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Cultural and Morphological Variability in *Rhizoctonia solani* Isolates of different hosts of Assam

KEYWORDS: Cultural variability, Morphological variability, Rhizoctonia solani

Manashi Debbarma
Department of Plant Pathology, Assam Agricultural University, Jorhat- 785013, Assam

Pranab Dutta
Department of Plant Pathology, Assam Agricultural University, Jorhat- 785013, Assam

ABSTRACT *Rhizoctonia solani (teleomorph: Thanatephorus spp.) is a plant pathogenic fungus with a wide host range. It is best known to cause various plant diseases such as collar rot, root rot, damping- off, sheath blight, stem canker, web blight, and wire stem throughout the world. Morphological variability was studied in 6 isolates of R. solani having different hosts from Assam. Colony size, colony growth, colour and sclerotia formation (ring at periphery, peripheral or scattered), location (surface) and texture (smooth or rough) varied in these isolates.*

Rhizoctonia solani (teleomorph: *Thanatephorus* spp.) is a plant pathogenic fungus with a wide host range and worldwide distribution causes severe damages in crops all around the world (Sneh *et al*., 1996) causing diseases on field and horticultural crops. *R. solani* is best known to cause various plant diseases such as collar rot, root rot, damping off, sheath blight, banded leaf and sheath blight, hypocotyl, stem canker, web blight, and wire stem throughout the world.

Isolates of *R. solani* Kühn are genetically diverse in their cultural, morphological and physiological characteristics as well as in their pathogenic range of host plants (Gonzalez *et al*., 2006; Kuninga *et al*., 1997). Because of a high degree of diversity in pathogenicity and morphology as well as in cultural, physiological characteristics the species complex of *R. solani* has been classified into different anastomosis groups (AGs) of which 13 have been described to date.

Materials and Methods

Diseased samples of different crops infected by *R. solani* showing typical symptom were collected from Assam (Instructional Cum Research farm and Experimental farm, Department of Horticulture, A.A.U, Jorhat) in air tight zip bag separately during survey. Collected sample were brought to the laboratory of Mycology Research Section, AAU, Jorhat and pathogens were isolated on PDA media. The details of sampling sites and crops are mentioned in Table 1.

To isolate the fungi, diseased sample showing typical symptom of *R. solani* infection were collected from the fields of Assam and Tripura were surface sterilized with 1 per cent sodium hypochlorite (NaOCl) solution and then rinsed with double distilled sterile water for twice. In a sterile petriplates, the diseased specimens were crushed and a small portion (size: 1-2 mm) of infected part with small healthy parts was transferred to culture plate containing PDA under aseptic condition. Inoculated plates were incubated at 25±1°C for 5-7 days. Plates were observed constantly for growth and development of associated micro-organisms. After five (5) days of incubation, the organisms were sub-cultured for purification by selecting the desired colonies. Pure culture of each isolate was made by transferring them to fresh PDA plates following the technique of hyphal tip culture. Isolated and purified cultures were maintained by periodical transferring in fresh PDA medium and storing in refrigerator at 4°C for further studies. A separate set of cultures were made on wax sealed slant and kept in freeze (-4°C). The basic cultural characteristics such as colony diam, colour and growth pattern were studied. The colour of colony was determined with the help of Royal Horticultural Society Colour Chart, London. The culture and key colour card was placed side by side and colour of the colony was observed from bottom side of the culture plate. Based on the mycelial pigmentation the cultures were assigned to different groups. Growth pattern was recorded by visual observation according to growth of hyphae-as abundant, aerial mycelium obscured surface mycelium and touched the cover of the petridish; moderate, aerial mycelium obscured surface mycelium without touching the cover, and slight-aerial mycelium did not obscure surface mycelium. Diam. growth rate was recorded after 24, 48 and 72h isolates were classified into three groups-fast, medium and slow. Growth was measured of the each isolate with three replications. Mycelium of 48 h old cultures was stained with aniline blue (0.5%) in lacto-phenol or only in water without any dyes and hyphal width was measured with five replications. Four days old fungal hyphae were mounted in water and observations were taken for type of septa, constriction and angle of branching. The number, size, colour, texture (smooth or rough), time taken for initiation of sclerotial formation, pattern of production (central, peripheral and scattered) and location of sclerotia formed were recorded. Diam of the sclerotia was measured in respect of randomly 10 sclerotia with the help of digital vernier Calipers. The colour of sclerotia was also determined.

Table 1: Details of samples collected for isolation of Rhizoctonia solani

Sl. No.	Source	Isolates	Location	GPS data
1	Rice (Oryza sativa)	RS-1	ICR Farm, Jorhat, Assam	N 26°43.005', E094°11.551'
2	Cowpea (Collar) (Vigna unguiculata)	RS-2	ICR Farm, Jorhat, Assam	N 26°42.981', E094°11.530'
3	Setaria (Setaria parviflora)	RS-3	ICR Farm, Jorhat, Assam	N 26°42.980', E094°11.545'
4	Greengram (Vigna radiata)	RS-4	ICR Farm, Jorhat, Assam	N 26°42.985', E094°11.235'

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5	Long pepper (Piper longum)	RS-5	Experimental Farm, Dept. of Horticulture, Assam	N 26°43.224', E094°11.445'
6	Maize (Zea mays)	RS-6	ICR Farm, Jorhat, Assam	N 26°43.161', E094°11.540'

Table 2: Cultural characteristics of different isolates of Rhizoctonia solani on PDA medium

Sources	Isolates	Colony character	Growth pattern	Radial growth (mm in 72h)
Rice (*Oryza sativa*)	RS-1	Greyed yellow-161 group(D)	Scarce	45.5*
Cowpea (Collar) (*Vigna unguiculata*)	RS-2	Greyed white group (A)	Scarce	42.0
Setaria (*Setaria parviflora*)	RS-3	Orange white -159 group (D)	Moderate	71.6
Greengram (*Vigna radiata*)	RS-4	White group 155 (B)	Scarce	47.6
Long pepper (*Piper longum*)	RS-5	Greyed yellow 161 group (C)	Abundant	90.0
Maize (*Zea mays*)	RS-6	Yellow group 9D (Canary yellow)	Abundant	90.0
CD(P=0.05)				0.56

A=Abundant; M=Moderate; S=Scarce

*Data are mean of three replications

Results and Discussion

Cultural variability:

Colony colour, growth pattern, and diam growth showed great diversity in all the isolates. Based on the colony pigmentation, all the isolates were assigned into 4 groups: white, yellow, grey, orange. One isolate was found white (RS-4) (Plate 4b), one isolate was found yellow (RS-6) (Plate 6b), three isolates were found grey (RS-1, RS-2 and RS-5) (Plate 1b, 2b and 5b) and one isolate was found orange (RS-3) (Plate 3b) (Table 2). Lal and Kandhari (2009) while studying variability of *R. solani* isolate found six isolates as light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown. Sunder *et al*. (2003) had also reported that colony colour ranged from brown, light brown, dark brown and yellowish brown. In the present study sometimes discolouration of growth media was also recorded. The discolorations of the growth media may be due to the production of pigments by the pathogen (Sunder *et al*., 2003). The difference in the intensity of the colour may also correspond to the amount of pigments released by respective isolate in the media.

On the basis of growth pattern, the isolates were categorized into three groups-abundant, moderate and scarce. Two isolates showed abundant growth (RS-5 and RS-6), one isolate was moderate (RS-3) and three isolates showed scarce growth (RS-1, RS-2 and RS-4). Similarly, Burpee *et al*. (1980) had also grouped the growth pattern of *R. solani* in to same three groups, *viz*., abundant, moderate and scarce.

Diam growth rate was recorded after 24,48 and 72 h. Isolates were classified into three groups-fast, medium and slow. Data presented in Table 2 showed highest radial growth (90 mm) by the isolates RS-5 and RS-6 (Plate 5b and 6b). This was followed by isolate RS-3 (71.6 mm). Isolate RS-1,RS-2 and RS-4 showed lowest radial growth (45.5 mm,42.0 mm and 47.6 mm) (Plate 1b,2b and 4b).

Morphological variability:

Compound microscope studies revealed that all the isolates of *R. solani* in present study characteristically having hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical importance. It was an obvious observation for the mycelial branching at right angles as a known feature of *R. solani* (Sneh *et al*., 1991).

Formation of sclerotia was observed in the petridish and classified into three groups: peripheral, scattered and ring at periphery. One isolate was found in ring at periphery (RS-1) (Plate 7a and b), two isolates were found scattered (RS-4 and RS-5) (Plate 7c and 7d) and one was found peripheral (RS-6) (Plate 7e) (Table 3). Singh *et al*. (1990) reported the sclerotial formation in the same manner i.e. central, peripheral or scattered.

Sclerotia was formed both in surface mycelium in four isolates (RS-1, RS-4, RS-5 and RS-6).

The texture of sclerotia, was classified into two groups-smooth and rough. In the smooth category, three isolates (RS-1, RS-5 and RS-6) and in the rough category one isolate (RS-4) was grouped (Table 3). Sclerotia may be absent in some *R. solani* isolates under certain cultural conditions therefore the absence of sclerotia does not automatically exclude a mycelium from *R. solani*.

Based on the pigmentation of the sclerotia, the isolates were assigned to 2 groups -willow green and greyed yellow. Willow green was observed in three isolates (RS-4, RS-5 and RS-6), greyed yellow in one isolate (RS-1) and no sclerotium was formed in two isolates (Table 3). Sinha and Ghufran (1988) reported that more variations in the type and colour of mycelium and size, colour, number and type of sclerotia among the isolates of *R. solani*. Hoa (1994) also reported that sclerotial colour ranged from brown, light/dark brown, black brown, chocolate brown, salmon and dark salmon.

There was a great diversity for time taken for initiation of sclerotia formation. It ranged from 9 to 18 d. The first sclerotial initiation was observed 9 days after inoculation by RS-1 (Plate 7a and 7b). Initiation of sclerotial production started in 11 days after inoculation for RS-4 (Plate 7c), 15 days for RS-5 (Plate 7d) and 18 days for RS-6 (Plate 7e). Isolates RS-2 and RS-3 did not produce any sclerotia till 35 days of its observation (Table 4). According to Meena *et al*. (2001) time taken for sclerotia formation ranged from 3-11 d.

The diam of sclerotia ranged from 1.4-2.5 mm (Table 4). According to Hoa (1994), the size of sclerotia, ranged from 0.85-3.05 mm. Basu *et al*. (2004) reported that sclerotial diam ranged from 0.23 to 1.91 mm and found that the abundance and size of sclerotia determine the virulence of an isolate. Dath (1985) and IRRI (1986) also reported that diameter of *R. solani* sclerotia ranged from 1 to 3 mm.

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Table 3: Formation, location, texture, and colour of sclerotia of different isolates of R. solani on PDA medium

Sources	Isolates	Pattern in petriplate	Location of sclerotia	Texture of sclerotia	Colour of sclerotia
Rice (*Oryza sativa*)	RS-1	Ring at periphery	Surface	Smooth	Greyed yellow-177 group(A)
Cowpea (Collar) (*Vigna unguiculata*)	RS-2	-	-	-	-
Setaria (*Setaria parviflora*)	RS-3	-	-	-	-
Greengram (*Vigna radiata*)	RS-4	Scattered/ Haphazards	Surface	Rough	Willow green
Long pepper (*Piper longum*)	RS-5	Scattered/ Haphazards	Surface	Smooth	Willow green
Maize (*Zea mays*)	RS-6	Peripheral	Surface	Smooth	Willow green

Table 4. Time taken for initiation of sclerotial formation, average diam of no. of sclerotia per Petri dish and hyphal width of different isolates of R. solani

Sources	Isolates	Initiation of sclerotia (d)*	Sclerotia/ plate** (no.)	Sclerotia (Av. diameter in mm)***	Hyphal diameter (µm)*** *
Rice (*Oryza sativa*)	RS-1	9	37	2.5	6.2
Cowpea (Collar) (*Vigna unguiculata*)	RS-2	-	-	-	5.6
Setaria (*Setaria parviflora*)	RS-3	-	-	-	5.8
Greengram (*Vigna radiata*)	RS-4	11	68	2.0	7.2
Long pepper (*Piper longum*)	RS-5	15	55	1.4	6.2
Maize (*Zea mays*)	RS-6	18	42	1.6	6.9
CD(P=0.05)				0.60	0.32

*Data are mean of three replications, **Data are mean of 20 replications, ***Data are mean of 10 replications, ****Data are mean of 10 replications

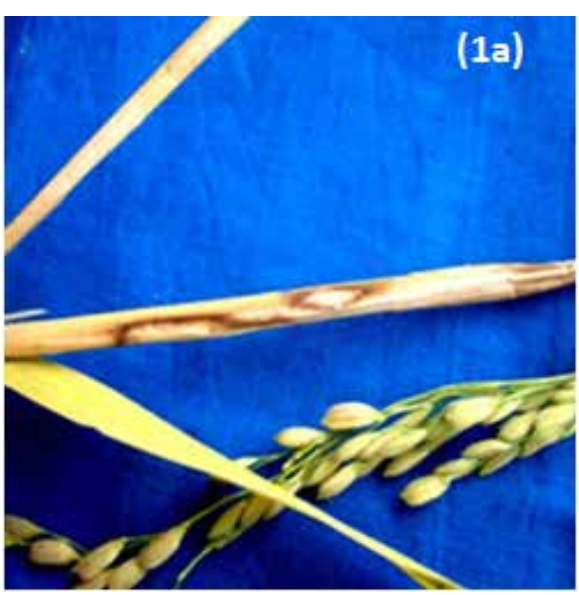

Plate 1a. Sheath blight of rice, Oryza sativa

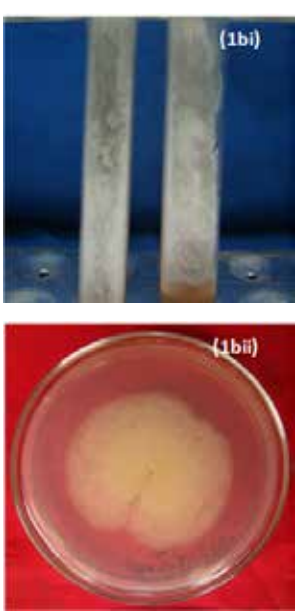

Plate 1b. Pure culture of Rhizoctonia solani (RS-1) in i. PDA slant, ii. PDA plate isolated from sheath blight of rice

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Plate 2a. Collar rot of cowpea, Vigna unguiculata

Plate 2b. Pure culture of Rhizoctonia solani (RS-2) in PDA plate isolated from Collar rot of cowpea

Plate 3a. Leaf blight of yellow bristle grass, Setaria glauca

Plate 3b. Pure culture of Rhizoctonia solani (RS-3) in PDA plate isolated from leaf blight of Yellow bristle grass

Plate 4a. Damping off of green gram, Vigna radiata

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Plate 4b. Pure culture of Rhizoctonia solani (RS-4) in i. PDA slant, ii. PDA plate isolated from damping off-of green gram

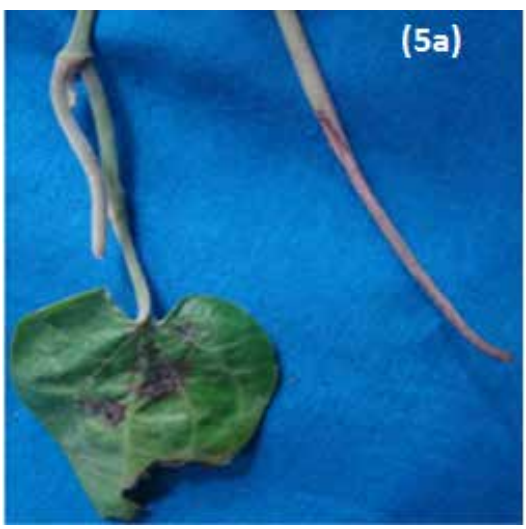


Plate 5a. Rhizoctonia blight of long pepper, Piper longum

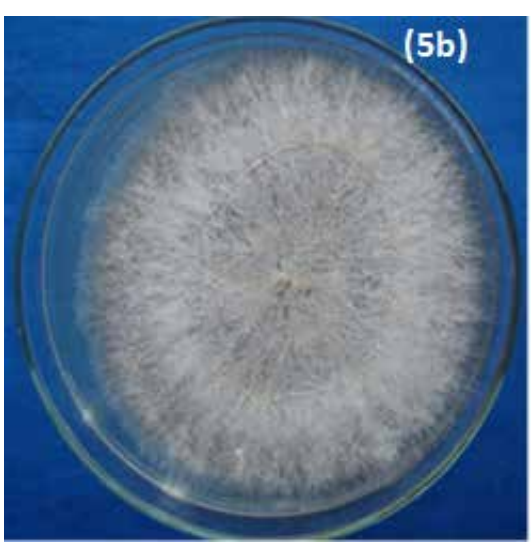


Plate 5b. Pure culture of Rhizoctonia solani (RS-5) in PDA plate isolated from Rhizoctonia blight of long pepper

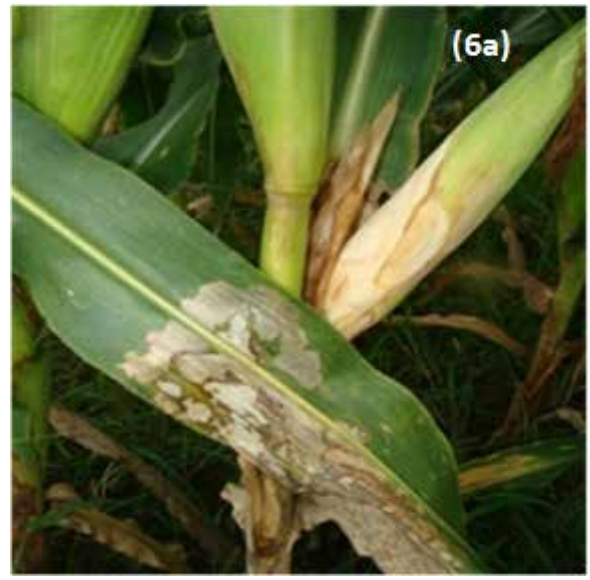


Plate 6a. Banded leaf and sheath blight of maize, Zea mays

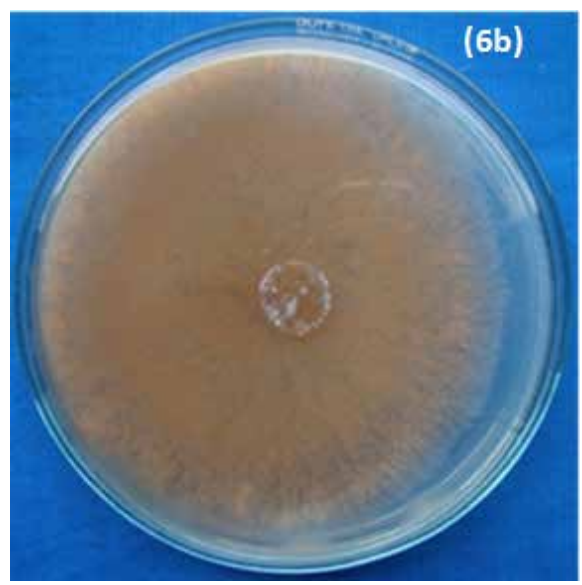
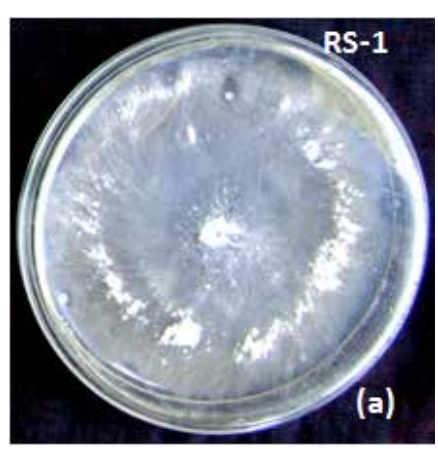


Plate 6b. Pure culture of Rhizoctonia solani (RS-6) in PDA plate isolated from banded leaf and sheath blight of maize



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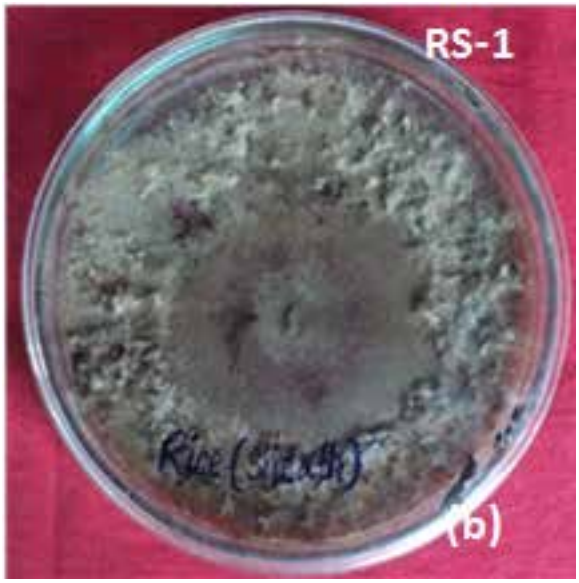

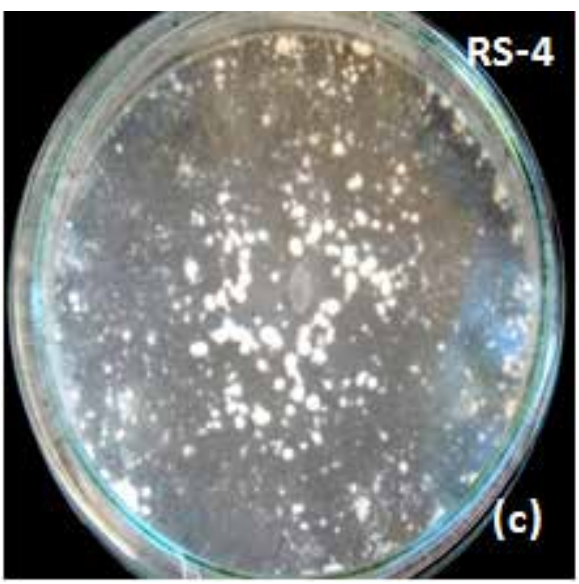

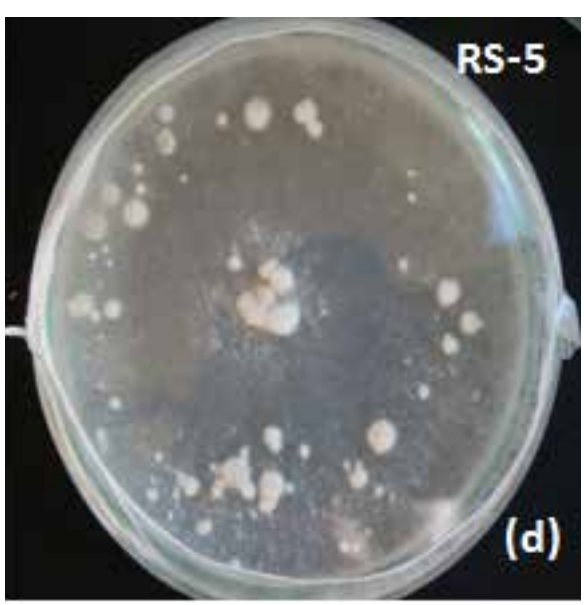

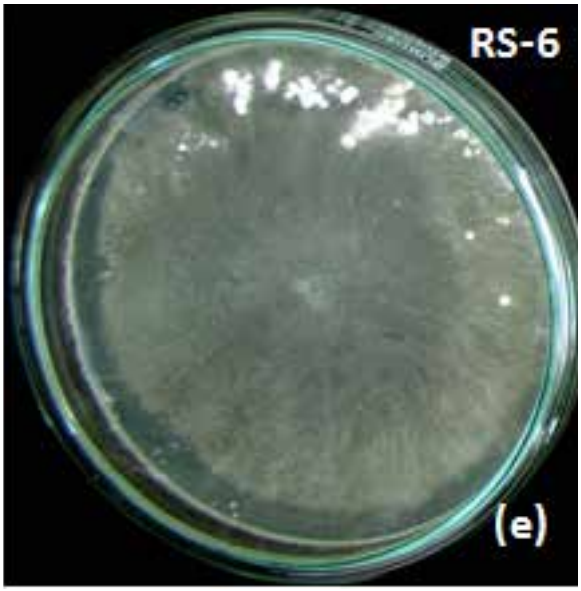

Plate 7(a-e). Initiation, formation and pattern of sclerotial formation by different isolates of R. solani in PDA media
a and b: RS-1, c: RS-4, d: RS-5, e: RS-6

Conclusion
It was observed that isolates have great diversity in cultural or morphological characters. *Rhizoctonia* represents a diverse group of fungi that differs in many significant features. Identification of *Rhizoctonia* isolates to some taxonomic level is of utmost importance for studying their epidemiology and control in different cropping systems.

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