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Patho-biochemical investigations on stem rot (*Sclerotinia sclerotiorum*) of Indian mustard (*Brassica juncea* L.)

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Abstract

The Indian mustard (*Brassica juncea* L.) is the main source of cooking oil in Asia. India is one of the leading oilseed *Brassicas* producing country in the world accounting for 11.12 per cent of the world's rapeseed-mustard production, and ranks third in the world next to China and Canada. *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal organism of stem rot (SR) of *Brassica* and over 500 host plants, is menace to cultivation of oilseed *Brassica* crops in the world. Infection occurs on leaves, stems and pods at different developmental stages, causing seed yield losses of up to 40%, as well as significant reductions in oil content and quality. In various bioassays, the partially purified toxic metabolites of the pathogen resulted in maximum wilting and chlorosis at 2:8 (Double Distilled Water: Partially Purified Toxin), and adversely affected the germination of seeds, and development of root and shoot of seedlings.

Key words: Indian mustard, *Sclerotinia sclerotiorum*, seedling vigour, toxin

The oil yielding *Brassica* crops grown in India include rai or raya or Indian mustard [*B. juncea* (L.) Czern. & Coss.], and rapeseed (*B. rapa* sp. *oleifera*] which cover an area of about 6.3 million hectares with an annual production of 7.4 million tonnes with an average productivity of 1176kg/ha (Kumar, 2014). More than thirty diseases occur on *Brassica* crops in India (Saharan *et al*., 2005). Infection by *Sclerotinia sclerotiorum*, a necrotrophic pathogen with a wide host range of cultivated and wild plant species, results in damage of the plant tissue, followed by cell death and development of soft rot or white mould (Purdy, 1979). Significant increase in the sclerotial population in the soil due to monocropping of rapeseed-mustard under irrigated conditions has made SR a very serious disease of oilseed *Brassica* crops in Rajasthan, Haryana, Punjab, Assam, West Bengal, Madhya Pradesh, Uttar Pradesh, and Bihar states in India (Saharan and Mehta, 2005). The production of oxalic acid by the pathogen is known to be an important factor in pathogenicity of *S. sclerotiorum (*Zhou and Boland, 1999).

Considerable evidence in the literature shows that fungal toxins play a major role in plant pathogenesis. Toxins, or telepathogens, are important metabolic products which partially or fully induce typical characteristic disease symptoms similar to those elucidated by a host specific toxin victorin produced by *Helminthosporium victoriae* (Samaddar and Scheffer, 1971). Toxins in low dosages, cause lesions on cell membranes hampering the cell permeability and thereby triggering increased leakage of electrolytes from the susceptible tissues (Thatcher, 1939). Evidence also exists that the cell free culture filtrates of twenty five geographical different *S. sclerotiorum* isolates induce toxic activity by producing characteristic foliar symptoms in Indian mustard (Sharma *et al.*, 2014). Partially-purified toxins from *Verticillium dahliae*, a wilt pathogen, were also capable of detecting losses of cell permeability in the susceptible, but not in the resistant, tissues of cotton hosts (Gour and Dube, 1985). Partially-purified toxins of *Fusarium oxysporum* f. sp. *cumini* have also been successfully used to screen cumin genotypes for resistance against wilt (Gour and Agarwal, 1988). The present studies, therefore, were conducted to determine disease producing capabilities of partially-purified *S.*

sclerotiorum toxic metabolites on detached leaves, seed germination, and seedling vigour.

A single sclerotium, or infected host tissue, was surface-sterilized in 5% sodium hypochlorite for 5 min, in 70% ethanol for 2 min, rinsed thrice with sterilized distilled water, blot-dried, and incubated on Potato dextrose agar (PDA) plates at 22±2 °C; newly produced sclerotia were harvested and stored in 5ml screw cap glass tubes at 4 °C (Sharma *et al.,* 2013).

The 100 ml Erlenmeyer flasks, each containing 25 ml sterilized Richard's medium (pH 6.5), inoculated with 4 mm diameter fungal pieces of 7-day old PDA grown cultures of *S. sclerotiorum,* were incubated for 15 days at 22±2 °C. The cell-free-clear culture filtrate was obtained by filtering mycelium through Whatman no 42 filter paper (Kumar *et al.,* 2013). To obtain partially-purified toxin (PPT), the culture filtrate was centrifuged at 3000 rpm for 20 min, fractionated using 70% ammonium sulphate until 25% saturation, stored at 4°C, and used for bio-assay following the method of Kumar *et al.* (2013).

Three to four fresh leaves of mustard (*Brassica juncea*), okra (*Abelmoschus esculentus),* brinjal (*Solanum melongena)* and twigs of chilli (*Capsicum annuam*) and tomato (*Solanum lycopersicum),* gently excised from 30-days old plants, were immersed in test tubes containing different dilutions (DDW: PPT 6:4, 4:6, 2:8) of partially-purified toxin solutions. All the treatments were run in four replications under similar conditions. Twigs/ leaves dipped in sterilized distilled water served as control. The symptoms produced on leaves were recorded following 0-5 point scale of Kumar *et al.* (2013) as follows:

Chlorosis: 0 = no chlorosis; 1 = slight chlorosis covering 1 % leaf area; 2= slight chlorosis with slight vein clearing; 3 = chlorosis increase and formation of primary lesions; 4 = formation of light brown patches; 5 = chlorosis covers more than 50 % leaf area.

Wilting: 0 = plant healthy; 1 = slight wilting; 2 = increased wilting with leaves showing some turgidity; 3 = leaf curling, increased wilting; 4 = leaves show complete curling; 5 = Leaves completely wilted and dried.

One hundred surface sterilized seeds of Indian mustard cv. Rohini were soaked in different dilutions (6:4, 4:6, 2:8) of PPT for 4 hr, were placed in Petri plates lined with sterilized moistened filter paper, and incubated at 22±2^{0}C until germination. Seeds treated with sterilized water served as control. The experiment was carried out in four replications. Per cent reduction in germination, root and shoot lengths was recorded after 7 days.

In all five plant species tested, 2:8 dilution of PPT produced the maximum chlorotic and necrotic symptoms. The PPT-produced chlorotic and necrotic symptoms invariably increased from 12 to 24 hrs with all three PPT dilutions (Table-1). On mustard and tomato leaves, 2:8 PPT dilution produced maximum wilting and chlorosis (5.0) after 24 hrs; on chilli, 2:8 PPT dilution also gave the maximum wilting and chlorosis rating of 5 after 24 hrs. On okra and brinjal leaves the wilting and chlorosis symptoms gradually increased to the maximum after 24 hours (Table-1). Seeds of susceptible Indian mustard cv. Rohini treated with PPT dilution of 2:8 and 6:4, inhibited germination by 36% and 16%, respectively. Similarly, PPT dilution of 2:8 also inhibited root and shoot lengths by 48.0 and 45.0% (Table 2).

Fungal pathogens are known to synthesize various kinds of secondary metabolites which play a key role in pathogenesis. In the present investigation, dilution of 2:8 induced maximum chlorosis and wilting which increased with time. Several workers have documented the properties and specificity of fungal toxins in host plants. Kramer *et al.,* (1989) observed similar results with the partially produced toxins by *A. alternata* and *Drechslera teres.* Kumar *et al.,* (2013) also reported similar findings with *A. alternata* and *Fusarium oxysporum* f.sp. *lycopersici* produced toxins. Effects of phytotoxic metabolites of *S. sclerotiorum* on cauliflower and cabbage leaves also resulted in highly susceptible and moderate reactions (Sharma *et al.,* 2004).

In the present studies, dilution of partially-purified toxins decreased the efficacy of toxic metabolites.

Table 1: Effect of different dilutions of partially-purified toxin(s) (PPT) produced by *S. sclerotiorum* on detached leaves.

Dilutions* (DDW:PPT)		Symptoms expressed by PPT			
		Wilting		Chlorosis	
		12 hour	24 hour	12 hour	24 hour
Mustard	6:4	3.5	4.0	3.0	3.5
	4:6	4.0	4.5	3.5	4.0
	2:8	4.5	5.0	4.0	4.5
Tomato	6:4	3.5	3.5	3.5	4.0
	4:6	4.0	4.5	4.0	4.5
	2:8	4.5	5.0	4.5	5.0
Brinjal	6:4	3.5	4.0	3.5	4.0
	4:6	4.0	4.5	4.0	4.5
	2:8	4.5	5.0	4.5	5.0
Chilli	6:4	2.5	3.0	3.0	4.0
	4:6	3.0	3.5	4.0	4.5
	2:8	3.5	4.0	4.5	5.0
Okra	6:4	3.0	4.0	3.5	4.0
	4:6	3.5	4.5	4.0	4.5
	2:8	4.0	5.0	4.5	5.0

* DDW=Double distilled water, PPT=partially purified toxin

Table 2: Effect of different dilutions of partially-purified toxin(s) (PPT) of *S.sclerotiorum* on seed germination and seedling vigour of Indian mustard cv. Rohini.

Pathogen	Dilutions** (DDW/PPT) (ml)	Seed germination		Seedling vigor			
		Germination per cent	Per cent inhibition over control	Root length (cm)	Per cent inhibition of root length over control	Shoot length (cm)	Per cent inhibition of shoot length over control
S.Sclerotiorum	6:4	76.0s	16.0	6.7	25.0	6.8	15.0
	4:6	68.2	24.0	5.8	33.0	5.6	30.0
	2:8	57.9	36.0	4.5	48.0	4.4	45.0
Control	10:0	90.0	-	8.7	-	8.0	-

Similar dilution effects were also reported by Niranjana and Shetty (1998) in sorghum cultivars treated with culture filtrates of *F. moniliforme*. Adverse effects of *S. sclerotiorum*-produced toxic metabolites on seed germination and seedling vigour of Indian mustard have also been reported previously (Sharma *et al.,* 2014). The earliest effects of toxin on susceptible hosts are the alterations in the cell permeability (Samaddar and Scheffer, 1971). Development of reproducible detached-leaf bioassay technique with a precise concentration of partially-purified toxins may prove very useful in screening large number of genotypes against several toxin-producing pathogens under laboratory conditions in a short time.

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