Eco. Env. & Cons. 28 (May Suppl. Issue) : 2022; pp. (S434-S442)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i03s.062

Cultural and morphological characterization of *Rhizoctonia solani*, responsible for Seedling blight and Dry Root rot of Lentil

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(Received 20 October, 2021; Accepted 2 December, 2021)

ABSTRACT

The experiments were aimed to know the Cultural and morphological variation of the important fungal pathogens of the lentil crop under Terai zone of West Bengal region and to find out the pathogenic variability. The isolate RHS-3 from Pundibari region show best growth on any of the media tested. Richard's medium gave the maximum number of sclerotia per Petri plate (9 cm) followed by the PDA and Cornmeal gave the minimum number of sclerotia. In case of liquid broth medium, the potato dextrose broth has produced the maximum fresh weight of the mycelium (7.453 g) followed by the Richard's broth medium (3.524 g) and the Asthana hawkers broth supports the less growth and produced the less fresh weight of mycelium (1.49 g) followed by the Czapek dox broth medium (1.590 g). Among the media, Richard's medium gave the bigger sclerotia (2.159 mm) followed by Asthana and hawkers medium (1.814 mm) and in PDA, small sized sclerotia were produced (1.195 mm) followed by Cornmeal agar medium (1.263 mm).

Key words : *Rhizoctonia solani, Lentil, Dry root rot*

Introduction

Lentil (*Lens culinaris*) occupies a unique position in the world of agriculture due to its high protein content and special capacity for fixing atmospheric nitrogen. It is the oldest pulse crop in the world as it has been grown for 8000 years ago. It derives its name 'Lens' from the lens shaped seeds. Lentil is considered to have its primary area of diversity in south-west Asia and Mediterranean region. The important lentil-growing countries of the world are India, Canada, Turkey, Bangladesh, Iran, China, Nepal, and Syria. In India, Lentil is mostly grown in northern plains, central and eastern parts of India. The major Lentil producing areas are situated in Madhya Pradesh, Uttar Pradesh, Bihar, and West Bengal. These states together account for 80-90% of total area and production of Lentil (Rao *et al.,* 2011).

Lentil plants are affected by wide range of plant pathogens. Among the different pathogens, fungal diseases being the most important. These diseases decrease the productivity of crop through infection and damage to leaves, stems, roots and pods and reduce the marketability by discoloring seed. Lentil

suffer from attack of a number seed borne diseases such as vascular wilt, collar rot, root rot and stem rot, rust, powdery mildew and downy mildew, which are caused by *Fusarium oxysporum f.sp. lentis, Sclerotium rolfsii*, *Rhizoctonia solani, Uromycis fabae*, *Erysiphe polygoni* and *Peronospora lentis*, respectively (Khare *et al*., 1979; Sugha *et al*., 1991; Singh and Tripathy, 1999).

Seed decay, Damping-off and Seedling blight caused by *Rhizoctonia solani* Kuhn can restrict the establishment and early growth of lentil crop. Infected seedlings either fail to emerge or lesions girdle the taproot near the soil surface, causing the seedlings to collapse. This reduces plant populations, creates uneven stands, and increases the potential for weed invasion. *Rhizoctonia solani* also causes root rot, which limits plant vigour and yield by reducing nutrient and water uptake and symbiotic nodulation (Hwang *et al.,* 1994).

Materials and Methods

Collection of the pathogen

The plants showing typical symptoms of Root rot of lentil were collected from five different villages (Khalishamari, Chhotosalbari, Pundibari, Latapata and Nisigani) of Terai Zone of West Bengal in paper bags to avoid saprophytic contamination and taken to the Plant Pathology laboratory of Uttar Banga Krishi Viswavidyala for further study and storage.

Isolation of the pathogens

The collected plant samples were surface sterilized with Mercuric chloride (0.1%) solution and again washed thoroughly with sterilized distilled water. The root samples, with symptoms were cut into small pieces along with some healthy tissue and put on to the sterilized blotting paper to remove excess water. The root bits were placed in 9 mm Petri plates containing Ko and Hara medium specific for *Rhizoctonia solani* under laminar air flow, and incubated at 27±1^{0}C for 7 days. After incubation, these isolates were transferred in slants for future use.

Purification of the pathogen

Hyphal tip culture technique was followed to maintain genetic purity of the test pathogen (Rangaswami and Mahadevan, 2004). The mycelial bit was transferred to PDA plates and incubated at 28±1°C for 10 days. In cultural studies, mycelial discs of 5 mm diameter from 3 days old cultures of each isolate were transferred into different sterilized culture media. While transferring the mycelial disc, it was place in the center of the plate and plates were incubated for 5 days at 28±1^{0}C. Cultural characteristics such as colony diameter, colour and growth pattern were studied. The colony colour was determined with help of Munsell's soil colour chart (Munsell, 1954). The cultural characters under the study also includes – radial growth, weight of sclerotia, size of sclerotia in solid media and fresh weight, dry weight of mycelium, number and size of sclerotia in liquid broth media were studied.

Media Used

To study the colony diameter, growth rate, five different media *i.e.,* three synthetic media (Asthana and Hawkers, Czapek's dox agar and Richard's agar), one semi synthetic medium (PDA) and one natural medium (Corn Meal Agar medium) were used and data recorded after 48 hrs. of inoculation at 28±2^{o}C. A total of five broth media *viz.* (Asthana and Hawkers, Potato Dextrose broth, Czapek's Dox broth, Corn Meal broth and Richard's broth) were tested for biomass (fresh and dry weight) production. Mycelial disc of size 5mm of each isolate were inoculated in flask containing 125 ml of broth medium. After incubation period, mycelial mat filtered through Whatman No.41 filter paper to remove the liquid broth. The weight of dried mycelium recorded by subtracting the weight of filter paper from the total weight recorded.

Results

Characterization of *Rhizoctonia solani*

Five isolates of *Rhizoctonia solani* were isolated from 5 different villages of Pundibari region and each isolate was given codenames (Table 1). The colony characters (colour and growth behaviour) of the mycelium of each isolate were clearly shown in the Plate 1. The data regarding the radial growth of the mycelium in the potato dextrose agar medium was shown in the Table 1.

Characterization on different media

The data presented in Table 2 revealed that radial growth of different isolates of *R. solani* were significantly varied when recorded at 48 hrs of the inoculation. Cornmeal agar was found best for growth

and development (Table 2). Comparatively, Cornmeal agar medium supported the best growth of *Rhizoctonia solani* followed by Asthana and Hawkers medium (Table 2). The radial growth of mycelium was more in these two media but the thickness of mat of mycelium is very less. The maximum radial growth was achieved by the Isolate RHS-3 on Cornmeal agar medium (6.00 cm) and minimum radial growth achieved by the Isolate RHS-4 in Richard's medium (2.76 cm). The Isolate RHS-3 achieved the maximum radial growth (4.45 cm) in all the media tested and Isolate RHS-4 achieved the minimum radial growth (3.52 cm).

Days to formation of sclerotia by different isolates of *R. solani*

All isolates showing a significant variation in time required (in days) to formation of sclerotia. Table 2 revealed that the Isolate RHS-3 (10.46 days) and Isolate RHS-4 (9.067 days) taken the maximum number of days for formation of sclerotia and the Isolate RHS-1 form the sclerotia very early (in 6.86 days). The Isolate RHS-3 took the maximum number of days (in 12.66 days) to form the sclerotia in Richard's media and the Isolate RHS-1 taken the minimum number of days (in 5.667 days) to form the sclerotia in Czapek dox agar medium. The for-

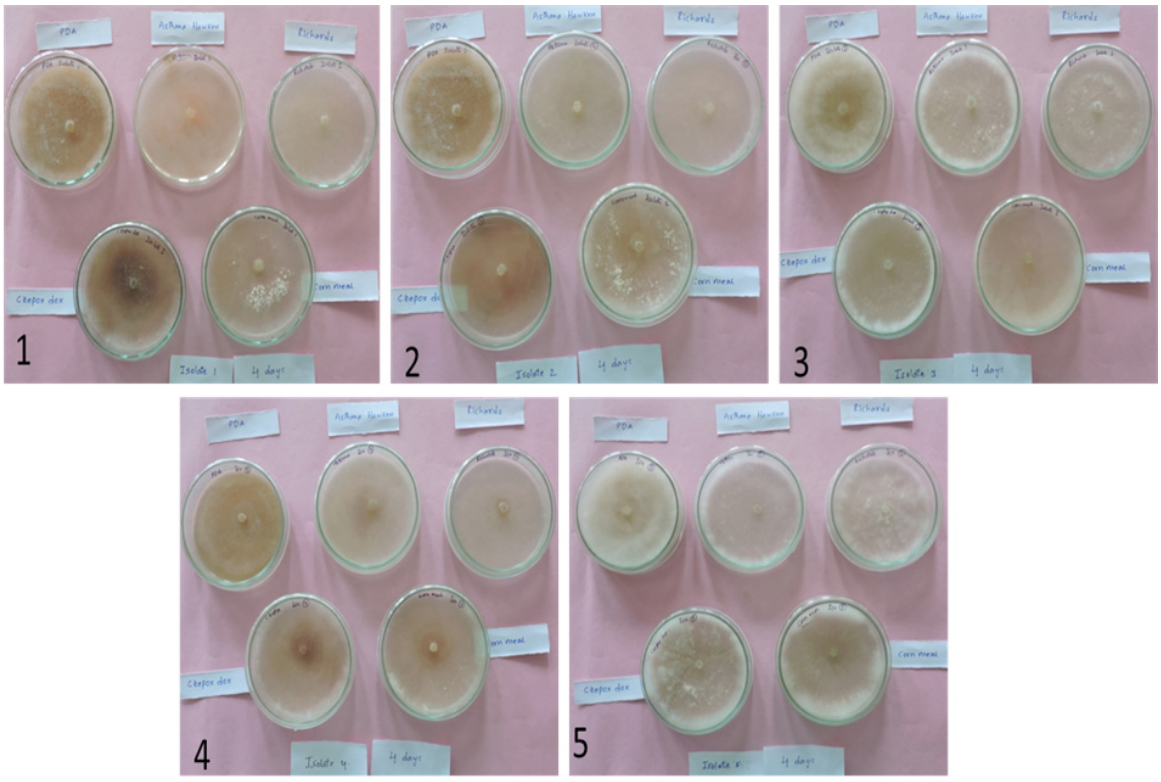

Plate 1. Growth of *Rhizoctonia solani* isolates on different media
1. Isolate RHS-1, 2. Isolate RHS-2, 3. Isolate RHS-3, 4. Isolate RHS-4 and 5. Isolate RHS-5

Table 1. Radial growth of the mycelium (cm) of different isolates of the *Rhizoctonia solani* in Potato Dextrose Agar (PDA) medium (2 days after inoculation)

Village name	Isolate	[N]Mean	Colony colour	Colony morphology
Khalishamari	RHS 1	2.933	White colony	Circular with suppressed growth
Chhotosalbari	RHS 2	3.900	White colony	Circular with profuse growth
Pundibari	RHS 3	4.167	Light brown	Circular to irregular, profuse growth
Latapata	RHS 4	2.800	Milky White	Circular suppressed growth
Nisigani	RHS 5	3.400	Milky white	Circular suppressed growth

*Mean of all three replications

Table 2. Effect of culture media on radial growth and days to formation of sclerotia of *Rhizoctonia solani*

Isolate	*Radial growth (cm) on different media						* Days to formation of sclerotia					
	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean
RHS 1	2.93	4.73	3.90	3.17	4.70	3.89	6.33	5.67	7.33	6.67	8.33	6.87
RHS 2	3.90	3.90	4.93	3.50	5.43	4.33	6.67	9.67	6.33	6.00	7.00	7.13
RHS 3	4.17	3.90	5.27	2.93	6.00	4.45	11.67	12.00	7.33	12.67	8.67	10.47
RHS 4	2.80	3.83	4.23	2.77	4.00	3.53	12.00	7.67	8.00	9.67	8.00	9.07
RHS 5	3.40	4.90	3.70	3.53	5.77	4.26	11.67	9.00	10.33	6.00	8.00	9.00
Mean	3.44	4.25	4.41	3.18	5.18		9.67	8.80	7.87	8.20	8.00	
SE(m±)	a= 0.095		b= 0.095		a×b= 0.212		a= 0.140		b= 0.140		a×b= 0.313	
CD(P=0.01)	a= 0.270		b= 0.270		a×b= 0.604		a= 0.398		b= 0.398		a×b= 0.891	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

mation sclerotia is very early in Asthana and hawker's medium (in 7.867 days) followed by the Cornmeal agar medium (in 8 days) and the formation of sclerotia is very late in PDA (in 9.667 days).

Number of sclerotia per plate

Table 3 revealed a significant variation existed for the number of sclerotia formed per plate among the isolates and also among the media. The Isolate RHS-2 produced the maximum number sclerotia per plate (57) followed by the Isolate RHS-1 (46.667) and the isolate RHS-5 formed the minimum number of sclerotia (30.993). Among the media, Richard's medium gave the maximum number of sclerotia (75.33) followed by the PDA (49.80) and Cornmeal agar medium gave the minimum number of sclerotia (20.66) followed by the Asthana and hawker's medium (25.80). Among the isolates, the Isolate RHS-3 formed the maximum number of sclerotia (141.667) in Czapek dox agar medium followed by the Isolate RHS-2 in PDA (105) and the Isolate RHS-3 produced the minimum number of sclerotia (9.667) in Cornmeal agar medium.

Fresh weight of sclerotia per plate

Table 3 revealed a significant variation for the fresh weight of sclerotia formed per plate. Among the isolates, the Isolate RHS-1 produced the maximum fresh weight of sclerotia (0.226 g) followed by the Isolate RHS-2 (0.223 g) and minimum fresh weight of sclerotia was produced by the Isolate RHS-5 (0.146 g) followed by the Isolate RHS-3 (0.162 g). Among the media, Richard's medium gave the maximum fresh weight of sclerotia (0.595 gm) followed by the Czapek dox agar medium (0.137 g) and the Asthana and hawker's medium gave the minimum fresh weight of sclerotia (0.043 g) followed by the PDA (0.118 g). Among the isolates, the Isolate RHS-1 produced the maximum fresh weight of sclerotia in Richard's medium (0.849 g) followed by the Isolate RHS-2 in the same Richard's medium (0.691 g) and the Isolate RHS-3 produced the minimum fresh weight of sclerotia in Cornmeal agar medium (0.016 g) followed by Isolate RHS-1.

Dry weight of sclerotia

Table 4 revealed that a significant variation in dry weight of sclerotia formed per plate. Among the isolates, the Isolate RHS-3 produced the maximum dry weight of sclerotia (0.165 g) followed by the Isolate RHS-2 (0.148 g) and minimum dry weight of sclerotia was produced by the Isolate RHS-5 (0.082 g) followed by the Isolate RHS-4 (0.123 g). Among the media, Richard's medium gave

Table 3. Effect of culture media on number of sclerotia and fresh weight of sclerotia of *Rhizoctonia solani*

Isolate	* Number of sclerotia per petri plate (9 mm diameter)						* Fresh weight of sclerotia (g)					
	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean
RHS 1	53.00	19.67	28.67	95.67	36.33	46.67	0.104	0.107	0.044	0.849	0.026	0.226
RHS 2	124.00	19.00	56.33	61.33	24.33	57.00	0.308	0.036	0.048	0.691	0.034	0.223
RHS 3	12.00	141.67	22.00	33.33	9.67	43.73	0.066	0.273	0.046	0.407	0.016	0.162
RHS 4	38.33	18.67	9.67	105.00	23.00	38.93	0.059	0.232	0.053	0.448	0.030	0.164
RHS 5	21.67	28.33	12.33	82.33	10.00	30.93	0.053	0.039	0.026	0.579	0.036	0.146
Mean	49.80	45.47	25.80	75.53	20.67		0.118	0.137	0.043	0.595	0.028	
SE (m±)	a= 2.538		b= 2.538		a×b= 5.674		a= 0.013		b= 0.013		a×b= 0.029	
CD (P=0.01)	a= 7.229		b= 7.229		a×b= 16.165		a= 0.037		b= 0.037		a×b= 0.083	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

Table 4. Effect of culture media on dry weight of sclerotia and size of sclerotia of *Rhizoctonia solani*

Isolate	* Dry weight of sclerotia (g)						* Size of sclerotia (mm)					
	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean	Potato dextrose agar	Czapek's Dox agar	Asthana and Hawkers	Richard's agar	Cornmeal agar	Mean
RHS 1	0.062	0.085	0.035	0.428	0.022	0.126	1.523	2.133	1.673	2.437	1.203	1.794
RHS 2	0.161	0.027	0.039	0.486	0.029	0.148	1.033	1.363	1.583	3.090	1.343	1.683
RHS 3	0.157	0.245	0.033	0.375	0.015	0.165	1.330	1.370	1.600	1.870	1.070	1.448
RHS 4	0.047	0.184	0.041	0.326	0.016	0.123	1.133	1.677	2.210	1.750	1.543	1.663
RHS 5	0.036	0.029	0.020	0.293	0.030	0.082	0.953	1.113	2.003	1.647	1.157	1.375
Mean	0.093	0.114	0.033	0.381	0.022		1.195	1.531	1.814	2.159	1.263	
SE(m±)	a= 0.016		b= 0.016		a×b= 0.036		a= 0.062		b= 0.062		a×b= 0.139	
CD(P=0.01)	a= 0.046		b= 0.046		a×b= 0.103		a= 0.177		b= 0.177		a×b= 0.397	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

the maximum dry weight of sclerotia (0.381 g) followed by the Czapek dox agar medium (0.114 g) and the Cornmeal agar medium gave the minimum dry weight of sclerotia (0.022 g). Among the isolates, the Isolate RHS-2 produced the maximum dry weight of sclerotia in Richard's medium (0.486 g) followed by the Isolate RHS-1 in the same Richard's medium (0.428 g) and the Isolate RHS-3 produced the minimum dry weight of sclerotia in Cornmeal agar medium (0.015 g).

Size of sclerotia

The data presented in the Table 4 revealed that there was significant variation for the size of sclerotia. Among the isolates, Isolate RHS-1 produced the maximum size of sclerotia (1.794 mm) followed by the Isolate RHS-2 (1.683 mm) and the Isolate RHS-5 produced the small sized sclerotia (1.375 mm) followed by the Isolate RHS-3 (1.448 mm). Among the media, Richard's medium gave the big sized sclerotia (2.159 mm) followed by Asthana and hawkers medium (1.814 mm) and in PDA, small sized sclerotia were produced (1.195 mm) followed by Cornmeal agar medium (1.263 mm). Among the isolates, the Isolate RHS-2 produced the big sized sclerotia in Richard's medium (3.090 mm) followed by the Isolate RHS-1 (2.437 mm) in the same Richard's medium and Isolate RHS-5 produced the small sized sclerotia (0.953 mm) in PDA (Table 4).

Fresh weight of mycelium

The data presented in the Table 5 revealed that significant variation for the fresh weight of mycelium was found among the all isolates. The Isolate RHS-3 produced the maximum fresh weight of mycelium (3.854 g) followed by the Isolate RHS-4 (3.819 g). Among the broth media, Potato dextrose broth produced the highest fresh weight of mycelium (7.453 g) followed by the Richard's broth medium (3.524 g). Asthana and hawker's broth medium produced the least fresh weight of mycelium (1.499 g) followed by the Czapek dox broth medium (1.590 g). Among the isolates, Isolate RHS-5 produced the maximum fresh weight of mycelium in Potato dextrose broth (9.431 g) followed by Isolate RHS-2 (8.680 g) in the same medium.

Dry weight of mycelium

Table 5 revealed a significant variation for the dry weight of mycelium among all isolates. Among the isolates, the Isolate RHS-5 produced the maximum

Table 5. Effect of liquid broth media on fresh weight and dry weight of mycelium of *Rhizoctonia solani*

Isolate	* Fresh weight of mycelium (g)					* Dry weight of mycelium (grams)				
	Potato dextrose broth	Czapek's Dox broth	Asthana and Hawkers	Richard's broth	Cornmeal broth	Mean	Potato dextrose broth	Czapek's Dox broth	Asthana and Hawkers	Richard's broth	Cornmeal broth	Mean
RHS 1	6.451	0.788	0.740	3.188	3.608	2.955	1.410	0.302	0.150	0.796	0.863	0.704
RHS 2	8.680	1.136	0.459	3.800	1.844	3.184	2.627	0.415	0.135	1.063	0.604	0.969
RHS 3	7.755	0.901	1.566	4.314	4.735	3.854	2.190	0.413	0.305	1.300	0.894	1.021
RHS 4	4.947	3.694	3.266	3.855	3.332	3.819	1.490	1.533	0.864	0.807	0.825	1.104
RHS 5	9.431	1.430	1.464	2.460	2.642	3.485	3.274	0.630	0.428	0.971	0.724	1.206
Mean	7.453	1.590	1.499	3.524	3.232		2.198	0.659	0.377	0.987	0.782	
SE(m±)	a= 0.151		b= 0.151		a×b= 0.338		a= 0.070		b= 0.070		a×b= 0.157	
CD(P=0.01)	a= 0.431		b= 0.431		a×b= 0.963		a= 0.200		b= 0.200		a×b= 0.447	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

dry weight of mycelium (1.206 g) followed by the Isolate RHS-4 (1.104 g). Among the broth media, Potato dextrose broth produced the highest dry weight of mycelium (2.198 g) followed by the Richard's broth medium (0.987 g). Asthana and hawker's broth media gave least dry weight of mycelium (0.377 g) followed by the Czapek dox broth medium (0.659 g). Among the isolates, Isolate RHS-5 produced the maximum dry weight of mycelium in Potato dextrose broth (2.198 g) followed by Isolate RHS-2 (2.627 g) in the same medium. The biomass of the mycelium of the *Rhizoctonia solani* is found to be highly influenced by nutritional status of the medium (Table 4 and Table 5).

Number of sclerotia

A significant variation existed for the number of sclerotia formed per 150 ml of broth media among the all isolates, which is presented in the Table 6. The Isolate RHS-2 produced the maximum number sclerotia per 150 ml of broth (84.60) followed by the Isolate RHS-1 (78.40) and the Isolate RHS-5 formed the minimum number of sclerotia (42.40). Among the media, Potato dextrose broth medium produced the maximum number of sclerotia (121.40) followed by the Richard's broth medium (73.40) and Asthana and hawker's broth medium produced the minimum number of sclerotia (18.46) followed by the Czapek dox broth medium (43.33). Among the isolates, the Isolate RHS-2 formed the maximum number of sclerotia (171.33) in Potato dextrose broth medium and the Isolate RHS-5 produced the minimum number of sclerotia (11.33) in Czapek dox broth medium.

Size of sclerotia

The data presented in the Table 7 revealed a significant variation for the size of sclerotia. The Isolate RHS-4 produced the maximum number sclerotia per 150 ml of broth (2.251 mm) followed by the Iso-

Table 6. Effect of liquid broth medium on number of sclerotia of different isolates of R. solani at 28±1^{0}C

	*Number of sclerotia per 150 ml of broth media					
Isolate	Potato dextrose broth	Czapek's Dox broth	Asthana and Hawkers	Richard's broth	Cornmeal broth	Mean
RHS 1	147.33	62.33	15.33	90.00	77.00	78.40
RHS 2	171.33	82.33	19.00	85.67	64.67	84.60
RHS 3	121.00	23.67	18.67	32.33	20.67	43.27
RHS 4	85.00	37.00	21.67	108.33	48.33	60.07
RHS 5	82.33	11.33	17.67	50.67	50.00	42.40
Mean	121.40	43.33	18.47	73.40	52.13	
SE(m±)	a= 2.769		b= 2.769		a×b= 6.192	
CD(P=0.01)	a= 7.890		b= 7.890		a×b= 17.642	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

Table 7. Effect of liquid broth medium on size of sclerotia of different isolates of R. solani at 28±1^{0}C

*Size of sclerotia (mm)						
Isolate	Potato dextrose broth	Czapek's Dox broth	Asthana and Hawkers	Richard's broth	Cornmeal broth	Mean
RHS 1	1.110	1.483	2.337	2.577	3.333	2.168
RHS 2	1.097	1.303	1.643	2.183	2.760	1.797
RHS 3	1.953	1.973	1.603	1.983	2.450	1.993
RHS 4	1.617	2.157	2.793	1.913	2.773	2.251
RHS 5	2.447	1.553	1.693	2.163	2.323	2.036
Mean	1.645	1.694	2.014	2.164	2.728	
SE(m±)	a= 0.115		b= 0.115		a×b= 0.257	
CD(P=0.01)	a= 0.1023		b= 0.327		a×b= 0.732	

a= Isolate, b= Media, a×b= Interaction, * Mean of three replications

late RHS-1 (2.168 mm) and the Isolate RHS-2 formed the smallest size of sclerotia (1.797 mm) followed by Isolate RHS-3 (1.993 mm). Among the media, Cornmeal broth medium gave the maximum size of sclerotia (2.728 mm) followed by the Richard's broth medium (2.164 mm) and Potato dextrose broth medium gave the smallest size of sclerotia (1.645 mm) followed by the Czapek dox broth medium (1.694 mm). Among the isolates, the Isolate RHS-1 formed the maximum size of sclerotia (3.333 mm) in Cornmeal broth medium followed by the Isolate RHS-4 in the same medium (2.773 mm) and the Isolate RHS-2 produced the smaller size of sclerotia (1.097 mm) in Potato dextrose broth medium.

Discussion

The results *i.e.,* characterization of *Rhizoctonia solani* is in accordance with the Bupree *et al.,* (1980) which he classified the isolates in to three groups based on the colony morphology. Based on the colony colour, the isolates were grouped in to three groups i.e., white colony, light brown and milky white. Two isolates (RHS 1 and RHS 2) showed white colour colony and two isolates (RHS 4 and RHS 5) showed milky white colour colony. Sunder *et al*. (2003) had reported varied brownish pigmentation of mycelial structure. Corn meal agar medium supports the best radial growth followed by the Asthana hawkers medium and Ricahrds medium supports the least radial growth. These findings are in contrast with the findings of Singh *et al.,* 1974. Sharma *et al*. (2013) reported that maximum sclerotia of *R. solani* produced by Czapek's Dox agar followed by Corn meal agar and Potato dextrose agar.

Tiwari and Khare (2002) reported that Czapek's Dox Agar was best for sclerotial production. Among the media, Richard's medium gives the maximum number of sclerotia (75.33) followed by the PDA (49.80) comparative to the all the media tested and Cornmeal gave the minimum number of sclerotia (20.66) followed by the Asthana and hawkers medium (25.80). These results were also in contrast with the results of Tiwari and Khare (2000). But one isolate RHS 3 has produced maximum number of sclerotia (141) in Czapek dox medium.

In case of fresh weight of sclerotia, Richard's medium produced the maximum number of sclerotia and maximum fresh weight of sclerotia followed by the Czapek dox medium and minimum fresh weight of sclerotia was observed in cornmeal agar medium. Richard's medium produced the maximum number of sclerotia and the weight of sclerotia also more in Richard's medium because may be the size of sclerotia and number of sclerotia are more in Richard's medium compared to sclerotia produced in other media. In case of dry weight of sclerotia, same trend is followed for the dry weight of sclerotia as like fresh weight of sclerotia.

Among the media, Richard's medium gave the bigger sclerotia (2.159 mm) followed by Asthana and hawkers medium (1.814 mm) and in PDA, small sized sclerotia were produced (1.195 mm) followed by Cornmeal agar medium (1.263 mm). These findings are highly contrasting to the findings of Singh *et al*., 2019 as he noticed that cornmeal agar medium produced the bigger size sclerotia. Our findings are highly in accordance with the Kumar *et al*. (2014), observed bigger sclerotia in Richard's Agar (4.95) followed by Asthana and Hawkers (3.95), Yeast Extract Dextrose agar (3.65), Malt extract agar (2.45) and Brown's agar (2.40). Results indicating that the size of the sclerotia may vary from 1.37mm to 1.79 mm in thickness with some exceptions. Among the isolates, the Isolate RHS-2 produced the bigger sclerotia in Richard's medium (3.090 mm) followed by the Isolate RHS-1 (2.437 mm) in the same Richard's medium and Isolate RHS-5 produced the small sized sclerotia in PDA (0.953 mm) followed by the Isolate RHS-2 in the same medium PDA (1.033 mm). In case of liquid broth media, Potato dextrose broth produced the maximum number of sclerotia and the isolate RHS-2 produced the greater number of sclerotia. The cornmeal broth medium produced the bigger sized sclerotia. The similar findings have been reported by other workers (Meena and Chattopadhyay, 2002).

In case of liquid broth medium, the potato dextrose broth has produced the maximum fresh weight of the mycelium (7.453 g) followed by the Richard's broth medium (3.524 g) and the Asthana hawkers broth supports the less growth and produced the less fresh weight of mycelium (1.49 g) followed by the Czapek dox broth medium (1.590 g). These findings are in accordance with the Lalan Sharma *et al*., (2013) as he found that Czapek dox broth produced the less fresh weight. Among the isolates, the Isolate RHS-3 produced the maximum fresh weight of mycelium (3.854 g) comparative to the all the isolates tested followed by the Isolate RHS-4 (3.819 g), the reason is due to profuse mycelium and having more water holding capacity. The

dry weight of the mycelium followed the same trend as like fresh weight of the mycelium.

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