respectively. The per cent loss of viscosity was maximum at 360 minutes at ranges of PH 7.0 and 420 minutes for pH 4.0 and pH 9.2. The enzymatic activity in culture filtrate started at 60 minutes at all the three pH levels. The production of cellulase enzyme by a strain of Myrothecium sp. was determined by Kassim (1983), Reddy (1998) and Okunowo (2010).

Influence of culture filtrate of M. roridum on the activity of Polygalacturonase (PG) at different PH levels

Culture filtrate of M. roridum influenced pectin activity of P G at different PH levels (Table 2). The per cent loss in viscosity was very high (90.67%) at PH 7.0 as compared to 80.01 per cent and 79.65 per cent at PH 9.2 and PH 4.0, respectively. The per cent loss of viscosity was maximum at 780 minutes at pH 7.0 followed by PH 9.0 and PH 4.0 at 900 and 720 minutes, respectively. PG enzymatic activity starting at 60 minutes reached maximum at 780 minutes.

Similar works by Lucas and Sherwood (1966) revealed that the brown spot of fungus A. alternata readily produces Polygalacturonase, Pectin esterase and Cellulase (Cx) when cultured on modified Richard's solution with cellulose and starch as carbon source. On pectin salts medium, the isolates produced Polygalacturonase, Pectin esterase and Pectinlyase. The pectin lyase was most active on pectin at PH 9.2

Umana and Ikotum (2000) investigated in-vitro the three pathotypes of Choanephora cucurbitarum from Abelmoschus esculentus, Amarenthus hybridus and vigna unguiculata for production of enzyme. They found endo-polygalacturonase was the predominant enzyme at a lower PH of 4.0-6.0, with the highest activity at PH 5.0. The endo-Pectate lysate which has an optimum PH of 8.0 occurred less often. The exo- polygalacturonase had a lower PH of between 3.0 and 5.0. The amount of enzymes produced varied with the age of the culture.

Influence of culture filtrate of Myrothecium roridum on attached leaf of soyabean

Culture filtrate of Myrothecium roridum on attached leaf of soyabean resulted in slight necrosis and browning of leaf after 6 days which changed to darker color after 10 days (Table 3 & Plate 1).

Maximum appearance of the necrosis and death of the plant (100 % leaf area cover) was recorded after 15 days.

Mukarami and Shirata (2005) found that the culture filtrate of Myroth-

ecium roridum consisted of toxin Myrotoxin B of Trichothecenes that causes necrosis on leaf of Mulberry which is probably the reason for necrosis in soyabean in the present experiment.

Influence of culture filtrate of Myrothecium roridum on detached leaf of soyabean

All three methods (pin prick, leaf scratch and plain leaf) of inoculation of detached leaves of soyabean by the culture filtrate of M. roridum affected the leaf surface more in comparison to the control (Table 4).

Uniform brown colour appeared on the surface of the leaf in 3 days by leaf scratch method while pin- prick and plain leaf method took 5 days and 7 days, respectively. Brown surface darkened and chlorotic area got enlarged in 9, 11 and 15 days by leaf scratch, pin-prick and plain leaf, respectively while no symptom was produced in control leaf. 100 percent leaf area turned blackish brown in leaf scratch method at 11 days while 80.00 per cent and 55.00 per cent blackish brown colouration were recorded at 15 and 20 days in pin-prick and plain leaf method, respectively.

Kuti et al. (1989) determined susceptibility of leaves of muskmelon for necrotic and chlorotic symptoms after inoculating the leaves by culture filtrate of the M. roridum by detached method. Mukarami et al., (1998) observed necrotic and browing symptoms on mulberry plant leaves by culture filtrate of Myrothecium roridum by detached methods. Mmbaga (2010) used culture filtrate of M. roridum on detached leaves of Garden Hydrangea where necrotic lesions appeared 4-5 days after inoculation of 5 mm mycelial plugs.

Influence of culture filtrates of Myrothecium roridum on germination of soyabean seeds tested by standard blotter method

Total mortality of the seeds were recorded in seed + blotter treated (100%) followed by blotter treated (90%) (Table 5). The total mortality in seed treated alone was 85%. Minimum mortality (15%) and maximum germination (87%) were recorded in broth treated control.

Reduction, delayed in germination and death of seeds due to the application of spore suspension of M. roridum was recorded by Duke (1980) on cultivated varieties of cotton seeds. The similar work was done by Siddaramaiah et al., (1979) who reported the culture filtrate of Alternaria. carthami Chowdhary inhibited seed germination up to 50 percent, with toxic effect to all 25 varieties.

Table 1: Influence of culture filtrate of Myrothecium roridum on the activity of Carboxymethyl cellulase (CMC) at different PH levels

Sl. No.	Time interval (in minutes)	Per cent loss in viscosity at different $_{P}$H level		
		$_{P}$H 4.0	$_{P}$H 7.0	$_{P}$H 9.2
1.	0	0.00	0.00	0.00
2.	60	45.19	60.16	39.65
3.	120	56.69	65.45	50.94
4	180	60.14	71.00	68.03
5.	240	65.89	75.55	68.17
6.	300	71.19	80.33	70.74
7.	360	81.09	88.57	73.88
8.	420	85.54	88.57	77.16
9.	480	85.54	88.57	77.16

Table 2: Influence of culture filtrate of Myrothecium roridum on the activity of Polygalacturonase (PG) at different PH levels

S. No	Time interval (in minutes)	Per cent loss in viscosity at different pH level		
		$_{P}$H 4	$_{P}$H 7	$_{P}$H 9.2
1.	0	0	0	0
2.	60	40.12	20.99	15.65
3.	120	50.37	21.06	17.01
4.	180	55.23	25.62	20.67
5.	240	65.78	33.93	35.15
6.	300	67.43	50.06	38.23
7.	360	69.09	57.56	45.55
8.	420	70.43	59.40	50.15
9.	480	70.56	66.53	53.45
10.	540	72.08	75.87	55.04
11.	600	73.73	77.48	56.00
12.	660	77.62	79.88	60.32
13.	720	79.65	80.58	66.15
14.	780	79.65	90.67	75.73
15.	840		90.67	77.56
16.	900		90.67	80.01
17.	960			80.01

Table 3: Influence of culture filtrate of Myrothecium roridum on attached plant leaf of soyabean.

S. No.	Disease range	Disease development after inoculation (in days)
1.	Initiation of necrosis and browning of the leaf	6
2.	Brown colour changes to darker color	10
3.	Maximum appearance of necrosis (100 % leaf area cover) and death of the plant	15

Table 4: Influence of culture filtrate of Myrothecium roridum on detached leaf of soyabean.

S. No.	Inoculation method	Symptoms developed after inoculation in days		
		Appearrance of uniform brown colour of leaf surface	Enlargement of chlorotic and necrotic area	Appearance of blackish brown of leaf surface
1.	Pin- prick method	5	11	After 15 day s 80% of leaf area appeared blackish brown
2.	Leaf scratch method	3	9	After 11 days 100% of leaf area appeared blackish brown
3.	Plain leaf method	7	15	After 20 days 55% of leaf area appeared blackish brown
4.	Control	No symptoms appeared.	-	

Table 5: Influence of culture filtrate of Myrothecium roridum on germination of soyabean seed (tested by standard blotter method)

S. No.	Treatment	Germination (%)	Percent mortality of seeds		
			Before germination	After germination	Total mortality
1.	Seed treated	30	70	15	85
2.	Blotter treated	40	60	30	90
3.	Seed + blotter Treated	25	75	25	100
4.	Control	87	13	2	15

A



B



C



Plate 1: Influence of culture filtrate on attached plant leaf of soyabean

A – Healthy plant of soyabean
B – Initiation of necrosis and browning
C – Dead plant

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