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Variation in growth and oxalic acid production by different crop isolates of *Sclerotium rolfsii* Sacc.

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Six different culture media were used to study variation in growth characteristics of ten (10) different crop isolates of *S. rolfsii viz.,* chickpea, cotton, wheat, isabgol, groundnut, sugar beet, tuberose, mango, tomato and bottle gourd and the results indicated that the growth rate of all the isoaltes were different on different media. The mean growth rate of these isolates were in the range of 16.49 to 22.24 mm/day. On Browns agar medium some *S. rolfsii* isolate formed the basidial stage. The oxalic acid production by these fungus was not correlated to biomass (mycelial mat) produced by *S. rolfsii* and varied significantly among different crop isolates. Production of oxalic acid was maximum during 14-21 days of mycelial growth of *S. rolfsii.* The culture filtrate of nine different crop isolates containing oxalic acid when tested on chickpea seedings exhibited variation in the mortality percentage of chickpea seedling. The mortality percentage was higher due to culture filtrate of sugar beet isolate followed by bottle gourd isolate, which was more due to more amount iof oxalic acid persent. The oxalic acid produced by *Sclerotium rolfsii* was non host specific.

Key words: *Sclerotium rolfsii,* variability, growth character, oxalic acid

INTRODUCTION

Sclerotium rolfsii (teleomorph : *Athelia rolfsii* (Curzi) Tu & Kimbrought) is a devastating soil borne plant pathogenic fungus, which is prevalent in warm temperate and subtropical regions of the world. The pathogen has a wide host range of over 500 plant species throughout the world, mostly infecting dicotylendonous plant and few monocotyledonous species. The pathogen mostly causes collar rot and root rot diseases (Punja, 1985).

Oxalic acid, a metabolite of S. *rolfsii,* is known to play a role in pathogenesis of this fungus (Kirtzman *et al.,* 1977). The oxalic acid sequesters the calcium in the host cell wall thereby favouring the pectic enzymes secreted by the pathogen to hydrolyze the pectate in the middle lamella more rapidly to cause the collar rot/root rot symptoms. Ansari and Agnihotri (2000) have found positive correlation between oxalic acid production and virulence of the isolates of *S. rolfsii.*

The available literature revealed that less work has been done on comparative variation among *S. rolfsii* isolates infecting different host in relation to oxalic acid production and therefore, the study has been undertaken to carry out detailed investigation on the variation among *Sclerotium rolfsii* isolates with regard to growth characteristics and oxalic acid production.

MATERIALS AND METHODS

Isolation of Sclerotium from its native host and its pathogenicity

Sclerotium rolfsii inciting collar rot disease in chickpea, cotton, wheat, isabgol, groundnut, tuberose, mango, tomato and bottle gourd were iso-

lated from respective host plants/seedlings which were collected from diverse geographic locations of Western Maharashtra on potato dextrose agar medium. Thus ten isolates of *S. rolfsii* were obtained and named according to respective infected host plant and also identified and deposited at Indian Type Culture Collection Centre (ITCC), New Delhi. The source host, year of collection, geographic location and ITCC identified number is given in Table 1

Growth rate and growth characteristics of Sclerotium rolfsii isolates on different media

Ten isolates of *Sclerotium rolfsii* were grown on six different media to study growth rate and growth pattern. The media *viz.,,* corn meal agar, water agar, Brown's agar, Richard's agar, Czapex dox agar and potato dextrose agar were used. Twenty ml of sterilized medium was poured into each sterilized Petri plates. Five mm disc of the test fungus were cut with the help of sterilized cork borer from the margin of 4 to 5 days old culture grown on PDA Petri plate and placed in the centre of each Petri plates. Three replications of each isolate were on various media made and the inoculated Petriplates were incubated at 28 ± 2°C. The data on growth of each isolate was recorded daily on various media. Variation in the colony diameter, types of fungaL growth, intensity of sclerotia, days to from the sclerotia and formation of basidial stage were also recorded.

Estimation of oxalic acid content in cultural filtrate of S.rolfsii

Richard's broth (KNO_3,10 g; KH_2PO_4 ,5 g; Mg SO_4. $7H_2O$, 2.5 g; Sucrose 35 g; Distilled water 1000 ml) was used for production and quantification of oxalic acid . Fifty m1 Richard's broth was poured in 250 ml Erlenmeyer flasks and sterilized at 121°C for 20 min. Each flask was inoculated with individual isolate of *S.rolfsii* (by putting 5mm(diam) mycelial disc of 7 days old culture grown on PDA plates) and incubated at 25 ± 1°C for 7,14 and 21 days. The broth containing mycelial growth was filtered through Whatman filter paper No.1 to remove the mycelial mass. The filtrate was centrifuged at 10000 rpm for 10 min. to remove the mycelial fragment, if any. To 10 ml cell free culture filtrate , 8ml of calcium chloride-acetate buffer (pH 4.5) was added and mixed thorughly. The mixture was allowed to stand overnight at room temperature and then centrifuged at 10000 rpm for 10 min. The supernatant was discarded and residue was washed with 10 ml of 50% acetic acid (saturated with calcium oxalate) and centrifuged. The residue now obtained was dissolved in 10 ml of 4N H_2SO_4 and transferred to 100 ml flask. This was heated at 80°C on a water bath.while hot, it was titrated with 0.02 N potassium permanganate until a faint pink colour persisted. Three replications were made for each isolate and mean value was calculated. For oxalic acid quantification, the amount of oxalic acid present in the culture filtrate was calculated as 1ml of 0.02 N potassium permanganate reacted with 1.265 mg of oxalic acid (Mahadevan and Sridhar, 1986).

Effect of culture filtrates of S. rolfsii on chickpea seedlings

The effect of culture filtrates of eight different isolates of *Sclerotium rolfsii* was studied for mortality of chickpea seedlings. For this 5 mm(diam) disc from 7 days old culture of *Sclerotium rolfsii* was individually multiplied on 50 ml sterilized potato dextrose broth in 250 ml Erlenmeyer flask. After 15 days of incubation at 28 ± 2°C, the liquid of each flask was decanted and filtered through Whatman filter paper No.1 aseptically. The culture filtrate thus obtained was diluted at 50 per cent concentration. Fifty ml of the diluted cultural filtrate i.e. fungal metabolite / oxalic acid of each isolate was tested individually for their effect on chickpea seedlings. Seven days old seedlings of chickpea (previously grown on towel paper) were placed in each test tube containing diluted fungal metabolities and each treatment was replicated ten times. Control was maintained with sterilized water and potato dextrose broth instead of filtrate. All the test tubes were kept at ambient temperature. Observations were recorded on mortality of seedling and type of symptoms produced due to fungal metabolities after 7th day of the experiment.

RESULTS

Cultural variation among different isolates of Sclerotium rolfsii

The growth rate of *Sclerotium rolfsii* was found to be influenced by the medium on which it grew. It was evident (Table 2) that potato dextrose agar and corn meal agar were favourable media for the growth of the fungus as compared to other media. Minimum growth of the fungus was observed on

Richard's agar followed by water agar, Brown's agar and Czapex dox agar media. In general all the *S. rolfsii* isolates varied in their growth rate. The mean growth rate of these isolates was in the range of 16.49 to 24.24 mm/day. The mean growth rate of cotton isolate was maximum (22.24 mm/day) followed by wheat, isabgol and chickpea, whereas it was minimum for sugar beet isolate.

It was further evident that one medium favoured the growth of certain isolates of *Sclerotium rolfsii* whereas another medium favoured the growth of other isolates. For example PDA favoured the growth of cotton and wheat isolates whereas the growth of chickpea, isabgol, groundnut, sugar beet, tuberose, mango, tomato and bottle gourd isolates were favoured by corn meal agar medium. It was noteworthy to mention that the type of medium not only affected the growth rate of the *Sclerotium rolfsii* mycelium but also affected its growth pattern (Table 3). Potato dextrose agar and corn meal agar produced profuse growth whereas other media produced scarce or highly scarce growth. In general potato dextrose agar, corn meal agar and

Table 1: Source host, geographic region and ITCC number of *Sclerotium rolfsii* isolate

Source (host) of *Sclerotium rolfsii*	Year of *collection*	Location	ITCC allocated no.		Species identified
			ID No.	Reference *no.*	
Chickpea	2006	M.P.K.V., Rahuri	7157.08	6416	*Sclerotium rolfsii*
Soybean	2006	M.P.K.V., Rahuri	7156.08	6415	*Sclerotium rolfsii*
Groundnut	2005	Narayangaon	7162.08	6420	*Sclerotium rolfsii*
Sugar beet	2006	M.P.K.V., Rahuri	7163.08	6421	*Sclerotium rolfsii*
Wheat	2006	M.P.K.V., Rahuri	7158.08	6417	*Sclerotium rolfsii*
Tomato	2007	M.P.K.V., Rahuri	7168.08	6426	*Sclerotium rolfsii*
Bottle gourd	2006	Narayangaon	7165.08	6423	*Sclerotium rolfsii*
Cotton	2006	M.P.K.V,. Rahuri	7161.08	6419	*Sclerotium rolfsii*
Tuberose	2005	AC, Pune	7160.08	6418	*Sclerotium rolfsii*
Mango	2005	Ac, Pune	7164.08	6422	*Sclerotium rolfsii*

water agar produced raised mycelial growth whereas on Czapex dox agar and Brown's agar the mycelia tended to produce branched growth curved at the end. It was interesting to note that browns agar medium induced the basidial stage in the fungus (Table 4.) *Sclerotium rolfsii* isolates of

Table 2: Variation in growth rate of *S.rolfsii* on different media

Sclerotium rolfsii Growth characteristics	Growth pattern and Scierotia development on					
	Potato Dextrose Agar	Corn meal Agar	Richards Agar	Czapex Dox Agar	Brown's Agar	Water Agar
Growth	Profuse	Profuse	Highly scarce	Scarce	Scarce	Highly scarce
Mycelium	Highly raised	Highly raised	Not raised	Submerged	Highly submerged	Raised upward filamentous
Mycelial structure	Cottony or filamentous	Filamentous	Branched slightly curved at end	Filamentous, irregular	Centered bit portion raised upward	Very less and irregular
Sclerotia development	Sclerotia developed profusely	Sclerotia developed profusely	No sclerotia development	No sclerotia body development	No sclerotia development	No sclerotial body development

wheat, isabgol, groundnut, tuberose, mango, soybean, and bottlegourd, formed the basidial stage in the fungus, whereas in other isolates the basidial stage was not observed indicating the variabil-

ity in the fungus for the formation of basidial stage.

Oxalic acid content of Sclerotium rolfsii

Different crop isolates of *S.rolfsii* exhibited variation in the amount of oxalic acid production (Table 5). There was increased in the oxalic acid production in all the isolates of *Sclerotium rolfsii* up to 14 days. At 21 days the production of oxalic acid decreased in the isolates of chickpea, mango, bottle gourd and wheat while, it increased in the isolates of sugar beet, isabgol, tuberrose, cotton, groundnut and tomato. The minimum amount of oxalic acid produced at 7 days was by tomato isiolate (0.434 mg per ml) whereas the maximum amount of oxalic acid produced was by cotton isolates whereas the maximum amount of oxalic acid produced was by bottle gourd isolate (2.870 mg/ml). On 21 days the minimum amount of oxalic acid was observed in cotton isolates (1.750 mg/ml) whereas the maximum amount of oxalic acid was observed in sugar beet isolates (3.330mg /ml). The amount of oxalic acid was statistically superior in some of the isolates over the others. It was interesting to note that the biomass production constantly increased in all the Isolates of *Sclerotium rolfsii* upto 21 days, however the production of oxalic acid in scome of the isolates decreased at 21 days. This shows that the tyoung mycenlia have got fair capacity to produce oxalic acid than the older or edged mycelia.

Table 3: Effect of different media on growth characteristic and sclerotial development in *Sclerotium rolfsii*

	Mean *S.rolfsii* colony diameter(mm) per day on					
S. rolfsii Isolate of	PDA	CMA	RA	CZA	BA	WA
Chickpea	26.90	28.41	9.50	20.91	22.33	12.50
Cotton	35.58	30.00	13.00	22.33	19.83	12.75
Wheat	35.83	30.00	11.00	22.33	15.25	12.37
Isabgol	28.00	29.83	13.92	20.33	14.33	17.37
Groundnut	23.41	26.67	8.50	17.92	14.33	12.83
Sugarbeet	23.91	26.83	8.83	16.16	10.66	12.58
Tuberose	27.00	29.75	13.50	20.58	10.50	12.50
Mango	22.08	24.83	9.67	17.25	12.66	14.33
Tomato	24.50	29.00	10.08	18.08	16.83	12.16
Bottle gourd	22.50	26.33	8.75	17.25	12.33	13.50
Mean	26.97	28.16	10.67	19.31	14.90	13.29

PDA = Potato dextrose agar CMA = Corm meal agar CZA = Czapex dox agar
RA = Richards agar BA = Browns agar WA = Water agar

Table 4: Detection of basidial stage in different isolates of *Sclerotium rolfsii* on browns agar media

Sclerotium rolfsii Isolate of	Basidial stage present (+) / Absent (-)
Chickpea	–
Cotton	–
Wheat	+
Isabgol	+
Groundnut	+
Sugarbeet	–
Tuberose	+
Mango	+
Tomato	–
Bottle gourd	+

Different isolates of *Sclerotium rolfsii* produced different amount of oxalic acid and this oxalic acid was responsible for causing symptoms like drooping of the seedlings, yellowing and finally mortality of the test plants i.e. chickpea. More the amount of oxalic acid content in the culture filtrate more was the mortality percentage of the chickpea seedlings (Table 6). Sugar beet isolate producing 3.330 mg $ml^{-1}$of oxalic acid caused 100 per cent seedlings mortality where as cotton isolate producing 1.520 mg ml^{-1} of oxalic acid caused 53.83 per cent seedlings mortality. It was found that 2.40 mg ml^{-1} of oxalic acid produced by mango and chickpea isolate caused 73.00 per cent mortality; whereas 2.20 mg ml-1 of oxalic acid produced by isabgol and tomato caused 66 per cent mortality indicating the amount of oxalic acid is proportional, with mortality percentage of the seedling. Further it is observed that the oxalic acid produced by *Sclerotium rolfsii* was non host specific i.e. the oxalic acid produced by isolates of sugar beet, mango, wheat, cotton, groundnut, tomato, bottle gourd etc, could cause the wilting and mortality in chickpea plant that of like the chickpea isolates . The biochemical profile of the culture filtrate of all isolates revealed a distinct difference in their oxalic acid content.

DISCUSSION

In this study it was observed that the potato dextrose agar and corn meal agar favoured the production of sclerotial bodies whereas on Richard's agar, Czapex dox agar, Brown agar and water agar there was no development of sclerotial bodies indicating that source of nutrient was important factor for the formation of sclerotial bodies of the fungus. It was noted that the medium where carbohydrate source was available induced sclerotial production whereas the medium devoid of carbohydrate did not produced sclerotia.

Mathur and Sorbhoy (1976) recorded maximum

growth of *S. rolfsii* on Richards medium followed by Dox's, modified Dox's, Czapex's, modified Brown's and Asthana and Hawker's medium. On the basis of dry weight results they reported that and pathogenic characters. Ahmed and Kulkarni (1966) also reported basidial development of *S. rolfsii* on PDA containing charcoal. Thus the present study are in line with the above workers in

Table 5: Oxalic acid content of different crop isolates of *Sclerotium rolfsii* in cultural filtrate at 7,14 and 21 DAI

Isolate of S. rolfsii	Fungal biomass Produced (gm) in Richards broth at days			Amount of oxalic acid content in cultural filtrate (mg/ml) at days		
	7	14	21	7	14	21
Chickpea	0.192	0.508	0.740	1.508	2.800	2.480
Sugar beet	0.106	0.456	0.521	1.201	2.240	3.330
Isabgol	0.404	0.611	1.021	1.362	1.660	2.250
Mango	0.181	0.432	0.583	0.927	2.450	2.430
Tuberose	0.315	0.406	0.516	1.550	1.850	2.070
Bottle gourd	0.255	0.604	1.134	1.738	2.870	2.600
Wheat	0.435	0.524	1.068	1.455	2.450	2.180
Cotton	0.323	0.589	0.624	0.601	1.040	1.570
Groundnut	0.241	0.473	0.681	1.482	2.200	2.320
Tomato	0.305	0.617	0.800	0.434	1.299	2.062
S.E.m ±	0.026	0.068	0.072	0.069	0.090	0.125
CD at 5%	0.076	0.199	0.213	0.204	0.265	0.367
C.V. (%)	4.7	5.6	6.7	5.1	6.1	4.9

the Richards medium was the best but it had poor sclerotia formation. Prasad *et al.* (1986) reported the influence of nutritional factor on growth of *S. rolfsii. Radwan et al.* (1987) reported production of fewer sclerotia on chickpea dextrose tapioca (CDT) and potato dextrose tapioca (PDT) media as compared to PDA and maximum number of sclerotia were formed when medium was supplemented with peptone. Nene and Sheila (1995) also tested CDT and PDA for the growth of *Sclerotium rolfsii* and found that the fungus differeed in sclerotial production according to media. Fewer sclerotia were produced on CDT and PDT as compared to PDA. Harnandez and Yasla (1997) reported variability among *S. rolfsii* isolates in cultural, morphological

Table 6: Effect of oxalic acid (culture filtrates) of *Sclerotium rolfsii* isolates on chickpea seedling.

Culture filtrate of *S. rolfsii* isolate	Amount of oxalic acid content (mg/ml) in culture filtrate at 21 days	Mortality % of chickpea seedling due to oxalic acid (after 7 days)
Chickpea	2.480	73.33
Sugarbeet	3.330	100.00
Mango	2.430	73.33
Wheat	2.180	60.00
Cotton	1.520	53.83
Groundnut	2.320	73.33
Isabgol	2.250	66.66
Tomato	2.290	66.66
Bottle gourd	2.600	80.00
Control (PDA broth)	0.0	0.0

concerned with the type of medium affects the growth pattern, sclerotial formation and basidial development of *S.rolfsii.*

The results also confirmed that the fungal metabolite of *Sclerotium rolfsii*, i.e. oxalic acid, was responsible for wilting and mortality symptoms, and further the amount of oxalic acid available was proportionately responsible for mortality percentage. Bateman and Beer (1965) reported that both the production of exogenous oxalic acid and the secretion of pectic enzymes are involved in pathogenes by *Sclerotium rolfsii.* Maxwell and Bateman (1968) studied the conditions that influence oxalate accumulation and the pathway for its biosynthesis. They showed that glyoxylate was oxidized to oxalate by the action of a nicotinamide adenine dinucleotide (NAD) requiring enzyme glyoxylate dehydrogenase.

Punja and Jenkins (1984) reported that the isolates of *Sclerotium rolfsii* varies in oxalic acid production in the culture. Ferrar and Walker (1993) postulated that the action of oxalic acid enhances the success of the pathogen by suppressing the host defence mechanisms. Ansari and Agnihotri (2000) characterized 44 isolates of *S. rolfsii* from soybean and classified them into 4 groups of the basis of quantity of oxalic acid produced. Sharma *et al.,* (2002) demonstrated marked variation in HPLC profiles of the exudates and culture filtrates of all the *Sclerotium rolfsii* isolates. They observed

a distinct difference in the production of phenolic acids and oxalic acid.

Maximum production of toxin (oxalic acid) was recorded after 14 days of incubation. Shukla and Pandey (2006) observed maximum oxalic acid after incubation for 14 days and calculated correlation coeffifcient after performing correlation studies between the growth and toxin production. The biochemical profile of the culture filtrate of all isolates revealed a distinct difference in their oxalic acid content. In our study some isolates produced maximum oxalic acid up to 14 days whereas other isolates produced maximum amount up to 21 days. The amount of oxalic acid was related to the time taken for expression of wilting symptoms and mortality percentage in the seedling. Also observed positive correlation between oxalic acid production and the virulence of the isolate.

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