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Morpho-cultural and pathogenic variabilities in *Sclerotium rolfsii* isolated from chickpea fields

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Abstract
Collar rot incited by *Sclerotium rolfsii* is a serious disease of chickpea in Central India. It causes plant mortality mostly in early stage of crop and significantly affect the varietal yield potential in the region. In present study, Morphological, cultural and pathogenic variabilities of fifteen isolates of isolated from chickpea fields across Madhya Pradesh were investigated. The isolates showed significant variation for cultural growth and sclerotial characteristic. Isolates i.e. SR 6, SR 8, SR 10, SR 11 and SR 12 (isolated from Damoh, Panna Dindori, Satna and Rewa, respectively) were very fast growing as recorded 90 mm radial growth within 5 days after inoculation. The shape of sclerotia showed variation from Spherical (SR 1, SR SR 2, SR 3, SR 6, SR 7, SR 8, SR 10, SR 11, SR 14 and SR 15) to Irregular (isolates SR 4, SR 5, SR 9, SR 12 and SR 13). The arrangement of sclerotia on media surface also showed great variation from peripheral (SR 1, SR 5, SR 9, SR 10, SR 11 and SR 15), scattered over the plate (SR 2, SR 7, SR 8), scattered and peripheral (SR 3, SR 4, SR 6, SR 13 and SR 14) and central and peripheral (SR 12), respectively. In case of pathogenic variability, all the isolates recorded post emergence mortality of chickpea seedling within 30 days of sowing. The maximum average post emergence mortality 68.60% was observed by SR 4 (Seoni) followed by SR 6 (Damoh, 67.72%) and SR 10 (Dindori, 50.82%).

Keywords: Collar rot, *Sclerotium rolfsii*, chickpea and variability

Introduction
Chickpea (*Cicer arietinum* L.) is third most important grain legume in the world. India is the leading chickpea-producing country with 73.3% of the world acreage and 67.4% of the production (FAOSTAT, 2014) [5]. In India, chickpea is majorly grown in Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh and together these contribute 90 and 91% of the area and production of chickpea in the country (Singh 2010). Chickpea a good source of carbohydrates, proteins, several vitamins and minerals, and also it is free of cholesterol (Wood and Grusak, 2007, Chibbar *et al*., 2010) [23, 4].

The biotic and abiotic stresses affect stable and high yields of chickpea crop worldwide. Collar rot caused by soil borne fungus *Sclerotium rolfsii* is a major chickpea disease favoured by soil moisture, high soil temperature (25-30 ^{0}C) and low organic matter (Mathur & Sinha, 1968) [11] and can cause 55-95% mortality of chickpea seedlings (Gurha & Dubey 1982 and Sharma and Ghosh, 2017) [6, 18]. Outbreak of the disease is also influenced by the presence of susceptible hosts and enough inoculum of virulent isolates. Collar rot is a fast spreading and destructive disease of chickpea with having wide host range (Aycock, 1966 and Kotasthane *et al.*, 1976) [2, 18]. It is reported that collar rot can cause 10-30% yield loss in India (Maurya *et al.* 2008) [12]. In Andhra Pradesh, the incidence of this disease is reported to be increased from 6.31 to 12.21% in one year of period (Nagamani *et al*., 2015) [13]. It is being noticed that the incidence of collar rot has been reported continuously from farmer's field of Madhya Pradesh. However, the severity of collar rot has been influenced by farmer practices, cropping pattern, varietal adoption and prevailing weather parameter (Azhar *et al*, 2006; Maurya *et al*., 2018) [3, 12]. Variability in pathogen of collar rot may also plays important role in its distribution and various level of severity among farmers field. Morphological variability of isolates based on mycelia characters and sclerotial parameters in *Sclerotium rolfsii* has been reported by different scientists on different hosts (Shukla and Pandey, 2007; Rakholiya and Jadeja, 2011; Sharma *et al*., 2013; Manjunath *et al*., 2014; Manu *et al*. 2018; Poonam *et al*., 2018; Srividya *et al*., 2018) [20, 16, 19, 9, 10, 14, 22]. The present investigation was undertaken to determine the variabilities in isolates of collar rot pathogen collected from prominent chickpea growing areas of Madhya Pradesh.

The most variable and highly pathogenic isolates could be utilized in screening of chickpea germplasm for identification of resistant gene/genotypes.

Materials and Methods

Sample collection, pathogen isolation and identification

Plants showing typical symptoms of collar rot of chickpea were collected from different fields across the Madhya Pradesh during Rabi 2018-19 and 2019-20 (Figure 1 and Table 1). In the present study 15 isolates of *Sclerotium rolfsii* were isolated from different chickpea growing district *viz*; Jabalpur, Katni, Narsingpur, Seoni, Chhindwara, Damoh, Sagar, Panna, Mandla, Dindori, Satna, Rewa, Sidhi, Harda and Hoshangabad of Madhya Pradesh under five Agro-climatic zones (Table 1). The collar rot infected chickpea plants collected during disease survey were initially washed with tap water. The infected part of collar region showing typical symptoms of the disease was cut into small pieces surface sterilized with 0.5% sodium hypochloride solution for 30 seconds and transferred to sterile discs of blotting paper. The dried bits were subsequently transferred onto sterile potato dextrose agar medium in Petri plates under aseptic conditions. They were incubated at 26±2°C for three days for the growth of the fungus. The fungus was further purified by hypal tip method under aseptic conditions (Rangaswamy, 1972). Total 15 isolates of *S. rolfsii* were purified and used for further studies. The isolated fungus was identified as *Sclerotium rolfsii* based on the cultural and morphological characters as given by Punja *et al*., 1982 [15]. Characterization was based on morphological characters such as colony morphology, mycelial growth rate, scleortial number, size and colour.

Cultural and morphological variability

Twenty ml sterile molten potato dextrose agar (PDA) was poured into sterilized 90 mm petri plates. The individual isolate growth was taken out with the help of cork borer (5 mm) discs and placed at the center of the petri plates and incubated at 27±2 °C. Observations were taken for radial growth, colony morphology along with sclerotial characters such as sclerotial initiation, size, shape, colour and density of sclerotia per plate. Morphological variation of the colonies on PDA was observed after 3 and 5 days of incubation and the sclerotia characters was taken after 25 days of incubation.

Mass Multiplication of isolates for pathogenic variability testing

Sorghum grains were soaked into 2% sucrose water solution for overnight. Thereafter it was slightly boiled, drained of excess water and air dried. One hundred gram this sorghum grains were filled in 250 ml conical flasks and sterilized in autoclave with 15 p.s.i. at 120.6 °C for 15 minutes. Sterilized sorghum gain in flask were inoculated with 4-5 mycelial discs (5mm) taken from the periphery of the 4 days old culture of *S. rolfsii* grown on PDA. The inoculated flasks incubated in BOD incubator at 25 ±2 °C for 20 days.

Proving Pathogenicity test in pots

Pot experiment was conducted to determine the pathogenic variability of fifteen isolates by using sterilized soil and pots (pots size about 45 x 30 cm). Pots were surface sterilized with 4% sodium hypochloride and soil by autoclaving at 15 psi for 20 minutes and pots. Twenty five days old mass multiplied culture (20 gm) of each isolate separately was mixed thoroughly in each pot. Then apparently healthy, surface sterilized chickpea seeds of four variety i.e. JG-16, BG-212, L550, and JG-315 separately were sown in pots inoculated with isolates. Plants in pots without inoculum served as control. Enough soil moisture was maintained by adding sterilized water as per requirement throughout the period in each pot. Plant mortality was recorded at 15 and 30 Days after sowing in all four v Arieties.

Results and Discussion

Morphological colony variations

The mycelial colony characters (growth, colony colour and appearance) of *S. rolfsii* isolates on PDA are presented in Table 2. The mycelial colour was varied from extra white (isolate SR 1, SR 6, SR 10, SR 11, SR 13 and SR 14) to dull/ cottony white (isolate SR 3, SR 4, SR 5, SR 7, SR 8, SR 9, and 15) and light (isolate SR 2 and SR 12), respectively. The mycelial growth was varied from fast (isolate SR 2, SR 3, SR 5, SR 7, SR 9, SR 13 and SR 15) to very fast (isolate SR 6, SR 8, SR 10, SR 11and SR 12) and moderately (isolate SR 1, SR 4 and SR 14), respectively. The colony pattern was varied from compact (isolate SR 1, SR 2, SR 5, SR 6, SR 11, SR 13 SR 14 and SR 15) to fluffly (isolate SR 3, SR 4, SR 7, SR 8, SR 9, SR 10 and SR 12). All the isolates were fast growing and the isolate (SR 6, SR 8, SR 10, SR 11 and SR 12) were very fast growing and covered full petriplate (90 mm) within 5 days after inoculation (Figure 2).

The shape of sclerotia showed maximum variation from Spherical (isolates; SR 1, SR 2, SR 3, SR 6, SR 7, SR 8, SR 10, SR 11, SR 14 and SR 15) to Irregular (isolates SR 4, SR 5, SR 9, SR 12 and SR 13), respectively. The arrangement of sclerotia on media surface showed great variation from peripheral (isolates SR 1, SR 5, SR 9, SR 10, SR 11 and SR 15), scattered over the plate (isolate SR 2, SR 7 and SR 8) scattered and peripheral in the isolates SR 3, SR 4, SR 6, SR 13 and SR 14) and Central and peripheral (isolates SR 12) respectively (Table 3). The number of sclerotia per plate showed great variation from isolate 1 to 15. The maximum sclerotia 456 per plate was observed at isolates SR 12 from Harda district followed by SR 14 (451), SR 3 (394), SR 4 (365), SR 5 (360.67), SR 6 (344), SR 7 (258.67), SR 8 (256), SR 9 (249), SR 10 (229), SR 11 (191), SR 12 (189), SR 13 (164), SR 14 (161) and SR 15 (157.67), respectively (Table 3, Figure 2).

The colour of sclerotia showed great variation from Blackish Dark Brown (isolates 1, 9 and 15), brown (isolate SR 5, SR 10 and SR 11), Dark Brown (isolates SR 4, SR 7, SR 12 and SR 13), light brown (isolate SR 2, SR 3, SR 6 and SR 8) and light orange (isolates SR 14) respectively (Table 3). Similar kind of variation in morphology of *Sclerotium rolfsii* were also observed by Srividya *et al*., 2018; Manu *et al* 2018; Manjunatha *et al*. 2014 [9, 10, 22]. The variation in sclerotial characters was also studied and the pattern was similar to our findings (Abida *et al.* 2008; Kumari and Ghatak, 2018; Poonam *et al*., 2018) [14, 8].

Assessment of pathogenicity test on pot culture method

The pathogenic variation for aggressiveness in causing mortality of chickpea plant was evaluated in inoculated soil on four different varieties and data on per cent plant mortality at 30 days after sowing was presented in Table 4 and Figure 3. The maximum average post emergence mortality (%) 68.60

was observed at isolate SR 4 from Seoni district followed by isolates SR 6 (67.72%), isolate SR 10 (50.82%), isolate SR 2 (45.39%), isolate SR 3 (45.38%), isolate SR 11 (45.28%), isolate SR 8 (45.22%), isolate SR 13 (44.10%), isolate SR 9 (43.16%), isolate- SR 5 (42.15%), isolate- SR 7 (40.36%), isolate SR 15 (39.94%), isolate SR 01 (39.73%), isolate SR 14 (33.76%) isolate SR 12 (30.70%) and untreated (3.80%) respectively. Pathogenic variation in isolates of *Sclerotium rolfsii* was also reported by Kumari and Ghatak, 2018 [8].

Table 1: Description of isolates collected for studies

S. No.	Districts	Blocks	Village	GPS Location		Name/ codes of Isolate	Year
				Latitude	Longitude		
1	Jabalpur	Panagar	Imaliya	23°15'01"	79°59'46"	SR-1	2018
2	Katni	Bahoriband	Khargawan	23°41'10"	80°05'41"	SR-2	2018
3	Narsingpur	Tendukheda	Jaitpur	23°09'19"	78°51'05"	SR-3	2018
4	Seoni	Chhapara	Payli Khurd	22°11'44"	79°17'03"	SR-4	2018
5	Chhindwara	Tamia	Jamunia Khurd	22°19'43"	78°38'55"	SR-5	2019
6	Damoh	Tendukheda	Pindrai	23°26'37"	79°32'12"	SR-6	2019
8	Sagar	Rehli	Samnapur Kalan	23°39'17"	78°59'44"	SR-7	2019
8	Panna	Amanganj	Amanganj	24°25'22"	80°02'28"	SR-8	2019
9	Mandla	Bichhiya	Sanjari	22°20'21"	80°48'19"	SR-9	2019
10	Dindori	Dindori	Dobhi Mal	23°06'39"	80°43'33"	SR-10	2019
11	Satna	Kotar	Goraiya	24°38'31"	81°01'28"	SR-11	2018
12	Rewa	Sirmour	Dihi	24°46'47"	81°20'04"	SR-12	2018
13	Sidhi	Rampur Naikin	Naikin	24°20'28"	81°25'35"	SR-13	2018
14	Harda	Khirkiya	Basantpura	22°07'46"	76°52'28"	SR-14	2018
15	Hoshangabad	Bankhedi	Kemdhana	22°44'35"	78°29'45"	SR-15	2018

Table 2: Cultural variability among isolates collected from different districts of M.P.

Isolates	Colony Characters			Mean Radial Growth (mm) 5DAI
	Growth	Colony Colour at 5 DAI*	Colony Pattern	
SR-1	Moderate	Extra White	Compact	81.00
SR-2	Fast	Light White	Compact	79.00
SR-3	Fast	Dull White	Fluffy	85.33
SR-4	Moderate	Dull White	Fluffy	79.00
SR-5	Fast	Cottony White	Compact	80.00
SR-6	Very Fast	Extra White	Compact	90.00
SR-7	Fast	Dull White	Fluffy	82.33
SR-8	Very Fast	Cottony White	Fluffy	90.00
SR-9	Fast	Cottony White	Fluffy	79.67
SR-10	Very Fast	Extra White	Fluffy	90.00
SR-11	Very Fast	Extra White	Compact	90.00
SR-12	Very Fast	Light White	Fluffy	90.00
SR-13	Fast	Extra White	Compact	80.33
SR-14	Moderate	Extra White	Compact	79.67
SR-15	Fast	Dull White	Compact	80.00
CD (p=0.05)				3.9684
SEM±				1.4361

*DAI- Days after Inoculation

Table 3: Variabilities among isolates for sclerotial morphology and number

Sclerotial Characters						
S. No.	Isolate	Days ot Sclerotial formation	Shape of Sclerotia	Arrangement on media surface	Number of sclerotia per plate at 25 DAI	Colour of Sclerotia
1	SR-1	10.00	Spherical	Peripheral	258.67	Blackish Dark Brown
2	SR-2	13.00	Spherical	Scattered all over plate	164.00	Light Brown
3	SR-3	11.67	Spherical	Scattered and peripheral	394.00	Light Brown
4	SR-4	10.33	Irregular	Scattered and peripheral	365.00	Dark Brown
5	SR-5	10.00	Irregular	Peripheral	161.00	Brown
6	SR-6	15.00	Spherical	Scattered and peripheral	344.00	Light Brown
7	SR-7	13.00	Spherical	Scattered all over plate	157.67	Dark Brown
8	SR-8	11.67	Spherical	Scattered all over plate	189.00	Light Brown
9	SR-9	10.67	Irregular	Peripheral	229.00	Blackish Dar Brown
10	SR-10	9.67	Spherical	Peripheral	249.00	Brown
11	SR-11	11.00	Spherical	Peripheral	191.00	Brown
12	SR-12	9.00	Irregular	Central and peripheral	456.00	Dark Brown
13	SR-13	14.67	Irregular	Scattered and peripheral	360.67	Dark Brown
14	SR-14	11.67	Spherical	Scattered and peripheral	451.00	Light Orange

15	SR-15	9.67	Spherical	Peripheral	256.00	Blackish Dark Brown
CD(p=0.05)		1.5527			48.192	

Table 4: Pathogenecity variability among isolates on different varieties.

S. No.	Isolate Number	Mortality till 30 DAS				Average post emergence mortality (%)
		L 550	BG 212	JG 16	JG 315	
1	SR-1	37.33	41.00	40.67	18.67	39.73
2	SR-2	51.67	43.67	41.00	22.33	45.39
3	SR-3	47.67	52.00	40.67	21.33	45.38
4	SR-4	71.67	68.33	56.67	26.67	68.60 (I)
5	SR-5	60.33	43.33	41.00	23.67	42.15
6	SR-6	63.67	54.33	68.67	30.33	67.72 (II)
7	SR-7	38.67	46.00	41.00	26.00	40.36
8	SR-8	45.33	39.33	43.67	33.00	45.22
9	SR-9	38.33	46.00	38.00	31.00	43.16
10	SR-10	49.67	50.00	38.00	36.00	50.82 (III)
11	SR-11	36.00	37.33	28.67	33.33	45.28
12	SR-12	31.33	30.67	32.00	25.67	30.70
13	SR-13	42.00	42.00	40.00	26.33	44.10
14	SR-14	30.67	34.00	23.67	32.67	33.76
15	SR-15	48.67	47.67	35.67	35.33	39.94
16	Untreated	5.67	2.67	1.33	2.33	3.80
	CD (p=0.05)	11.236	7.927	6.3183	13.073	

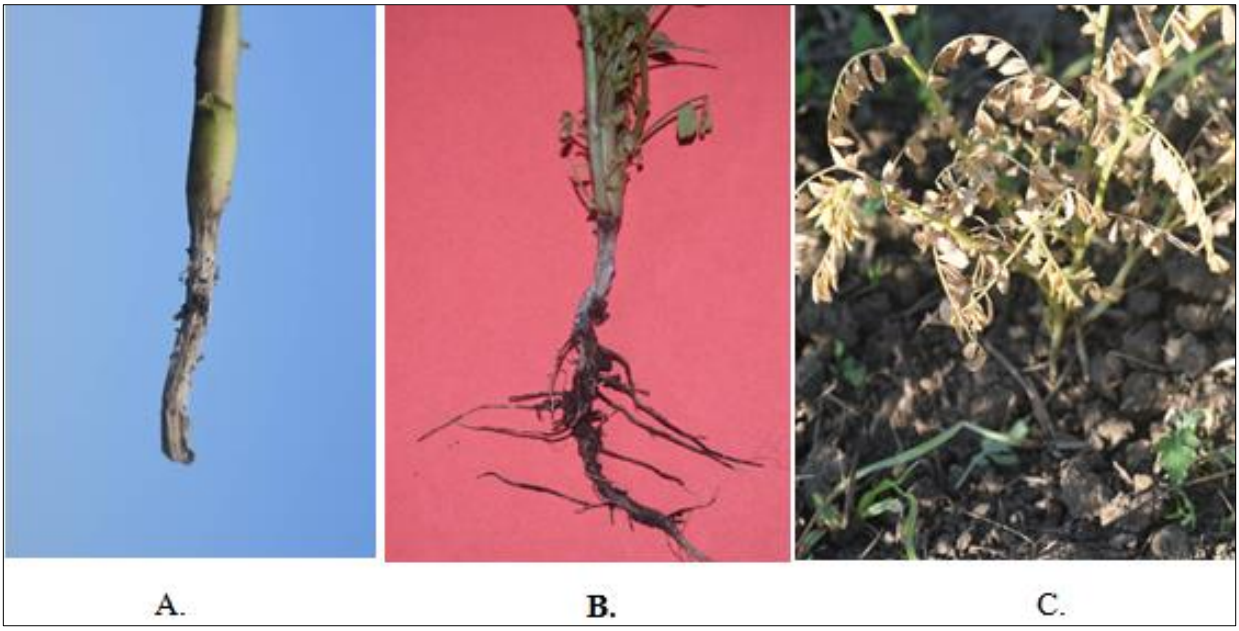

Plate 1: Collar rot affected plants **A** (Close-up of collar region showing girdling), **B (**Whole plant affected) and **C** (Field view of the plant)

Fig 2: Pathogenic variations: Showing different percentage of mortality by different isolates

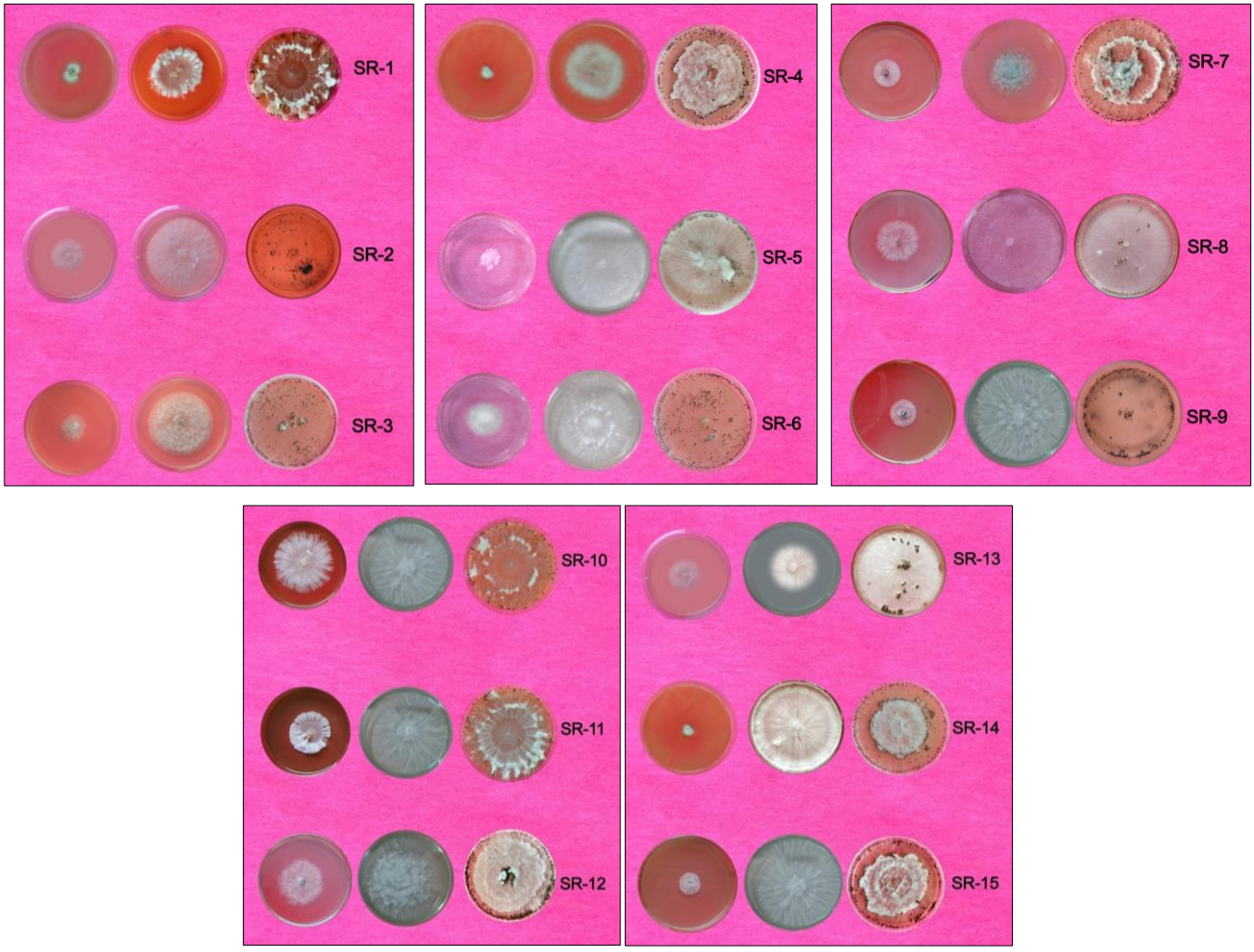

Fig 3: Cultural and morphological variation among isolates

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