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Cultural and Morphological Variabilty in *Rhizoctonia solani* Isolates of Different Rice Growing Areas of Chhattisgarh, India

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The sheath blight disease of rice caused by *Rhizoctonia solani* is an economically important disease in India. A survey was carried out to find the disease severity of sheath blight of rice and collected the disease samples from the different locations of Chhattisgarh. Fifty eight isolates of *R. solani* collected from various locations of Chhattisgarh were studied for their morphological and cultural variation. Cultural and morphological parameters of each isolate like growth pattern of mycelium, colony colour, Colony growth diameter (mm) at different intervals was recorded after inoculation of *R. solani* on to the sterilized PDA in 90 mm Petri plates incubated at 28 ± 2 0C in B.O.D incubator after 15 days [1]. The isolates were assigned code numbers such as RS1, where "RS" named *Rhizoctonia solani* and "1" denote the serial number of the isolate. The *R. solani* isolates RS1, RS2, RS3, RS4, RS5, RS12, RS19, RS34, RS50 and RS57 were recorded as fast growing isolates. The color of the fungal colony was varied from light brown to dark brown.

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Keywords: Rhizoctonia solani; 58 isolates; cultural variability; morphological variability; rice.

1. INTRODUCTION

"Rice (*Oryzae sativa* L.) is the staple food crop of over half of the world's population, and is also widely cultivated across the world, making it possibly the most valuable plant on earth" [2,3]. "It provides 20 percent of the world's supply of dietary energy followed by maize and wheat. Rice grows in at least 114 countries and more than 50 have a capacity of 100,000 tons or more per year. The production of rice to be adept by 2020 is 128 Mt. to feed the growing population in India. This crop also suffers due to number of diseases accounting for severe losses. Of the several factors known to destabilize rice yields, pests and diseases account for 30-40 percent crop losses. In Chhattisgarh, rice production is comparatively smaller than the national average production. A lot of fungal, bacterial, nematode, and viral diseases are attacked on rice. Serious incidences of diseases such as blast, sheath blight and bacterial blight have been reported from rice growing areas in Chhattisgarh regions" [2,3].

"The pathogen *Rhizoctonia solani* is most widely distributed causing diseases in many crops. *Rhizoctonia solani* causes stem rot, fruit rot, fruit and seed decay, foliar blight, damping off, crown rot and stem canker in various crops" [4,5]. Sheath blight is one of India's widespread and harmful rice diseases. Rice sheath blight disease is causing significant loss, particularly in areas where high yielding varieties are cultivated. *Rhizoctonia solani* (Perfect stage-*Thanatephorus cucumeris*) which causes rice sheath blight in both soil and water borne. Several workers reported, yield loss ranging from 20-50% in highly susceptible cultivars [6,7,8,9]. "Sheath blight disease of rice is an economically important in many rice growing regions. Its causes up to 50% yield loss under favorable condition around the world" [10,11]. "In Eastern Asia, it is reported that sheath blight disease of rice causes yearly yield loss up to 6 million tons of rice grain by affecting nearly 15-20 million ha of rice under irrigated condition" [11]. Many attempts have been made to organize *R. solani* isolates into groups on the basis of various morphological, cultural and pathological characteristics [12].

The earlier studies suggest that sheath blight pathogen *R. solani* was found homogenous in nature [13] but recent investigations revealed that the pathogen is more diverse than previously assumed [14-17] Keeping in view, the present investigation was carried out to determine the morphological and cultural variability in 58 isolates of *R. solani* from rice crop.

2. MATERIALS AND METHODS

2.1 Survey

The extensive survey was done and the diseased samples were collected from 58 farmer fields from 41 locations of (Mohad, Jungleswer, Somni, Mokhala, Dewada, Kaketara, Ratepayali, Ghumka, Odiya, Haldi, Chhichhanpahri, Tolagaon, Kanhe, Dharmapur, Surgi, Mohbhatta, Mahasamund, Saloni, Khadgaon, Sanesara, Gathala, Kumarda, Muretitola, Bhathasakri, Kutelikhurd, Jogidalli, Matri, Hirapur, Dhamtari, Surajpura, Nagdha, Singhola, Kirwai, Borsi, Pakhanjur, Narainpur, Lailunga, Jaspur, Korea, Utai and Pali) situated in fifteen districts i.e. Rajnandgaon, Bemetara, Mahasamund, Balod, Raipur, Dhamtari, Kabirdham, Gariyaband, Durg, Kanker, Narainpur, Raigarh, Jaspur, Korea, Korba of Chhattisgarh during kharif 2015-16 at maximum tillereing stage of rice crop under natural conditions.

2.2 Isolation and Purification of Pathogen

In the present study isolates were assigned code numbers such as RS1, where "RS" named *Rhizoctonia solani* and "1" denote the serial number of the isolate. "Similarly, the other fifty-eight isolates were also referred to as RS1, RS2, RS3, RS4, RS5, RS6, RS7, RS8, RS9, RS10, RS11, RS12, RS13, RS14, RS15, RS16, RS17, RS18, RS19, RS20, RS21 , RS22, RS23, RS24, RS25, RS26, RS27, RS28, RS29, RS30, RS31, RS32, RS33, RS34, RS35, RS36, RS37, RS38, RS39, RS40, RS41, RS42, RS43, RS44, RS45, RS46 , RS47, RS48, RS49, RS50, RS51, RS52, RS53, RS54, RS55, RS56, RS57, RS58 were listed. The sheath blight causing the *R. solani* pathogen was isolated and purified by a single hyphal tip / single sclerotial method. Cultures were kept in test tubes on sterile PDA slants maintained in 4°C to further investigate the variability" [18]. Similar results for isolation, purification and identification have been reported by Parmeter and Whitney [19].

2.3 Systematic Classification

"The causative agent of sheath blight, now commonly known as *R. solani* Kühn, and

Thanatephorus cucumeris (Frank) Donk, a teleomorph (perfect stage). The teleomorph of the pathogen Thanatephorus cucumeris belongs to the family of the Ceratobasidiaceae of the order Tulasnellales in the form class Hymenomycetes, subclass Holobasidiomycetidae of the class Basidiomycetes. The anamorph *R. solani* comes under the class Deuteromycotina, form class Deutromycetes and order Aganomycetales" [20].

2.4 Identification of the Test Fungus

"The isolated fungus was then identified based on the following morphological properties. *R. solani* does not form vegetative spores and is present as a mycelium and sclerotia. The isolate had typical characteristics of *R. solani*: (I) It creates a shade of brown hyphae. (II) Branches at right angles beside the distal septum of the cell in young hyphae. (III) formation of a septum in the branch beside the point of origin, (IV) narrowing at the branch point, dolipore septum, (V) moniloid cells, (VI) undifferentiated sclerotia and (VII) absence of rhizomorphs (VIII) clamp connection absent. Undifferentiated Sclerotia, aggregations of thick-walled cells, small (1-4 mm diameter) irregularly shaped brown to black structures" [21]. A similar result in identification was reported by Doman and Flentje [22], Sherwood [23].

2.5 Pathogenicity Test

After artificial inoculation with the susceptible Swarna variety, rules of Koch's postulates for fifty-eight isolates were described and the isolates were yet to confirmed. After 3 days of inoculation, all *R. solani* isolates showed their pathogenic potential and developed typical symptoms of the sheath blight in the susceptible Swarna variety as follows: Dark lesions were developed on the sheath near the water line. In the further growth phase of the infected plant, small sclerotia were developed. The sclerotia seen as round and light brown to dark brown on the affected sheath. Sclerotia were also formed in the hollow leaf internodes at maturity. When opening the infected parts, the sclerotia was clearly visible. The fungus was isolated again and compared to the original *R. solani* culture to meet Koch's postulates. The symptoms produced were consistent with the authentic reports from previous workers about rice sheath blight [24-26].

2.6 Determination of Cultural and Morphological Variability of *R. solani* Kuhn

Cultural and morphological variation was determined by analyzing colour, growth pattern, and growth rate in the colony. This experiment was conducted under *in vitro* condition in completely randomyzed design (CRD) with five replications during kharif 2016. For each replication, three plates were maintained. With the help of Munsel's Soil Color Map (Munsell Color Company, Inc. 1954), the color of the colony was calculated. Visual observation documented growth pattern based on growth of the aerial hyphae. Growing isolate's growth rate was measured, using plastic scale at 24 h, 48 h, 72 h and 96 h intervals. The systems proposed by Burpee et al*.* [27] were followed for the categorization of colony characteristics. Cultural and morphological parameters of each isolate like growth pattern, colony colour, Colony growth diameter (mm) at different intervals was recorded after inoculation of *R. solani* on to the sterilized PDA in 90 mm Petri plates incubated at 28 ± 2 0C in B.O.D incubator after 15 days [1]. Single sclerotial cultures of all the isolates were cultured separately on PDA and master cultures of these isolates were maintained on PDA slants in test tubes at 10+_20C [28]. The mycelial growth rate, however, was recorded after every 24 hours interval till the last Petri plate was completely colonized (96 hours). Five replications were maintained for each isolate.

3. RESULTS AND DISCUSSION

3.1 Cultural and Morphological Variation in *R. solani* Isolates of Different Rice Growing Areas of Chhattisgarh

The variability of *R. solani* isolates based on some cultural and morphological features was examined in the present study. Fifty-eight *R. solani* isolates collected from various locations in Chhattisgarh were taken for the study presented in the Materials and Methods section. Variability studies among these *R. solani* isolates were performed based on morphology, mycelial growth pattern and other cultural characteristics. With all isolates of *R. solani*, which were collected in different places, variations of the cultural and morphological properties were observed. Morphological properties *i.e.* growth rate, growth pattern and color of the mycelium *etc*. were observed. All of the isolates collected

were purified and confirmed according to the current species concept by *R. solani* [19]. The isolates differ in growth pattern, color of the colony and growth rate. The results is shown in Table 1 and Figs. 1, 2 and 3.

3.2 Growth Pattern

The data in Table 1 showed that significant variations in mycelial growth were observed from *R. solani* isolates collected at different locations in Chhattisgarh. Among the fifty-eight isolates, the *R. solani* fungus was divided into three classes based on the complete radial mycelial growth pattern. The isolated completion of the radial growth was recorded in the intervals of 24 hours, 48 hours, 72 hours and 96 hours and then divided into 3 groups: abundant (fast) covering petriplates (90 mm diameter) in 72 hours, moderately covering petriplates (90 mm diameter) in 96 hours, slightly covering petriplates (90 mm diameter) longer than 96 hours. Of the 58 isolates from *R. solani*, ten rapidly growing isolates RS1, RS2, RS3, RS4, RS5, RS12, RS19, RS34, RS50 and RS57 completed their radial growth within 72 hours after inoculation, and were found to be abundant grow and be categorized accordingly Group 1. Fourteen medium-sized isolates, namely RS6, RS10, RS13, RS14, RS15, RS16, RS17, RS18, RS25, RS26, RS39, RS41, RS43 and RS58, ended their radial growth within 96 hours after inoculation, with a moderate growth pattern exposed and divided into group 2, while the remaining thirty-four isolates, namely RS7, RS8, RS9, RS11, RS20, RS21, RS22, RS23, RS24, RS27, RS28, RS29, RS30, RS31, RS32, RS33, RS35, RS36, RS37, RS38, RS40, RS42, RS44, RS45, RS46, RS47, RS48, RS49, RS51, RS52, RS53, RS54, RS55 and RS56 grew very slowly and completed the complete radial growth after 96 hours of inoculation, which was considered slow growing was considered to have a slightly low growth pattern and should therefore be divided into group 3. Similarly, Burpee et al. [27] divided the growth pattern into the same three groups. The diameter growth rate was recorded after 24, 48 and 72 hours. The isolates were divided into three groups - fast, medium and slow. Ten isolates covered the entire plate (90 mm) in 48 hours. However, the RS-22 isolate did not cover the plate even after 72 hours. Fast and medium growing isolates were more pathogenic than the slow growing isolates. Cultural and morphological variations in *R. solani* have been reported by various workers, namely Singh et al. [29], Panja et al. [1] etc. Singh et al. [30] reported the AG-1 group from *R. solani*'s isolates, which showed different morphological properties in culture, were collected at five different endemic locations of sheath blight, namely Assam, Andhra Pradesh, Tamil Nadu and Andaman, India. Sclerotia was formed in 9 isolates in the aerial mycelium and in all isolates with the exception of RS-22 on the surface of the mycelium. The location of the sclerotia as aerial, surface and embedded was also described by Singh et al. [15] reports. Growth of this pathogen was good and very fast as in some isolates. Rajat et al, [31] found that all thirteen isolates showed wide morphological and cultural variations in terms of colours (white, whitish yellow, light cream and pale-yellow) colony behavior (upper touch lid and no-upper touch lid), radial growth (slow, moderate and fast growing), sclerotia colour (brown, light brown, dark brown, and deep dark brown).

3.3 Colony Colour

Of fifty-eight isolates, thirteen are isolates of (RS1, RS-6, RS-15, RS-16, RS21, RS-22, RS-27, RS-29, RS-33, RS-39, RS- 43, RS-51 and RS-57) *R. solani* consisted of brown colored thirty-eight isolates (RS-3, RS-5, RS-7, RS-8, RS-9, RS10, RS-11, RS -12, RS -13, RS-14, RS-17, RS-18, RS-19, RS-20, RS-23, RS-24, RS25, RS-26, RS-28, RS-30, RS-31 , RS-32, RS-35, RS-37, RS-38, RS-40, RS-42, RS44, RS45, RS-47, RS-48, RS-49, RS-50, RS-52 , RS-54, RS-55, RS-56 and RS-58) were colored light brown, six isolates (RS-2, RS-4, RS-34, RS-36, RS-41 and RS-46) were dark brown colored (Table 1). The discoloration of the growth media is mainly attributed to the production of pigments by the pathogen. The difference in the intensity of the color can also correspond to the amount of pigments released by the respective isolate in the media. Similarly, morphological characterizations of *R. solani* isolates based on the mycelium color in the Petri dish were carried out on the PDA medium [32,12,33,34]. Sunder et al. [35] also reported that the colony color was between brown, light brown, dark brown, and yellowish brown. The discoloration of the growth media is mainly attributed to the production of pigments by the pathogen. Other variations in the type and color of the mycelium as well as the size, color, number and type of sclerotia among the isolates from *R. solani* [36]. Mishra et al. [37] found that 22 *R. solani* isolates derived from rice were analyzed for their cultural, morphological and pathogenicity variability. The colonial appearance of the isolates was light brown and

of sparse color. Desvani et al. [38] found that colony colors were white and cream color. The mostly color colonies were white, only 24 isolates showed cream color colonies. Yaduman et al. [39] studies variability of 24 isolates of *R. solani*, and observed in different colours like, light yellow, whitish yellow, pale yellow, light cream yellow, yellow and cream yellow.

Table 1. Cultural and morphological variation in *R. solani* isolates of different rice growing areas of Chhattisgarh

Isolates	Growth Pattern			Colony colour	Colony growth diameter (mm) at different intervals				Mean
	Abundant*	Moderate	Slight		24h	48h	72h	96h	
RS1	Abundant	-	-	Brown	18.19	47.26	90.0	90.0	61.36
RS2	Abundant	-	-	Dark brown	23.19	50.06	90.0	90.0	63.31
RS 3	Abundant	-	-	Light brown	23.93	55.73	90.0	90.0	64.92
RS4	Abundant	-	-	Dark brown	20.39	53.33	90.0	90.0	63.43
RS5	Abundant	-	-	Light brown	24.19	66.33	90.0	90.0	67.63
RS 6	-	Moderate	-	brown	29.4	50.19	71.48	90.0	60.27
RS7	-	-	Slight	Light brown	15.26	36.26	54.46	74.32	45.08
RS 8	-	-	Slight	Light brown	6.33	15.19	26.53	39.79	21.96
RS 9	-	-	Slight	Light brown	8.35	16.0	22.33	30.73	19.35
RS10	-	Moderate	-	Light brown	23.67	49.23	69.79	90.0	58.17
RS11	-	-	Slight	Light brown	12.39	32.33	52.13	78.13	43.75
RS 12	Abundant	-	-	Light brown	22.59	49.46	90.0	90.0	63.01
RS 13	-	Moderate	-	Light brown	16.13	30.66	62.90	90.0	49.92
RS14	-	Moderate	-	Light brown	18.19	45.26	60.26	90.0	53.43
RS15	-	Moderate	-	Brown	18.99	47.46	72.06	90.0	57.13
RS16	-	Moderate	-	Brown	10.16	36.19	57.33	90.0	48.42
RS17	-	Moderate	-	Light brown	11.0	45.39	75.33	90.0	55.43
RS18	-	Moderate	-	Light brown	12.06	39.76	62.43	90.0	51.06
RS19	Abundant	-	-	Light brown	19.13	50.99	90.0	90.0	62.53
RS20	-	-	Slight	Light brown	12.20	24.07	40.13	54.20	32.65
RS21	-	-	Slight	brown	11.13	25.20	40.13	54.13	32.65
RS22	-	-	Slight	Brown	6.19	13.73	19.39	33.73	18.26
RS 23	-	-	Slight	Light brown	6.33	9.13	16.86	25.33	14.41
RS24	-	-	Slight	Light brown	5.19	15.26	26.19	41.26	21.98
RS25	-	Moderate	-	Light brown	23.19	46.19	65.26	90.0	56.16
RS26	-	Moderate	-	Light brown	22.33	41.25	62.33	90.0	53.98
RS27	-	-	Slight	Brown	16.19	41.86	60.19	75.60	48.46
RS28	-	-	Slight	Light brown	22.79	49.26	71.47	85.33	57.21
RS 29	-	-	Slight	Brown	20.33	40.33	61.26	80.13	50.51
RS30	-	-	Slight	Light brown	19.30	47.13	66.86	87.07	55.09
RS 31	-	-	Slight	Light brown	9.13	24.53	43.76	66.60	36.01
RS32	-	-	Slight	Light brown	6.80	10.47	18.87	26.46	15.65
RS33	-	-	Slight	Brown	2.13	17.07	23.73	32.26	18.80
RS 34	Abundant	-	-	Dark brown	20.50	50.26	90.00	90.00	62.69
RS 35	-	-	Slight	Light brown	10.23	17.19	28.39	39.26	23.77
RS 36	-	-	Slight	Dark brown	5.99	12.73	18.73	24.99	15.61
RS37	-	-	Slight	Light brown	0.0	9.26	17.79	24.06	12.78
RS38	-	-	Slight	Light brown	10.26	20.40	30.73	40.20	25.40
RS 39	-	Moderate	-	Brown	22.69	45.99	68.53	90.0	56.80
RS 40	-	-	Slight	Light brown	15.26	41.33	59.59	76.19	48.09
RS41	-	Moderate	-	Dark brown	16.73	44.06	60.19	90.0	52.75
RS42	-	-	Slight	Light brown	20.33	47.06	59.29	81.73	52.10
RS 43	-	Moderate	-	Brown	22.16	43.23	69.53	90.0	56.23
RS 44	-	-	Slight	Light brown	8.13	12.93	17.33	21.13	14.88
RS 45	-	-	Slight	Light brown	16.76	35.86	50.59	70.14	43.34

Isolates	Growth Pattern			Colony colour	Colony growth diameter (mm) at different intervals				Mean
	Abundant*	Moderate	Slight		24h	48h	72h	96h	
RS 46	-	-	Slight	Dark brown	9.80	21.20	32.93	45.00	27.23
RS 47	-	-	Slight	Light brown	16.93	33.6	54.53	73.33	44.60
RS 48	-	-	Slight	Light brown	9.32	20.13	32.33	43.60	26.35
RS 49	-	-	Slight	Light brown	10.19	25.26	35.33	44.36	28.79
RS 50	Abundant	-	-	Light brown	18.16	49.59	90.0	90.0	61.94
RS 51	-	-	Slight	Brown	10.66	23.99	36.13	49.39	30.04
RS 52	-	-	Slight	Light brown	16.53	30.67	51.13	77.73	44.02
RS53	-	-	Slight	Dark brown	16.33	45.19	64.26	84.33	52.53
RS 54	-	-	Slight	Light brown	15.20	36.00	53.26	74.13	44.65
RS55	-	-	Slight	Light brown	8.07	14.13	26.47	49.80	24.62
RS 56	-	-	Slight	Light brown	7.26	15.26	31.73	73.20	31.86
RS57	Abundant	-	-	brown	25.66	53.13	90.0	90.0	64.70
RS58	-	Moderate	-	Light brown	19.33	39.33	70.19	90.0	54.71

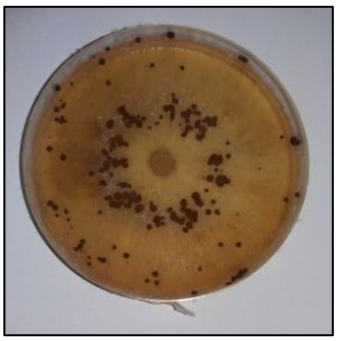
Abundant(Rapid)- Covering petriplate (90 mm dia.) in 72 hrs., Moderate-Covering petriplate (90 mm dia.) in 96 hrs., Slight- Covering petriplate (90 mm dia.) more than 96 hrs*

Morphological variability was studied in 25 isolates from different rice growing areas. Colony size, colony growth, colour and sclerotia formation (central, peripheral or scattered), location (aerial or surface) and texture (smooth or rough) varied in these isolates.

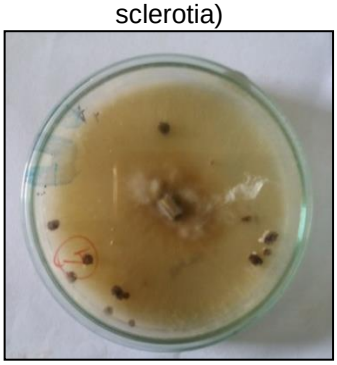
Morphological variability was studied by M. Lal and Janki Kandhari [40] in 25 isolates from different rice growing areas and found that Colony size, colony growth, colour and sclerotia formation (central, peripheral or scattered), location (aerial or surface) and texture (smooth or rough) varied in these isolates. Mohammad Najeeb Mughal et al. [41] isolated sheath blight pathogen from all 27 isolates and examined for their cultural and morphological characteristics, they found that the colour varied from light brown, greyish brown to dark brown and the isolates differed in their growth rate.

Isolate RS-1 (Scattered sclerotia)

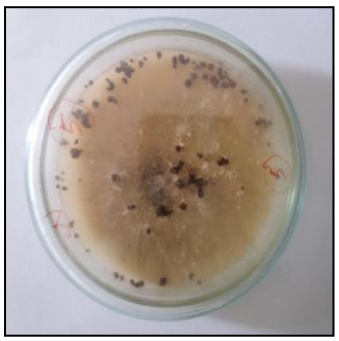
Isolate RS-2



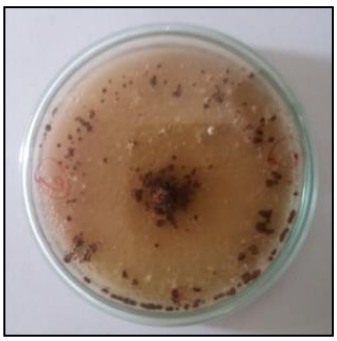
Isolate RS-3 (Central sclerotia)



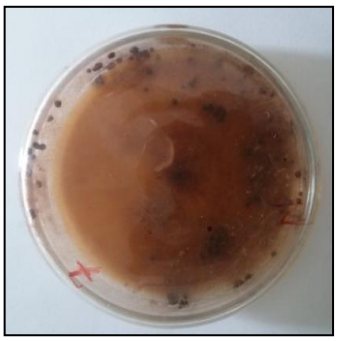
Isolate RS-4

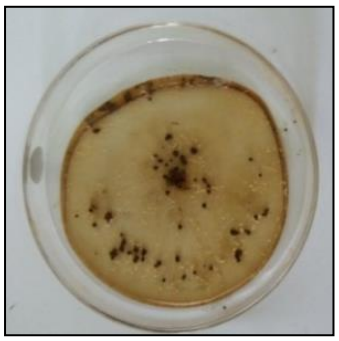


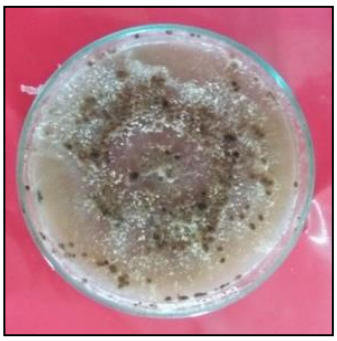
Isolate RS-5



Isolate RS-6


Isolate RS-7


Isolate RS-8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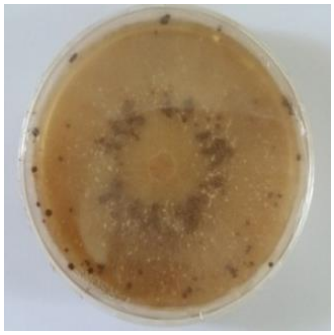
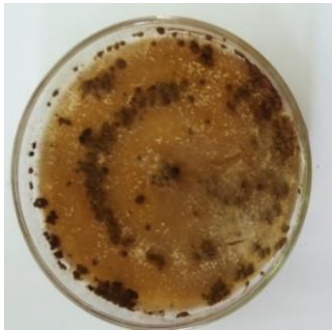
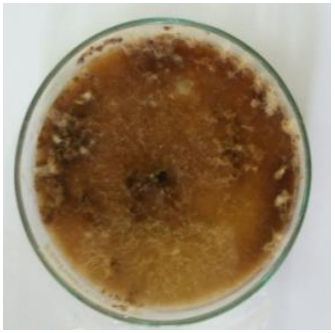
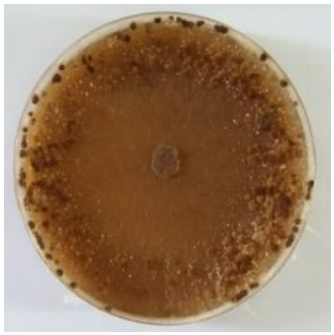
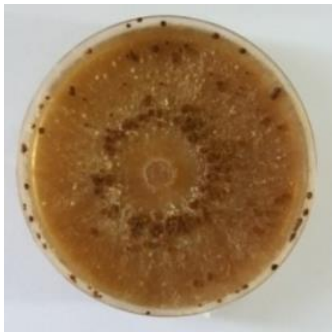
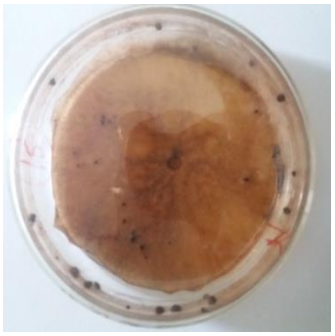

Isolate RS-10


Isolate RS-11

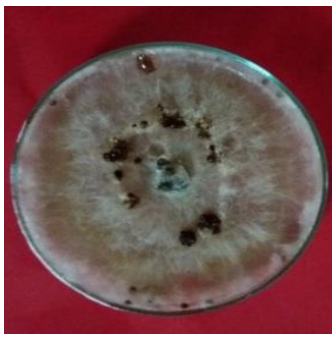

Isolate RS-12


Isolate RS-13 (Periferal, Abundant, sclerotia)


Isolate RS-14


Isolate RS-15


Isolate RS-16


Isolate RS-17

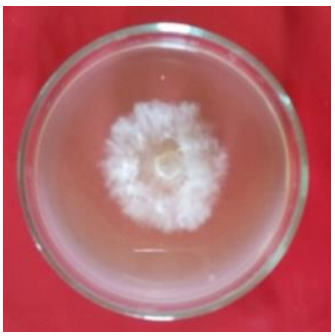
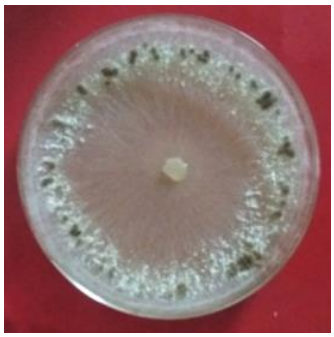
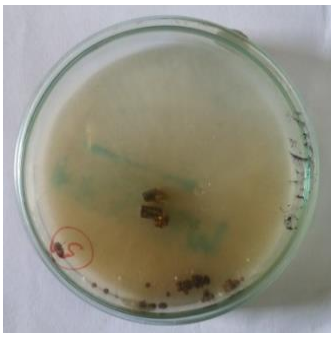
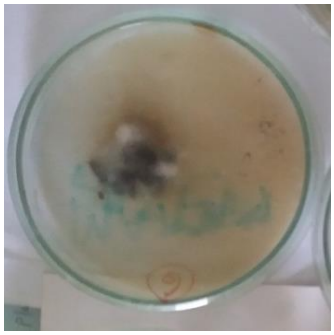

Isolate RS-18 (Slight growth)

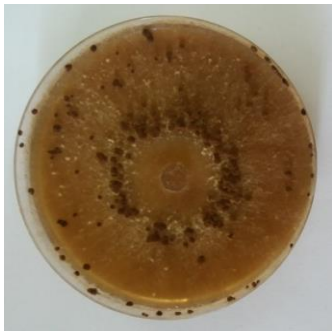
Fig. 1. Cultural and morphological characters of *R. solani*

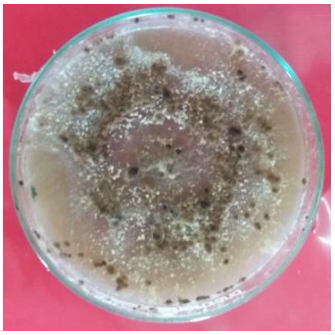

Isolate RS-19


Isolate RS-20

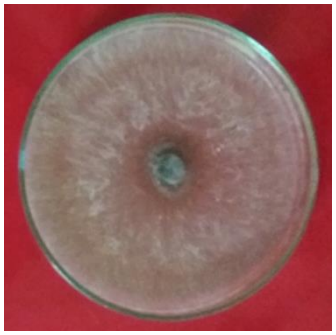

Isolate RS-22

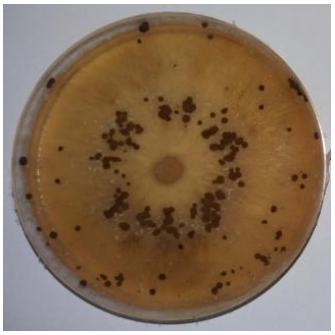

Isolate RS-23

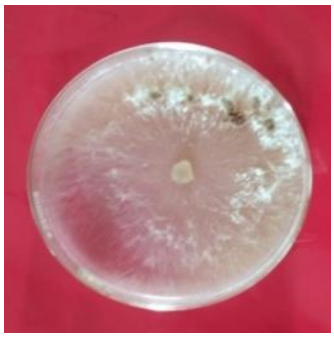

Isolate RS-24


Isolate RS-25 (Scattered Embedded, Rough)


Isolate RS- 29(Slight growth)


Isolate RS-30


Isolate RS-31


Isolate RS-32


Isolate RS-33

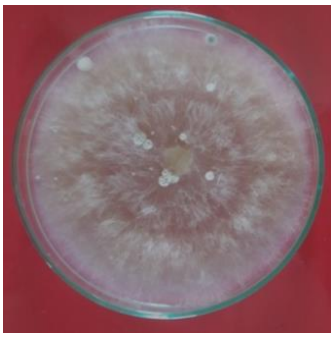
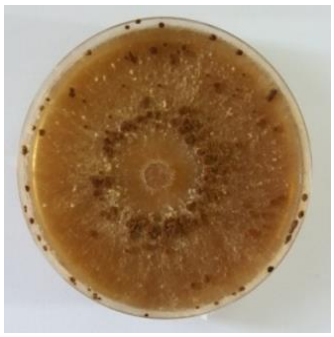
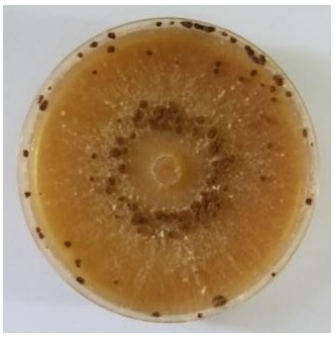
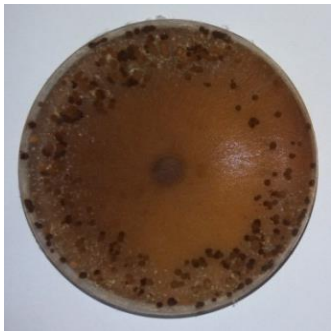

Isolate RS-35

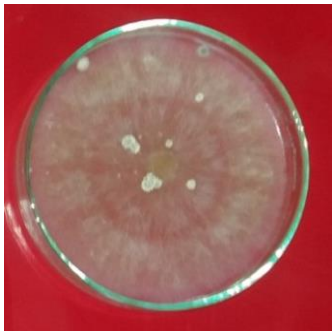
Fig. 2. Cultural and morphological characters of *R. solani*

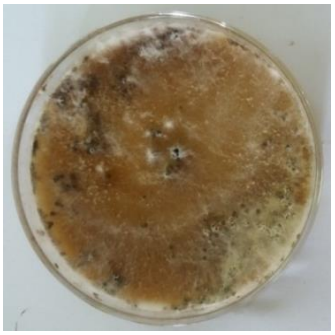

Isolate RS-36

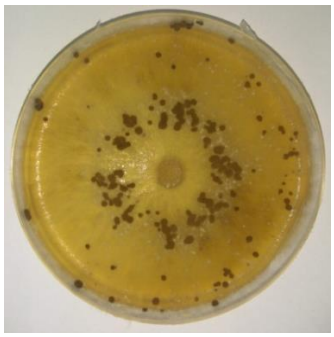

Isolate RS-37

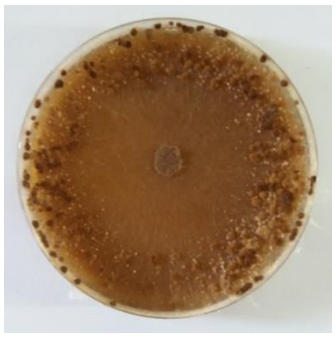

Isolate RS-39

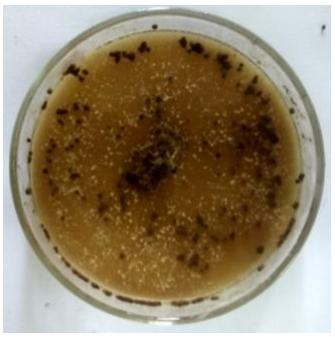

Isolate RS-40


Isolate RS-43

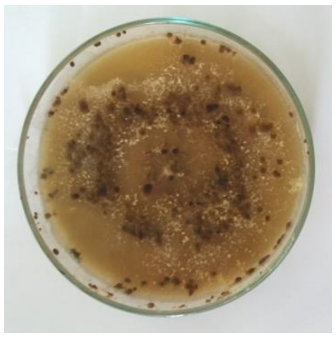

Isolate RS-44

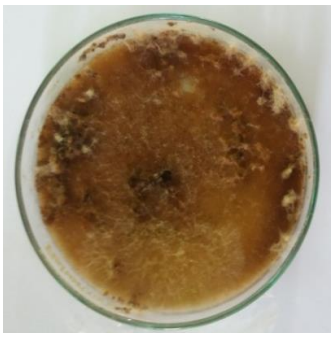

Isolate RS-45(Central Embedded)

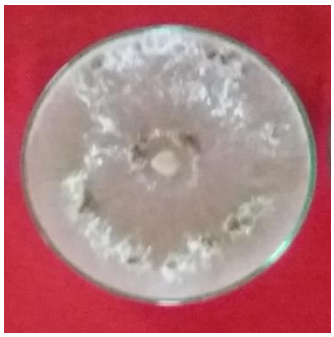

Isolate RS-46

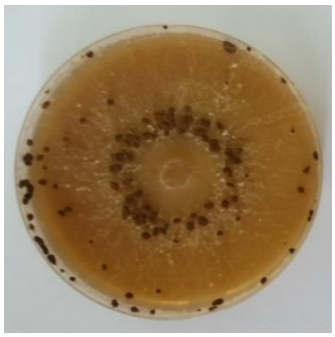

Isolate RS-47

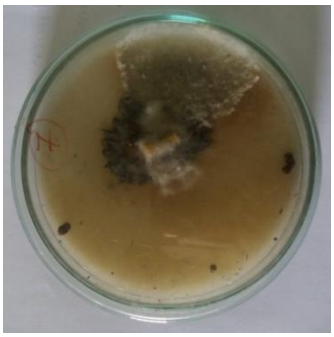

Isolate RS-48

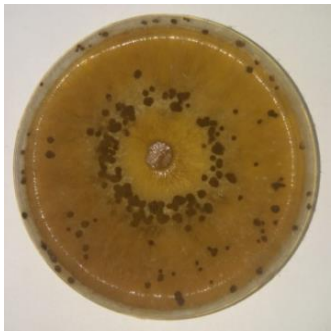

Isolate RS-49


Isolate RS-50


Isolate RS-51


Isolate RS-52


Isolate RS-54


Isolate RS-55


Isolate RS-57(Abundant growth)


Isolate RS-58 (Moderate growth)

Fig. 3. Cultural and morphological characters of *R. solani*

4. CONCLUSSION

R. solani isolates showed significant variability in their cultural and morphological properties. The isolates differ in colony color and growth pattern. The color varied from light brown, brown to dark brown. The variability helps in determining the level of resistance in the germplasm and in the selection of the parents in the crossing program.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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