Mycobiology 34(3): 114-119 (2006)

Variation in Biochemical Composition among Indian Isolates of *Sclerotinia sclerotiorum*

S. Ameer Basha[1], B. K. Sarma[1], K. P. Singh[2] and U. P. Singh[1]*

[1]*Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India*
[2]*College of Forestry & Hill Agriculture, G. B. Pant University of Agriculture and Technology, Hill Campus, Ranichauri-249199, India*
(Received May 3, 2006)

Biochemical variability among 20 Indian isolates of *Sclerotinia sclerotiorum* collected from different hosts/soil samples from different localities in India is reported. High Performance Liquid Chromatographic (HPLC) analysis of ethyl acetate fraction of culture filtrate, mycelia, sclerotia and sclerotial exudate showed 15~23 peaks but only 11 could be identified. They were tannic, gallic, oxalic, caffeic, vanillic, ferulic, *O*-coumeric, chlorogenic, cinnamic, salicylic and gentisic acids. The amount of phenolic compounds varied among the culture filtrates, mycelia, sclerotia and sclerotial exudates of *S. sclerotiorum*.

KEYWORDS: Biochemical composition, *Sclerotinia sclerotiorum*, Variability

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic pathogen with cosmopolitan in distribution and wide host range (Purdy, 1979). Some workers (Corsini and Tourneau, 1973; Jones and Cooke, 1977) reported biochemical variability in *S. sclerotiorum* particularly in the amount and kind of organic acids (oxalic, fumaric, mallic and succinic acids) which vary with the isolates and the medium. According to some workers (Chet and Henis, 1975; Willetts and Bullock, 1992; Willetts and Wong, 1980) the importance of sclerotia for survival and propagation of *S. sclerotiorum* and other sclerotium-forming fungi has stimulated numerous investigations into the structural makeup and developmental regulation of sclerotia.

Keates *et al.* (1998) reported that extracts of sclerotia of *S. sclerotiorum* contained 2 active constituents, D-glycero-pent-2-enono-1,4-lactone (D-erythroascorbic acid), and 5-O-(alpha-D-galactopyranosyl)-D-glycero-pent-2-enono-1,4-lactone. Starratt *et al.* (2002) demonstrated that young cultures of *S. sclerotiorum* isolate SS7 produced 1,8-dihydroxynaphthalene monoglucoside, a new natural product. It was previously shown to produce and excrete into agar medium in copious amounts of melanin precursor 1,8-dihydroxynaphthalene. When cultured in the presence of tricyclazole, such young cultures also accumulated two new monoglucosides of 1,3,8-trihydroxynaphthalene, as well as 1,8-dihydroxynaphthalene monoglucoside. Sharma *et al.* (2001) reported the activities of pectinases and pectin methylesterase enzymes in culture medium of *S. sclerotiorum* which were inhibited by phenolic compounds (m-coumaric, homovanillic and protocatechuic acid) present in the culture medium. Singh *et al.* (2004) analyzed ethyl acetate fraction of exudate of *S. sclerotiorum* with the help of High Performance Liquid Chromatography (HPLC) and showed that it consisted of tannic, gallic, ferulic and cinnamic acids along with many other unidentified compounds and the exudate showed antifungal activity against some parasitic as well as saprophytic fungi.

As much work has not been done about biochemical variability among the Indian isolates, the present experiments were conducted on the above aspects and the results are presented here.

Materials and Methods

Maintenance of *Sclerotinia sclerotiorum* isolates. Twenty isolates of *S. sclerotiorum* used in this study were collected from various hosts/soil samples from diverse geographic origins (Table 1). The isolates were further purified by growing single sclerotia from each colony grown on potato dextrose agar (PDA) (peeled potato 200 g, dextrose 20 g, agar 15 g, distilled water 1 *l*) slants.

Ethyl acetate fractionation of sclerotia, mycelia and culture filtrate of *S. sclerotiorum*. Twenty-five *ml* potato dextrose broth was poured in each of 100 *ml* capacity flasks. The mycelial disc from actively growing cultures of *S. sclerotiorum* was inoculated and allowed to grow for a week at 25 ± 2°C. The mycelial mat of the isolates from potato dextrose broth was harvested and washed three times with distilled water and placed on a pad of sterile filter paper to remove excess water. Two g of the mat from each isolate was thoroughly macerated separately in ethyl acetate in a pestle-mortar. The finely crushed material was collected in screw-capped bottles containing 5 *ml* ethyl acetate and kept overnight. The culture filtrate was filtered through Whatman No. 1 filter paper. An equal

*Corresponding author <E-mail: upneem@sify.com>

Table 1. Phenolic acid content ($\mu g/ml$) in culture filtrates of 20 isolates of *Sclerotinia sclerotiorum*

		Phenolic Acid										
Isolate	Host	Tannic	Gallic	Oxalic	Caffeic	Vanillic	Ferulic	*O*-coumeric	Chlorogenic	Cinnamic	Salicylic	Gentisic
1	*Abelmoschus moschatus*	14.8	–	783.86	–	10.98	181.95	0.19	–	–	–	–
2	*Amaranthus tristis*	0.17	–	616.75		–	–	19.85	46.77	–	–	–
3	*Brassica campestris var. toria*	–	1.30	22.03	–	3.14	61.69	4.26	9.50	1.61	0.829	–
4	*Brassica oleracea var. botrytis*	–	–	852.9	–	35.57	556.47	0.078	–	–	–	27.91
5	*Brassica oleracea var. botrytis*	–	–	367.21	–	17.23	300.29	–	–	–	–	32.43
6	*Cicer arietinum*	–	–	21.42	31.97	–	241.42	15.02	84.77	8.48	–	184.73
7	*Coriendrum sativum*	–	–	998.76	–	–	235.79	16.30	43.98	–	–	20.44
8	*Cucurbita pepo*	1.49	–	28.46	15.93	30.42	–	–	–	–	–	–
9	*Daucus carota*		–	637.15	–	0.29	249.79	2.37	238.63	–	–	60.08
10	*Gaillardia pulchella*	0.14	–	161.27	–	–	–	13.52	–	–	–	1.86
11	*Helianthus annus*		–	968.25	–	–	91.68	0.325	–	–	–	–
12	*Mentha arvensis*	0.05	–	72.47	3.11	–	45.62	1.35	–	0.101	–	–
13	*Papaver somniferum*	0.03	–	28.79	–	–	297.52	7.67	71.95	–	–	–
14	*Phaseolus vulgaris*	–	590.30	97.45	–	461.96	0.39	118.76	0.060	–	2.90	–
15	*Phaseolus vulgaris*	–	280.27	56.93	–	206.45	3.29	127.12	1.37	–	–	–
16	*Pisum sativum*	–	3.63	158.39	–	2.98	109.81	–	21.08	0.615	–	4.06
17	*Pisum sativum*		–	930.27	–	23.22	345.73	1.48		9.70	0.905	33.20
18	Soil	–	–	638.63	–	26	499.34	0.63	99.45	0.18	–	–
19	*Solanum melongena*	–	–	33.42	–	15.91	141	–	108	0.42	–	14.03
20	*Solanum melongena*	1.07	–	62.27	–	13.49	298.39	0.612	72.37	2.55	0.42	3.15

volume of ethyl acetate was mixed with culture filtrate separately, and after vigorous shaking in a separatory funnel, the ethyl acetate fractions of the culture filtrate were collected separately. The residue was extracted second time and the ethyl acetate fractions were pooled with the previous extract. The fractions were then evaporated under vacuum. Dried samples were resuspended in 1 *ml* HPLC grade methanol by vortexing and stored at 4°C for further analysis.

Exudate depletion experiment. Mycelium of an actively growing (5-day-old) culture of *S. sclerotiorum* grown PDA medium was cut with a 5 mm diameter sterile cork borer and transferred to the center of Petri dishes containing PDA and incubated at 25 ± 2°C. The plates were observed regularly for the formation of exudate destined for sclerotium formation. The exudate in the form of fine droplets was discerned after 6~10 days. They were removed by sucking with a sterilized capillary tube, collected in sterile culture tubes and stored at 4°C. Sclerotial development following exudate depletion was consistently observed and the fresh weight and size of 100 mature exudate-depleted sclerotia were measured and compared with the completely matured non-depleted sclerotia of the same age. The whole experiment was repeated thrice.

Collection of sclerotia was done from different isolates grown on PDA and the matured sclerotia was collected after a week. One g of sclerotia from each isolate was taken separately and crushed in a pestle-mortar in the same way as the ethyl acetate fractions of mycelia described earlier.

HPLC analysis. High performance liquid chromatography (HPLC) of the samples was performed with HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with two Shimadzu LC-10 AT*VP* reciprocating pumps, a variable UV-VIS detector (Shimadzu SPD-10 AT*VP*) and a Winchrom integrator (Winchrom). Reverse phase chromatographic analysis was carried out in isocratic conditions using RP C-18 HPLC column (250 × 4.6 mm id, particle size 5 μm, Luna 5 μ C-18 (2), Phenomenex, USA) at room temperature. Running conditions included injection volume: 5 μl, mobile phase: methanol-0.4% acetic acid (80 : 20, v/v), flow rate: 1 *ml*/min, detection at 290 nm and attenuation response 0.03 AUFS. Samples were filtered through membrane filters (Pore size 0.20 μm, Millipore) prior to injection in sample loop. Tannic, gallic, oxalic, caffeic, vanillic, ferulic, chlorogenic and cinnamic acids were used as internal and external standards. Phenolic compounds present in the samples were identified by comparing retention time (Rt) of the standards and by the co-chromatography. Contents of phenolic acids were calculated by comparing peak areas of reference compounds with those in the samples run under similar elution conditions.

Results

HPLC analysis of culture filtrate. HPLC analysis of the culture filtrate of different isolates of *S. sclerotiorum* revealed 4~14 peaks consistently. Out of these, some were identified on the basis of their retention time (Rt.) as well as by co-injection with the standard compounds. Among

Table 2. Phenolic acid contents (μg/g) in mycelium of 20 isolates of *Sclerotinia sclerotiorum*

		Phenolic Acid										
Isolate	Host	Tannic	Gallic	Oxalic	Caffeic	Vanillic	Ferulic	*O*-coumeric	Chlorogenic	Cinnamic	Salicylic	Gentisic
1	*Abelmoschus moschatus*	2.61	6.57	255.93	–	5.18	105.13	–	14.04	0.35	–	1.86
2	*Amaranthus tristis*	0.26	9.55	–	–	–	23.48	–	0.15	–	–	–
3	*Brassica campestris var. toria*	2.76	6.78	37.89	–	0.98	16.56	12.34	2.23	–	–	0.12
4	*Brassica oleracea var. botrytis*	0.97	4.92	–	–	2.04	90.78	–	–	–	–	–
5	*Brassica oleracea var. botrytis*	2.78	12.23	78.92	–	6.23	32.0	3.67	0.56	–	–	–
6	*Cicer arietinum*	1.05	40.06	–	–	–	103.95	–	–	2.62	1.10	120.83
7	*Coriendrum sativum*	1.50	4.66	425.51	–	0.13	117.37	–	25.78	0.35	0.85	11.10
8	*Cucurbita pepo*	3.27	1.78	98.34	–	0.09	27.83	12.67	7.90	–	–	–
9	*Daucus carota*	1.33	16.95	–		–	32.25	2.41	4.51	0.043	–	–
10	*Gaillardia pulchella*	2.78	5.08	–	1.92	–	175.47	–	–	3.50	5.57	56.22
11	*Helianthus annus*	0.84	3.57	271.27	–	0.06	37.67	–	–	–	2.56	–
12	*Mentha arvensis*	2.88	11.95	9.59	–	14.52		–	35.29	–	0.22	23.47
13	*Papaver somniferum*	6.07	24.01	32.44	–	–	330.19	–	2.44	–	2.50	
14	*Phaseolus vulgaris*	1.78	–	–	4.61	11.28	–	0.066	–	–	–	36.25
15	*Phaseolus vulgaris*	0.45	–	–	0.79	23.21	–	0.27	–	–	–	23.47
16	*Pisum sativum*	3.62	8.59	–	2.97	0.027	54.97	0.058	16.07	0.32	0.177	0.37
17	*Pisum sativum*	3.69	13.28	–	–	–	26.30	–	1.012	–	–	–
18	Soil	0.30	2.56	–	–	–	29.83	0.08	0.67	0.039	–	–
19	*Solanum melongena*	60.35	1.93	–	–	–	28.68	–	26.48	–	–	–
20	*Solanum melongena*	5.23	19.69	–	–	0.02	87.24	–	0.68	–	–	–

these peaks, six consistently appeared in culture filtrates of most of the isolates. The peaks identified were of tannic (Rt.2.76 min), gallic (Rt.2.86 min), oxalic (Rt.3.03 min), caffeic (Rt.3.12 min), vanillic (Rt.3.26 min), ferulic (Rt.3.30 min), o-coumeric (Rt.3.55), chlorogenic (Rt.4.16 min), cinnamic acids (Rt.4.46 min) and gentisic acids (Rt.6.62). Out of 20 isolates, gallic acid was detected in 4 isolates, oxalic in all, vanillic in 13, ferulic in 17, o-coumeric in 16, cinnamic in 8, caffeic in 3, tannic in 7 and chlorogenic acid in 12 isolates. The major component of culture filtrates was oxalic acid and its amount varied from 21.42 to 998.76 $\mu g/ml$ in ethyl acetate fractions. Ferulic acid ranged from 0.39 $\mu g/ml$ (*Phaseolus vulgaris*) to 556.47 $\mu g/ml$ (*Brassica oleracea* var.. *botrytis*) while, it was absent in 3 isolates. Caffeic acid ranged from 3.11 $\mu g/ml$ (*Mentha arvevs*) to 31.97v (*Cicer arietinum*) being absent in 16 isolates. Cinnamic acid was 0.101 $\mu g/ml$ in *Mentha arvevs* collected from Ranichauri and 9.70 $\mu g/ml$ in *Pisum sativum* isolate from Lucknow and not detected in 8 isolates. Gallic acid was 1.30 $\mu g/ml$ in *Brassica campestris* var. *toria* isolate and 3.63 $\mu g/ml$ in *Pisum sativum* isolate. Vanillic acid was 0.29 to 461.96 $\mu g/ml$ in some isolates. Eleven isolates showed chlorogenic acid while six isolates had tannic acid. Gentisic acid was found in 10 isolates (1.86 to 184.73v) whereas salicylic acid was present in very low amount (0.42 to 2.90 $\mu g/ml$) in three isolates (Table 1).

HPLC analysis of mycelium. Out of 20 isolates, gallic acid was detected in all the isolates except in two, oxalic in 8, vanillic in 12, ferulic in 17. o-coumeric in 8, cinnamic in 7, caffeic in 4 and tannic acid in all the isolates and chlorogenic in 14 isolates. The major component of mycelium was oxalic acid (425.51 $\mu g/ml$) in isolate of *Coriendrum sativum*. Ferulic acid was 23.48 $\mu g/ml$ in isolate of *Amaranthus tristis* and 330.19 $\mu g/ml$ in *Papaver somniferum* isolate while absent in 2 isolates isolated from *Phaseolus vulgaris*. Caffeic acid amounting to 1.92 $\mu g/ml$ was observed in isolate from *Gaillardia pulchella* and 4.61 $\mu g/ml$ in *Pisum sativum* isolate. However, caffeic acid was absent in rest of the isolates. Cinnamic acid was 0.039 $\mu g/ml$ in the soil isolate and 3.50 $\mu g/ml$ in *Gaillardia pulchella* isolate but absent in 13 isolates. Gallic acid was 1.93 $\mu g/ml$ in isolate from *Solanum melongena* and 40.06 $\mu g/ml$ in *Cicer arietinum* isolate. Vanillic acid was 0.02 $\mu g/ml$ in isolate from *Solanum melongena* and 23.21 $\mu g/ml$ in *Phaseolus vulgaris*. Tannic acid was present in all the isolates of *S. sclerotiorum*. Gentisic acid was detected in 9 isolates. It ranged from 0.12 $\mu g/ml$ to 120.83 $\mu g/ml$ whereas salicylic acid was discerned in very low amount (0.17 to 5.57 $\mu g/ml$) in 7 isolates (Table 2).

HPLC analysis of sclerotia. HPLC analysis of sclerotia of different isolates of *S. sclerotiorum* revealed 4~15 peaks consistently. Out of these, 11 were identified as mentioned above. Gallic and ferulic acids were present in all the isolates, oxalic in 7 and vanillic acid in 6 isolates, o-coumeric in 11 isolates, caffeic in 9 and chlorogenic acid in 11 isolates, tannic in all the isolates except in one isolate infecting *Pisum sativum*, salicylic and gentisic acids in 6 and 4 isolates, respectively. Major component of sclerotia was ferulic acid. Most of the phenolic compounds varied in quantity in different isolates (Table 3).

Table 3. Phenolic acid contents (μg/g) in sclerotia of 17 isolates of *Sclerotinia sclerotiorum*

		Phenolic Acid										
Isolate	Host	Tannic	Gallic	Oxalic	Caffeic	Vanillic	Ferulic	*O*-coumeric	Chlorogenic	Cinnamic	Salicylic	Gentisic
1	*Abelmoschus moschatus*	2.12	11.09	1.67	–	5.16	172.01	0.303	–	10.24	2.62	–
2	*Amaranthus tristis*	3.26	46.30	–	0.30	–	631.77	76.17	–	0.035	6.44	12.88
3	*Brassica campestris var. toria*	4.74	23.61	–	0.13	–	73.05	8.415	15.91	1.15	1.23	–
4	*Brassica oleracea var. botrytis*	0.267	18.75	14.08	0.301	–	197.92	–	0.970	0.52	–	–
6	*Cicer arietinum*	9.06	30.90	–	–	–	42.15	–	12.68	2.11	–	–
7	*Coriendrum sativum*	1.34	15.90	–	–	12.01	127.57	8.81	–	3.71	0.06	–
9	*Daucus carota*	3.24	11.21	16.78	–	3.67	60.23	17.21	5.63	–	–	–
10	*Gaillardia pulchella*	1.39	12.21	155.16	–	–	170.32	4.92	–	0.094	0.78	–
12	*Mentha arvensis*	0.64	19.76	16.43	–	1.27	31.61	1.26	2.76	–	–	–
13	*Papaver somniferum*	3.36	22.89	12.26	–	–	99.18	2.87	0.65	–	–	1.32
14	*Phaseolus vulgaris*	0.422	10.72	–	4.07	–	107.19	1.71	1.60	0.35	–	3.93
15	*Phaseolus vulgaris*	8.21	13.67	21.73	0.51	–	36.41	7.65	2.16	–	–	–
16	*Pisum sativum*	6.12	37.19	–	0.06	–	305.31	–	3.67	2.74	–	–
17	*Pisum sativum*	–	26.32	–	0.05	–	351.37	1.62	–	3.70	–	23.55
18	Soil	0.218	19.10	–	0.24	0.10	152.73	–	21.04	2.80	–	–
19	*Solanum melongena*	1.65	15.13	–	–	–	113.35	–	14.47	1.28	–	–
20	*Solanum melongena*	1.94	18.96	–	0.15	0.144	85.42	–	–	1.57	7.31	–

Table 4. Phenolic acid content (μg/ml) in sclerotial exudates of 17 isolates of *Sclerotinia sclerotiorum*

		Phenolic acid						
Isolate	Host	Gallic	Oxalic	Vanillic	Ferulic	*O*-coumeric	Chlorogenic	Cinnamic
1	*Abelmoschus moschatus*	–	40.30	2.09	24.51	0.026	–	–
2	*Amaranthus tristis*	0.45	16.81	0.513	12.52	–		–
3	*Brassica campestris var. toria*	–	21.61	1.67	7.37	–	–	–
4	*Brassica oleracea var. botrytis*	0.014	25.22	0.41	2.41	–	–	0.02
6	*Cicer arietinum*	0.018	18.69	0.25	3.84	0.09	–	–
7	*Coriendrum sativum*	0.96	9.67	1.21	9.27	0.97	–	–
9	*Daucus carota*	1.76	11.32	–	2.96	0.62	–	–
10	*Gaillardia pulchella*	–	4.69	0.69	6.23	–	–	–
12	*Mentha arvensis*	–	11.21	–	9.21	0.04	–	–
13	*Papaver somniferum*	2.76	23.91	1.89	14.59	0.91	0.57	–
14	*Phaseolus vulgaris*	0.017	–	–	21.39	–	–	–
15	*Phaseolus vulgaris*	0.27	21.61	0.76	13.23	–	–	–
16	*Pisum sativum*	0.67	6.76	2.06	4.21	0.05	–	–
17	*Pisum sativum*	0.19	18.21	–	11.41	–	–	–
18	Soil	0.39	–	0.135	6.196		1.23	0.10
19	*Solanum melongena*	–	48.92	0.645	6.25	0.84	0.41	–
20	*Solanum melongena*	–	36.16	0.76	16.34	–	0.96	–

HPLC analysis of exudates. HPLC analysis of sclerotial exudates of different isolates of *S. sclerotiorum* revealed 4~10 peaks consistently. Out of these, 7 were identified as mentioned above. Gallic acid was present in exudates of 11 isolates, oxalic acid in all except in two, vanillic in all except 4 isolates, ferulic acid in all the isolates, o-coumeric in 8, chlorogenic and cinnamic acids in 4 and 2 isolates respectively. Most of the phenolic compounds varied in quantity in different isolates (Table 4).

Discussion

Several workers (Cruikshank, 1983; Kohn, 1979; Petersen *et al.*, 1982; Russo and Van Etten, 1985; Scott, 1981; Tariq *et al.*, 1985; Willetts and Wong, 1980) have taken several characters like morphological, cytological, biochemical, cultural and epidemiological into consideration to characterize different species of *Sclerotinia*. The results on biochemical characteristics of the present experiment also revealed wide variation among Indian isolates of *S. sclerotiorum*. HPLC analysis of the exudate, mycelia, sclerotia and culture filtrate of all the individual isolate showed difference in phenolic and oxalic acid composition of exudate, mycelia, sclerotia and culture filtrate. Varied amounts of phenolic acids in different components of the *S. sclerotiorum* isolates further shows that this crite-

rion can also be taken into consideration for studying variability. It is interesting to note that salicylic acid which is also reported to induce resistance in host plants against pathogen attack (Gaffney *et al.*, 1993) was not detected in sclerotial exudates. In contrast, oxalic acid which is described to be responsible for pathogenicity of *S. sclerotiorum* (Ferror and Walker, 1993; Godoy *et al.*, 1990; Maxwell and Lumsden, 1970; Margo *et al.*, 1984; Marcino *et al.*, 1983; Noyes and Hanecock, 1981) was commonly found in the culture filtrates as well as in sclerotial exudates in most of the isolates. The presence of oxalic acid in culture filtrates and sclerotial exudates of *S. sclerotiorum* has also been reported by some other workers (Ferror and Walker, 1993; Keets *et al.*, 1998; Maxwell and Lumsden, 1970; Tahmasebi *et al.*, 1998; Zhau, 1999).

Similarly, ferulic acid which is reported to be highly antifungal against another sclerotia forming pathogen *Sclerotium rolfsii* (Sarma and Singh, 2003) were also found in higher amounts in sclerotia compared to mycelia, culture filtrates and exudates of the isolates. However, its concentration was less than the inhibitory concentration (>500 *μg/ml*) (Singh *et al.*, 2002) except in the isolate from *Amaranthus tristis*, which may be considered more resistant towards ferulic acid than the rest of the isolates. Apart from the other criteria, the variation in phenolic acid profile in different parts of the pathogen may also be taken as a criterion for assessing variability in *S. sclerotiorum* isolates. It will be interesting to see if there is any correlation between the phenolic acid content in the pathogen and its pathogenicity as well as its ability to survive in soil.

Acknowledgement

S. Ameer Basha is highly grateful to the Council of Scientific and Industrial Research, New Delhi, India for providing Senior Research Fellowship during Ph.D. research work.

References

Corsini, D. L. and Letourneau, D. 1973. Organic acid metabolism in *Sclerotinia sclerotiorum*. *Arch. Microbiol.* **90**: 59-64.

Chet, I. and Henis, Y. 1975. Sclerotial morphogenesis in fungi. *Ann. Rev. Phytopathol.* **13**: 169-192.

Cruikshank, R. H. 1983. Distinction between *Sclerotinia* species by their pectic zymograms. *Trans. Brit. Mycol. Soc.* **80**: 117-119.

Deng, F., Melzer, M. S. and Boland, G. J. 2002. Vegetative compatibility and transmission of hypovirulence - associated dsRNA in *Sclerotinia homoeocarpa*. *Can. J. Pl. Pathol.* **24**: 481-488.

Dutton, M. V. and Evans, C. S. 1996. Oxalate production by fungi: Its role in pathogencity and ecology in the soil environment. *Can. J. Microbiol.* **42**: 881-895.

Ferror, P. H. and Walker, J. R. L. 1993. O-Diphenol oxidase inhibition - an additional role of oxalic acid in the phytopathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. *Physiol. Mol. Pl. Pathol.* **43**: 415-522.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**: 754-756.

Godoy, G., Steadman, J. R., Dickman, M. B. and Dam, R. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenecity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. *Physiol. Mol. Pl. Pathol.* **37**: 179-191.

Humpherson-Jones, F. M. and Cook, R. C. 1977. Induction of sclerotium formation by acid staling compounds in *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. *Trans. Br. Mycol. Soc.* **68**: 413-420.

Keets, S. E., Loewus, F. A., Helms, G. L. and Zink, D. L. 1998. 5-0-(a-D-galactopyranosyl)-D-glycero-pent-2-enono-1,4-lactone : characterization in the oxalate-producing fungus *Sclerotinia sclrotiorum*. *Phytochemistry* **49**: 2397-2401.

Kohn, L. M. 1979. A monographic revision of the genus *Sclerotinia*. *Mycotoxon* **9**: 365-444.

Kull, L. S., Pedersen, W. L., Palmquist, D. and Hartman, G. L. 2004. Mycelial compatibility grouping and aggressiveness of *Sclerotinia sclerotiorum*. *Pl. Disease* **88**: 325-332.

Magro, P., Marciano, P. and Dilenna, P. 1984. Oxalic acid production and its role in pathogenesis of *Sclerotina sclerotiorum* (sunflower). *FEMS Microbiol. Letters* **24**: 9-12.

Marciano, P., Dilenna, P. and Magro, P. 1983. Oxalic acid, cell wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. *Physiol. Pl. Pathol.* **22**: 339-345.

Maxwell, D. P. and Lumsden, R. D. 1970. Oxalic acid production by *S. sclerotiorum* in infected bean and in culture, *Phytopathol.* **60**: 1395-1398.

Noyes, R. D. and Hanecock, J. G. 1981. Role of oxalic acid in the *Sclerotinia* wilt of Sunflower, *Physiol. Pl. Pathol.* **18**: 123-132.

Petersen, G. R., Russo, G. M. and Van Etten, J. L. 1982. Identification of major proteins in sclerotia of *Sclerotinia minor* and *Sclerotinia trifoliorum*. *Exp. Mycol.* **6**: 268-273.

Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: history, disease, symptology, host range, geographic distribution and impact. *Phytopathol.* **69**: 875-880.

Russo, G. M. and Van Etten, J. L. 1985. Synthesis and localization of development-specific protein in sclerotia in *Sclerotinia sclerotiorum*. *J. Bacteriol.* **163**: 696-703.

Sarma, B. K. and Singh, U. P. 2003. Ferulic acid may prevent infection by *Sclerotium rolfsii* in *Cicer arietinum*. *World. J. Microbiol. Biotech.* **19**: 123-127.

Scott, S. W. 1981. Serological relationships of three *Sclerotinia* species. *Trans. Brit. Mycol. Soc.* **77**: 674-676 .

Sharma, S., Harmandeep and Soni, G. 2001. Interaction of phenolic compounds with pectinases from *Sclerotinia sclerotiorum*. *Ind. Phytopathol.* **54**: 167-170.

Singh, U. P., Sarma, B. K., Singh, D. P., Maurya, S., Mishra, P. K. and Singh, H. B. 2004. Effects of exudate depletion on sclerotial development of *Sclerotinia sclerotiorum* and oxalic acid on the synthesis of phenolic acids in egg plant (*Solanum melongena*). *Z. Pflkrankh. PflSchutz* **111**: 371-379.

_____, _____, _____ and Amar Bahadur. 2002. Studies on exudate-depleted sclerotial development and effect of oxalic acid, sclerotial exudate and culture filtrate of *Sclerotium rolfsii* in induction of phenolic acids in chickpea (*Cicer arietinum*). *Can. J. Microbiol.* **48**: 443-448.

Starratt, A. N., Ross, L. M. and Lazarovits, G. 2002. 1,8-Dihydroxynaphthalene monoglucoside, a new metabolite of *Sclerotinia sclerotiorum*, and the effect of tricyclazole on its production. *Can. J. Microbiol.* **48**: 320-325.

Stephen, G. C., Valerie, E. S., Martin, B. D. and Low, P. S. 2000. Oxalic acid, a pathogencity factor for *S. sclerotiorum*, suppresses the oxidative burst of the host plant. *Pl. Cell.* **12**: 2191-2199.

Tahmasebi-Enferadi, S., Gomerancher, D., Baladini, M. and Vannozzi, G. P. 1998. Effect of *Sclerotinia sclerotiorum* (Lib.) de Bary culture filter on sunflower morphological characters, oxalic acid content and shikimate dehydrogenase activity. *Helia.* **21**: 81-95.

Tariq, V. N., Gutteridge, C. S. and Jeffries, P. 1985. Comparative studies of cultural and biochemical characteristics used for distinguishing species within *Sclerotinia*. *Trans. Brit. Mycol. Soc.* **84**: 381-397.

Vega, R. R., Corsini, D. and Le Tourneau, D. 1970. Nonvolatile organic acids produced by *Sclerotinia sclerotiorum* in synthetic liquid media. *Mycologia* **62**: 332-338.

Wang, S. Y. and Le Tourneau, D. 1972. Amino acids as nitrogen sources for growth and sclerotium formation in *Sclerotinia sclerotiorum*. *Trans. Br. Mycol. Soc.* **59**: 509-512.

Willets, H. J. and Wong J. A. L. 1980. The biology of *Sclerotinia sclerotiorum*, *S .trifoliorum* and *S. minor* with emphasis on specific nomenclature. *Bot. Rev.* **46**: 101-165.

_____ and Bullock, S. 1992. Developmental biology of sclerotia. *Mycol. Res.* **96**: 801-816.

Zhau Ting, G. L. 1999. Mycelial growth and production of oxalic acid by virulent and hypovirulent isolates of *Sclerotinia sclerotiorum*. *Can. J. Pl. Pathol.* **21**: 93-99.