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Effect of *Sclerotinia sclerotiorum* culture filtrate on seed germination and seedling vigour of Indian mustard (*Brassica juncea* cv. Rohini)

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Abstract

Culture filtrate of 25 *Sclerotinia sclerotiorum* isolates isolated from infected *Brassica* plants grown in nine states in India were tested for their effect on germination and seedling vigour of Indian mustard (*Brassica juncea*) cultivar Rohini; fresh and dry mycelial weight of all isolates grown in Richard's liquid medium were also recorded. All geographical isolates showed considerable variability in fresh and dry mycelial weight. Compared to the control, filtrates of all isolates reduced seed germination and length of radicle and plumule. Isolates SR 15 and SR 03 significantly reduced the germination (53.5 and 53.3%) and gave the maximum pre-emergence mortality (46.9 and 46.7%). However, isolate SR 19 gave the maximum seed germination (83.4%) and minimum pre-emergence mortality (16.7%).

Key words: *Culture filtrate, Sclerotinia sclerotiorum, seed germination, seedling vigour*

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most devastating and cosmopolitan fungal pathogen belonging to ascomycetes and capable of infecting more than 400 host plants in 75 families (Boland and Hall, 1994). In India, Sclerotinia rot is a threat to cultivation of oilseed Brassica in Rajasthan, Haryana and Punjab states of North India. The disease is important particularly in areas where mono-cropping is practiced. Disease incidence as high as 80 % has been recorded in some parts of Punjab, MP, UP and Haryana (Aggrawal *et al.*, 1997). A necrotrophic pathogen with cosmopolitan distribution and a wide host range, *S. sclerotiorum* shows a high level of intraspecific phenotypic variability (Purdy, 1979). Variability among *S. sclerotiorum* populations based generally on morphological differences in sclerotia, mycelial growth, and ascospores has also been reported (Attalah *et al.*, 2004; Harlton *et al.*, 1995; Okabe *et al.*, 1998). Fungal metabolites are substances discharged by fungi in their metabolic processes and are products of some amino acids, cyclic peptides, aromatic, phenols, terpenoids and plant growth regulators (Griffin, 1981; Madhosing, 1995). Culture filtrate from different pathogenic fungi has been reported to reduce seed germination and seedling vigour. Reduction in % seed germination of soybean seeds was observed in seeds soaked in filtrates of *Phomopsis phaseoli* (Hilty and Lee, 1988). Filtrate from mycelial cultures of *Verticillum alboatrum* was found to inhibit cell growth and reduced the viability of alfalfa (*Medicago sativa*) seeds (Frame *et al.*, 1991). Culture filtrates of *Fusaruim moniliforme*, *F. semitectum* and *F. oxysporum* are also known to reduce seed germination and inhibit root and shoot growth of sorghum. The present study was therefore, undertaken to determine the effect of culture filtrates of different geographical isolates of *Sclerotinia sclerotiorum* from *Brassica* on seed germination and seedling vigour of Indian mustard.

Materials and Methods

Sclerotinia sclerotiorum isolates

Twenty five *Sclerotinia sclerotiorum* isolates from infected *Brassica* plants were collected from Bihar, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Punjab, Rajasthan, UttaraKhand, Uttar Pradesh and West Bengal. *S. sclerotiorum* was isolated from infected plant tissues and designated

as SR-1 to SR- 25 (Table 1). For isolation, a single sclerotium or infected host tissue was surface-sterilized in 5% sodium hypochlorite solution for 5 min, in 70% ethanol for 2 min, rinsed thrice with steriled distilled water, blot-dried for 3 min and transferred into potato dextrose agar (PDA) plates incubated at 22±2°C, and newly produced sclerotia stored in 5ml screw cap glass tubes at 4°C (Atallah *et al.,* 2004).

Preparation of culture filtrate

Two discs of mycelial agar plugs (5 mm diameter) from the margin of 7 days old culture of each isolate were placed into autoclaved 100 ml Richard's liquid media ($MgSO_4$. $7H_2O$, 2.5 g/l, K_2HPO_4 5.0 g/l, KNO_3 10. 0g/l, $FeCl_3.6H_2O$, 0.02g/l and sucrose 50 g/l in double distilled water sterilized) in 250 ml conical flasks and incubated at 22±1°C on a rotary shaker at 100 rpm for 14 days. The mycelial mat was removed by filtering through a Whatman filter paper No.1 and heated at 100 °C for 2 min to inactivate enzymes (Vidhysekaran *et al.,* 1970). Conical flasks with autoclaved 100 ml Richard's liquid media without mycelial agar plugs were kept as control.

Variability in mycelial growth

Fresh mycelial weight was recorded. For dry mycelial weight, harvested mycelial mats were oven dried at 60 °C for 72 hours.

Effect of culture filtrate

Effect of *S. sclerotiorum* culture filatrates on germination and seedling vigour, one hundred seeds of *B. juncea* cv. Rohini were surface sterilized in 0.5% sodium hypochloride (NaClO) for 5 min., rinsed thrice in sterilized distilled water, placed on blotting paper in Petri plates, each watered with 10 ml of culture filtrate. Petri plates were sealed with parafilm, incubated in a growth chamber at 25±1°C and 80% relative humidity, and percentage of seed germination and length of radical and plumule recorded 72 h and 7 days after incubation, respectively. The experimental design was completely randomized (CRD) with four replications.

Results and Discussion

The growth rate of the isolates differed significantly. Maximum fresh mycelial weight was observed in SR 14 (13.9 g) followed by SR 08 (11.5 g), while SR 17 (1.3 g) produced the maximum dry mycelial weight; SR 15 yielded the minimum fresh (0.96 g) and SR 22 the minimum dry mycelial weight (0.3 g), respectively (Table 1). Compared to the control treatments, culture filtrates of isolates SR 03 and SR 15 significantly reduced seed germination (53.5 and 53.3%, respectively) and caused maximum pre-emergence mortality (46.9 and 46.7%, respectively); seed germination (83.4%) and pre-emergence mortality (16.7%) were least afftected by SR19 (Table 1). Post-emergence mortality was highest in SR 08 (80%) followed by SR 19 (76.7%); minimum post-emergence mortality was recorded in SR 02 (Table 1). Significant effect of culture filtrates was also observed on seedling vigour. For effect on lengths of radical and plumule, the culture filtrates of SR 08 and SR 14, and SR 05, 08, 09, 14, 15, 17, 20 and 22, respectively, proved to be most toxic (Table 1). Isolates SR 01 and SR 02 were least toxic in reducing lengths of radical and plumule, respectively (Table 1).

Variability in mycelial growth was observed among *S. sclerotiorum* isolates collected from different geographical regions in India. Morrall *et al.* (1972) also observed large variations in mycelial growth, numbers, shape, size and texture of sclerotia and other characteristics among 114 *Sclerotinia* spp. isolates collected from 23 hosts in Saskatchewan in Western Canada. Results of our study especially regarding variations in fresh and dry mycelial weight are also in agreement with the findings of Kohli *et al.* (1995) who reported remarkable phenotypic variability in mycelial growth rate, pigmentation, and amount of aerial mycelium in *S. sclerotiorum* populations, compared to the generally uniform appearance of isolates from canola in Canada and Norway. Willets and Wong (1980) also reported considerable variations in morphological characters among *S. Sclerotiorum, S. trifoliorum* and *S. minor* isolates.

The results of this study have shown that culture filtrates of different geographical isolates of *S. sclerotiorum* inhibit seed germination and seedling vigour including length of radical and plumule of Indian mustard. This shows that *S. sclerotiorum* produces toxic metabolites in the media in which they are grown. Pathogenic fungi may often damage their host plants by producing phytotoxins, which cause various symptoms including necrosis,

Table 1: Culture filtrate of different geographical isolates of *Sclerotinia Sclerotium* and their effect on seed germination and seedling vigour

Isolate no.	Fresh mycelial wt (g)	Dry mycelial wt (g)	Per cent seed germination	Pre-emergence mortality (%)	Post-emergence mortality (%)	Seedling vigour (mm)	
						Radicle	Plumule
SR 01	4.21	0.58	70.0 (56.79)	30.0 (33.21)	46.7 (43.11)	7.33	9.67
SR 02	1.65	0.53	63.3 (52.71)	36.7 (37.29)	26.7 (31.11)	5.33	10.67
SR 03	3.18	0.78	53.5 (47.01)	46.9 (43.22)	33.3 (35.24)	3.33	7.00
SR 04	5.40	0.89	76.7 (61.14)	23.3 (28.86)	46.7 (43.11)	2.12	3.67
SR 05	8.78	0.67	73.3 (58.89)	26.7 (31.11)	63.3 (52.71)	1.00	0.00
SR 06	6.42	1.16	63.3 (52.71)	36.7 (37.29)	50.0 (45.00)	2.67	3.67
SR 07	3.57	0.95	63.3 (52.71)	36.7 (37.29)	43.3 (41.15)	1.50	3.33
SR 08	11.51	0.74	80.0 (63.44)	20.0 (26.56)	80.0 (63.44)	0.10	0.10
SR 09	4.88	0.82	70.0 (56.79)	30.0 (33.21)	66.7 (54.76)	0.33	0.00
SR 10	4.25	1.26	70.0 (56.79)	30.0 (33.21)	56.7 (48.85)	5.67	4.00
SR 11	8.97	0.57	73.3 (58.89)	26.7 (31.11)	60.0 (50.77)	3.67	4.00
SR 12	4.68	0.75	66.7 (54.76)	33.3 (35.24)	43.3 41.15)	2.00	1.33
SR 13	5.23	0.85	76.7 (61.14)	23.3 (28.86)	63.3 (52.71)	4.67	2.67
SR 14	13.87	0.55	73.3 (58.89)	26.7 (31.11)	73.3 (58.89)	0.10	0.10
SR 15	0.96	0.52	53.3 (46.89)	46.7 (43.11)	33.3 (35.24)	1.33	0.00
SR 16	5.61	0.75	73.3 (58.89)	26.7 (31.11)	63.3 (52.71)	1.00	0.33
SR 17	9.00	1.27	66.7 (54.76)	33.3 (35.24)	63.3 (52.71)	0.67	0.00
SR 18	2.64	0.98	56.7 (48.85)	43.3 (41.15)	46.7 (43.11)	4.00	2.67
SR 19	3.30	0.78	83.3 (65.88)	16.7 (24.12)	76.7 (61.14)	1.00	0.33
SR 20	7.92	0.39	66.7 (54.76)	33.3 (35.24)	63.3 (52.71)	0.67	0.00
SR 21	2.91	0.73	70.0 (56.79)	30.0 (33.21)	43.3(41.15)	8.00	2.00
SR 22	7.14	0.33	70.0 (56.79)	30.0 (33.21)	66.7 (54.76)	0.33	0.00
SR 23	3.76	0.65	73.3 (58.89)	26.7 (31.11)	60.0 (50.77)	3.67	6.33
SR 24	4.33	1.04	76.7 (61.14)	23.3 (28.86)	73.3 (58.89)	0.33	0.33
SR 25	3.45	0.89	63.3 (52.71)	36.7 (37.29)	33.3 (35.24)	5.33	4.33
Control (Un inoculated media)	0.00	0.00	90.0 (71.56)	10.0	5.31	60.42	14.21
Control (with distilled water)	0.00	0.00	96.7 (79.53)	3.33	0.00	111.0	52.33
CD (at 5%)	0.886	0.168	1.633	1.633	1.735	2.554	2.510
CV	10.179	13.699	14.168	11.261	9.946	12.835	13.468

*Figures in parenthesis are angular transformed values

chlorosis, wilting, water soaking and eventually death of plants (Scheffer, 1983). Fungal filtrates from *Fusarium solani, F. oxysporum, Aspergillus. niger, A. flavus, A. terreus* and *Alternaria alternata* were reported to reduce germination of soybean seeds (Ibraheem *et al.,* 1987). The culture filtrate of *Penicillium citrinum, Fusarium moniliforme* and *F. equiseti* also adversely affected the seed germination of fennel (Sharma and Sharma, 1983). The inhibition of radicle and plumule growth especially in wheat grown from seeds treated with Fusarium head blight fungus has been also reported (Gilbert and Tekauz, 1995). Metabolites from six different fungi also adversely affected seed germination and seedling growth in coriander (Pant, 2011).

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