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Studies on variability of *Rhizoctonia solani* isolated from different hosts and its virulence on rice crop

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Abstract
The cultural and morphological features of *Rhizoctonia solani* were studied on growing them on PDA medium which was isolated from different host viz. rice, soybean, moongbean groundnut and maize. Five field isolates are characterized for cultural and morphological characteristics on PDA medium under *in vitro* condition. The isolates varied with respect to growth, colony characteristics and sclerotial morphology and it was found that the rice isolate was submerged to subdued type while the soybean and moongbean isolates produced aerial mycelium with strandy and subdued growth, and it was strandy and subdued in maize and groundnut isolate produced strandy and subdued mycelium. Various shades of dark brown mature sclerotia were observed in rice, soybean, moongbean whereas groundnut and maize isolates produced very dark greyish colored sclerotia. Marked differences in the shape and size of sclerotia were observed by the isolates of different hosts. In rice isolate, the sclerotia was big sized, globular and was myceloid. Maize isolate produced somewhat spherical to small oval shaped sclerotia. Soybean and moongbean isolate produced irregular shaped sclerotia with pitted surface. While the groundnut isolate generally produced irregular shaped sclerotia with smooth surface. *In vivo* virulence potential of five different field isolates of *Rhizoctonia solani* isolated from different hosts were studied against different varieties (Swarna, Maheshwari, TN-1, and HMT) of rice crop and it was found that the isolate of groundnut had maximum development of lesion followed by moongbean in Swarna variety. In Maheshwari and HMT variety, moongbean isolate showed maximum development of lesion and least was shown by maize isolate and maize isolate does not had any effect on HMT variety of rice crop. In TN-1 variety, groundnut isolate had maximum development of lesion followed by the isolates of rice and maize while the isolate of moongbean showed least reduction in lesion length.

Keywords: *Rhizoctonia solani*, *Oryzae sativa,* Swarna, Maheshwari, TN-1, and HMT

Introduction
Rhizoctonia solani Kühn is most widely distributed plant pathogen causing disease problem on many crops (Führer Ithurrart *et al*., 2004). *Rhizoctonia solani* can cause disease on at least 200 different plant species (Anderson, 1982; Salazar *et al*., 2000). Isolates vary considerably in pathogenicity and cultural characteristics not only of same host but also in the different host. Classification of *Rhizoctonia spp.* has evolved mainly from studies of isolates obtained from diseased plants.In nature, plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence.
To understand the present plant disease situations and to predict the possible future development it is essential to learn as much as possible about the variability in fungi that are pathogenic to plants. Morphological and pathogenic variations are known in many fungal pathogens (Kumar *et al.*, 1995). Due to variation in the distribution of *R. solani* across environments, it is important to know which isolate is most virulent within a given species especially to identify genetic resistance to highly virulent isolates. Keeping in view, the importance of the crop, severity of the disease and advantages of biological control, present investigation was taken up to study the variability in cultural and morphological characters of *R. solani* isolates.

Materials and Methods
General procedure followed
For each set of treatment, different replications were used in all *in vitro* studies. In general, in each petriplates about 15-20 ml of potato dextrose agar medium was poured, supplemented with streptomycin sulphate, in order to check the unwanted bacterial contaminations. Wherever growth studies were conducted, 5 mm disc of pure culture of *Rhizoctonia solani* was

used for inoculation of the medium in petriplates.
The inoculated petriplates were incubated in the BOD incubator at 27± 2^0C for maximum period of 15 days. Observations for the mycelial growth were recorded one, two, three, four, and five days after incubation and total number of sclerotia formed were counted 15 days after inoculation.

Studies on Symptomatology
Rice plants were observed at all the growth stages of crop from germination to maturity stage under field condition at the Research Farm, IGKV, Raipur for symptomatological studies.
Disease sample infected with *R. solani* were collected from maize, soybean, moongbean, groundnut and rice crops, planted at Research Farm. College of Agriculture, Raipur, during the *kharif* season 2013. Name of the host, disease and their collected parts are listed below:

Table 1: List of naturally infected hosts and their symptoms

S. No	Host	Botanical Name	Disease	Infected Plant Part
1	Rice	*Oryza sativa*	Sheath blight	Sheath
2.	Maize	*Zea mays*	Banded blight	Sheath
3.	Soybean	*Glycine max*	Aerial blight	Leaf
4.	Moongbean	*Vigna radiata*	Web blight	Leaf
5.	Groundnut	*Arachis hypogaea*	Web blight	Leaf

Isolation of the pathogen from its different hosts
The diseased plants exhibiting characteristic symptoms were collected from field and brought to the laboratory for isolation. The diseased parts were cut into small pieces by sterilized blade and surface sterilized with 1.0% Sodium hypochlorite for one minute followed by three washing with sterile water. These surface sterilized bits of infected leaf and sheath were placed in petriplates containing PDA. The petriplates were kept for incubation at 27±2^0C and whenever fungal colonies appeared, they were sub cultured and purified on PDA slants.

Pathogenicity Test
The healthy seedlings were collected from field and selected the healthy trifoliates of groundnut, soybean, moongbean and leaf of maize and rice for inoculation. All were surface sterilized with 1.0% sodium hypochlorite for one minute, after sterilization these healthy leaves were kept in big size desiccators and inoculated the mycelium disc of test fungus. Inoculation disc without fungal mycelium was served as control. After inoculation desiccators was sealed with the help of grease. Symptoms were observed after first day of inoculation.

***In-vitro* evaluation of cultural and morphological variation of *R. solani* isolates obtained from different hosts.**
Cultural characters of five field isolates of *R. solani* were studied on twenty ml sterilized Potato Dextrose Agar medium. Mycelial disc were transferred aseptically at the centre of each petri plates and incubated at 27±2º C for 15 days. The colour of the colony was determined with the help of Munsell´s soil colour chart (Munsell colour company, Inc. 1954).

***In-vivo* evaluation of virulence of *Rhizoctonia solani* isolated from different hosts on different varieties of rice crop (Swarna, Maheshwari, TN-1, HMT.)**
The virulence of five field isolates of different hosts were evaluated on varieties of rice; Swarna, Maheshwari. TN-1 and HMT. All the varieties were planted in Research Farm of Plant Pathology Department, COA, Raipur in randomized block design. The study was conducted in bunded rice field under irrigated conditions. At early booting stage the plant were inoculated with sclerotia of *R. solani* of five different hosts (rice, maize, soybean, moongbean and groundnut) by insertion placement method.
The observation for the development of lesion length and relative lesion length in rice were recorded at regular intervals. Relative lesion length was calculated by using formula-

$$\text{Relative lesion length (\%)} = L/H \times 100$$

Where,
L= Length in lesion in mm
H= Height of the plant in mm

Result and Discussion
Five field isolates were characterized for cultural and morphological characteristics on PDA medium after 72 hrs of incubation. Hyphal characteristics of all the isolates showed typical characteristics of *R. solani*.

Cultural and morphological characteristics
Five field isolates are characterized for cultural and morphological characteristics on PDA medium after 72 hrs of incubation and the purified culture were multiplied on PDA slants for further use and the original was preserved. For all the cultural and morphological variation studies, the isolates of rice from COA, Raipur were used for comparison.

Variation in color
Differentiation of the color shades produced by the sclerotium and vegetative growth, chart no. Hue 10 YR of Munsell´s soil color chart (Munsell colour company, Inc. 1954), was used for all the isolates. Vegetative growth of rice and maize isolates produces pale yellow brown shade (shade no.8/4 and 6/4 respectively) while it was light gray in soybean (7/1), moongbean (7/2), and groundnut (7/1) isolates. Colour variation was not observed within the isolates of the crop. Various shades of dark brown mature sclerotia were observed in rice, soybean, moongbean and groundnut isolates whereas maize isolates produced very dark grey colored sclerotia.

Appearance of vegetative growth
Appearance of vegetative growth in rice isolates was submerged to subdued type while the soybean and moongbean isolates produced aerial mycelium with strandy and subdued growth, and it was strandy and subdued in maize and groundnut isolates with no aerial mycelium.

Table 2: Variability in mycelial and sclerotial characteristics of *R. solani* isolates from different hosts on PDA medium.

Isolates	Cultural characteristic		Appearance of mycelial growth
	Colour of mycelial growth	Radial growth in mm	
Rice	Very pale brown	90	Mycelium with submerged to subdued and strandy growth
Maize	Yellow brown	45	Mycelium with submerged to subdued and strandy growth
Soybean	Light grey	66	Aerial mycelium with strandy and subdued growth
Moongbean	Light grey	71	Aerial mycelium with strandy and subdued growth
Groundnut	Light grey	61	Mycelium with strandy and subdued growth

SEm±= 1.88
C.D (P=0.05)= 5.92

Average of three replication

The isolates varied with respect to growth, colony characteristics and sclerotial morphology and it was found that the rice isolates was submerged to subdued type while the soybean and moongbean isolates produced aerial mycelium with strandy and subdued growth, and it was strandy and subdued in maize and groundnut isolates produced strandy and subdued mycelium. Various shades of dark brown mature sclerotia were observed in rice, soybean, moongbean whereas groundnut and maize isolates produced very dark greyish colored sclerotia.

Similar result on cultural and morphological variation in thirty five different isolates was found by Goswami *et al.* 2010, The result given below indicates that the radial growth of mycelium was significantly highest in rice isolates followed by moongbean isolates while the least radial growth was observed in maize isolates.

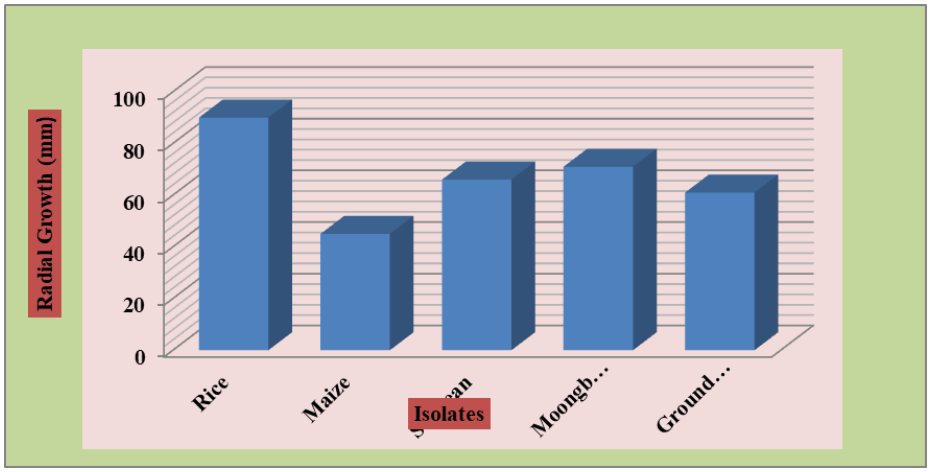

Fig 1: Radial growth of *R. solani* collected from different crops on PDA medium.

Sclerotial Morphogenesis

Sclerotia are asexual, multi-cellular, hard resting structure. The sclerotia of *R. solani* are comparised of clusters of melanin encrusted, thick walled cells rich on nutrients formed by increased branching from short thick lateral hyphae.

Morphological characters of sclerotia

Sclerotia of *Rhizoctonia* is a basic character by which the genus is distinguished from *Sclerotium*. Isolates from five different host when cultured on PDA showed differential effects. Abundant no. of sclerotia were produced by the isolates of rice followed by moongbean isolates while few sclerotia were produced by the isolates of soybean. However, average no. of sclerotia was produced by the isolates of maize and groundnut.

Shape of Sclerotia

Marked differences in the shape and size of sclerotia were observed by the isolates of different hosts. In rice isolates, the sclerotia were big sized, globular and were myceloid. Maize isolates produced somewhat spherical to small oval shaped sclerotia. Soybean and moongbean isolates produced irregular shaped sclerotia with pitted surface. While the groundnut isolates generally produced irregular shaped sclerotia with smooth surface.

Distribution of sclerotia

On potato dextrose agar medium, the sclerotial isolates of rice, maize and groundnut were scattered in all over the plate. The isolates of soyabean produced clumped sclerotia and situated at the center of the plate. However great number of scattered sclerotia was produced by the isolates of moongbean and also few sclerotia were also found on the upper surface of the plate.

Table 3: Shape, size and distribution of sclerotia of different host isolates.

Medium Used	Characters	Isolates				
		Rice	Maize	Soybean	Moongbean	Groundnut
Potato Dextrose Agar	Shape and size of sclerotia	Big sized, globular and were myceloid	Spherical to small oval shaped sclerotia	Irregular shaped sclerotia with pitted surface	Irregular shaped sclerotia with pitted surface	Irregular shaped sclerotia with smooth surface
	Distribution of sclerotia	Scattered all over the plate	Scattered all over the plate	Clumped sclerotia and situated at the center of the plate	Scattered sclerotia and also few sclerotia were also found on the upper surface of the plate	Scattered all over the plate

Table 4: Reaction of *Rhizoctonia solani* isolated from different hosts on different varieties of rice crop.

S. No.	Variety	Plant Height (mm)	Isolates of Different Hosts	Lesion Length (mm)	Relative Lesion Length(%)
1.	Swarna	900	Rice	70.0	7.77
		910	Maize	76.6	8.41
		880	Soybean	83.3	9.46
		890	Moongbean	96.6	10.85
		900	Groundnut	116.6	12.95
			SEm± C.D (P=0.05)	1.60 3.35	

All the isolates of different host were significantly differ in their reaction. In Swarna variety, maximum lesion length (116.6 mm) was recorded from groundnut isolates followed by moongbean, soybean and maize isolates. Minimum lesion length was recorded in rice isolates. However, the relative lesion length was highest in groundnut isolate followed by moongbean isolate while the least was observed in rice isolate.

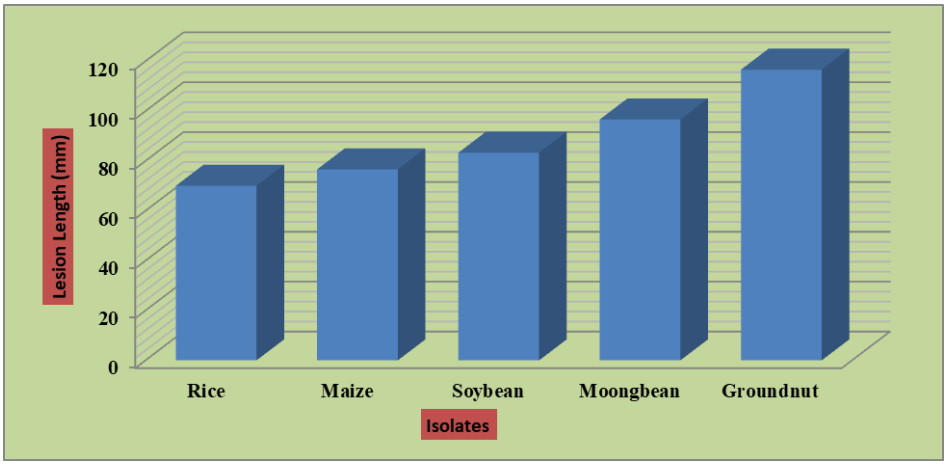

Fig 2: Reaction of *R. solani* isolated from different hosts on var. Swarna of rice crop.

Table 5

S. No.	Variety	Plant Height (mm)	Isolates of Different Hosts	Lesion Length (mm)	Relative Lesion Length (%)
2.	Maheshwari	900	Rice	33.3	3.70
		940	Maize	16.6	1.76
		910	Soybean	46.6	5.12
		890	Moongbean	80.0	8.89
		880	Groundnut	13.3	1.51
			SEm± C.D (P=0.05)	0.86 2.70	

All the isolates of different host were significantly differ in their reaction. In Maheshwari variety, maximum lesion length (80.0 mm) was recorded from moongbean isolates followed by soybean, rice and maize isolates. Minimum lesion length was recorded in groundnut isolates. However, the relative lesion length was highest in moongbean isolate followed by soybean isolate while the least was observed in groundnut isolate.

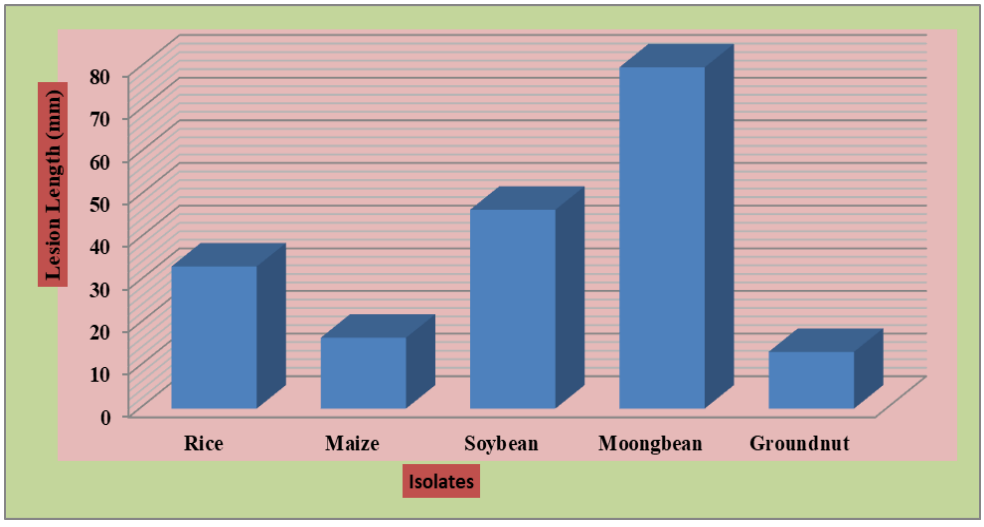

Fig 3: Reaction of *R. solani* isolated from different hosts on var. Maheshwari of rice crop.

Table 6

S. No.	Variety	Plant Height (mm)	Isolates of Different Hosts	Lesion Length (mm)	Relative Lesion Length(%)
3.	HMT	750	Rice	40.0	5.33
		770	Maize	00.0	0.00
		740	Soybean	43.3	5.85
		780	Moongbean	70.0	8.97
		800	Groundnut	33.3	4.16
			SEm± C.D (P=0.05)	1.03 3.25	

All the isolates of different host were significantly differ in their reaction. In HMT variety, maximum lesion length (70.0 mm) was recorded from moongbean isolates followed by soybean, rice and groundnut isolates. No infection was recorded in maize isolates. However, the relative lesion length was highest in moongbean isolate followed by soybean isolate.

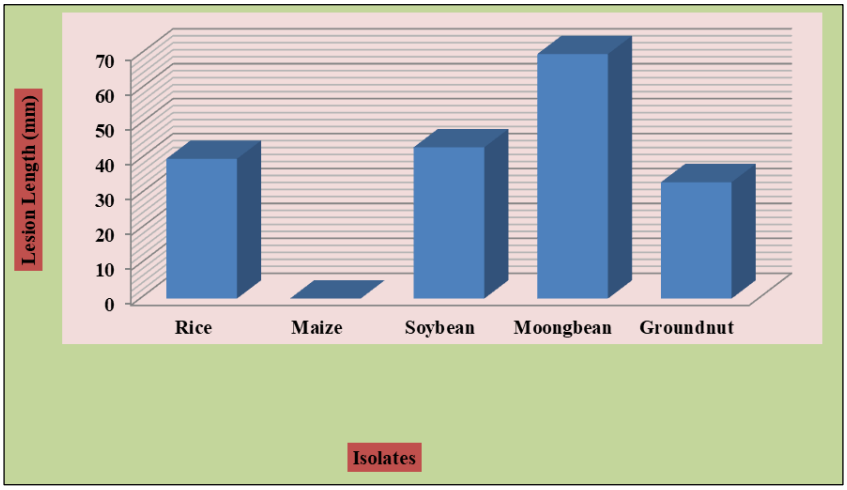

Fig 4: Reaction of *R. solani* isolated from different hosts on var. HMT of rice crop.

Table 7

S. No.	Variety	Plant Height (mm)	Isolates of Different Hosts	Lesion Length (mm)	Relative Lesion Length (%)
4.	TN-1	110	Rice	60.0	54.54
		120	Maize	60.0	50.00
		110	Soybean	53.3	48.45
		100	Moongbean	33.3	33.30
		110	Groundnut	83.3	75.72
			SEm± C.D (P=0.05)	1.65 5.21	

All the isolates of different host were significantly differ in their reaction. In TN-1 variety, maximum lesion length (83.3 mm) was recorded from groundnut isolates followed by rice and maize isolates. Minimum lesion length was recorded in moongbean isolates. However, the relative lesion length was highest in groundnut isolate followed by rice isolate while the least was observed in moongbean isolate.

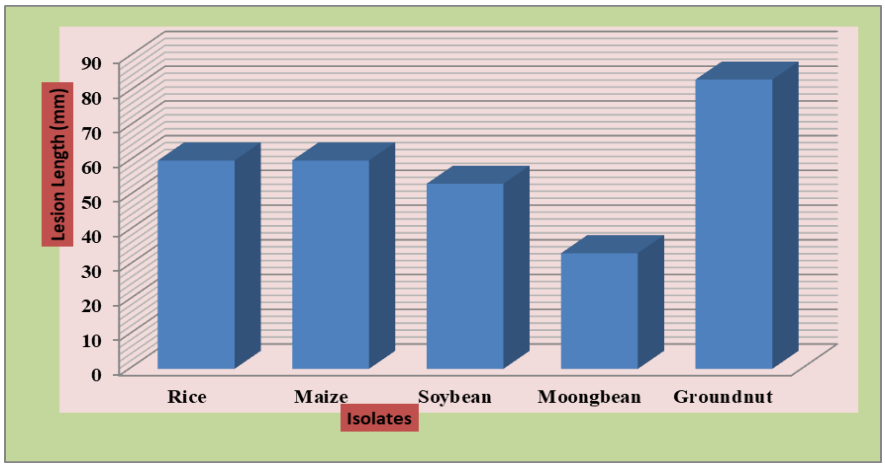

Fig 5: Reaction of *R. solani* isolated from different hosts on var. TN-1 of rice crop.

The results in above table are in agreement of Barid (1996), Goswami *et al.* 2010 [9]., collected *Rhizoctonia solani* isolates from soil of different agro-ecological zones of Bangladesh and also from infected plant parts of different crops and grasses and classified the isolates into five different cluster groups on the basis of morphological and cultural characters. After analyzing he found that there were no relations between the isolates with respect to their origin from where they were collected. It indicated that the diversity among the isolates was not correlated with their origin. In case of host range and pathogenicity among the five selected isolates of different cluster groups, the isolate JES-16 was an avirulent isolate. The isolate SYL-30 had narrow host range and a low virulent isolate. The isolates DIN-8 and GAZ-18 possessed wide host range and might be considered as virulent isolates. The isolate GAZ-9 was a highly virulent isolate with a wide host range.

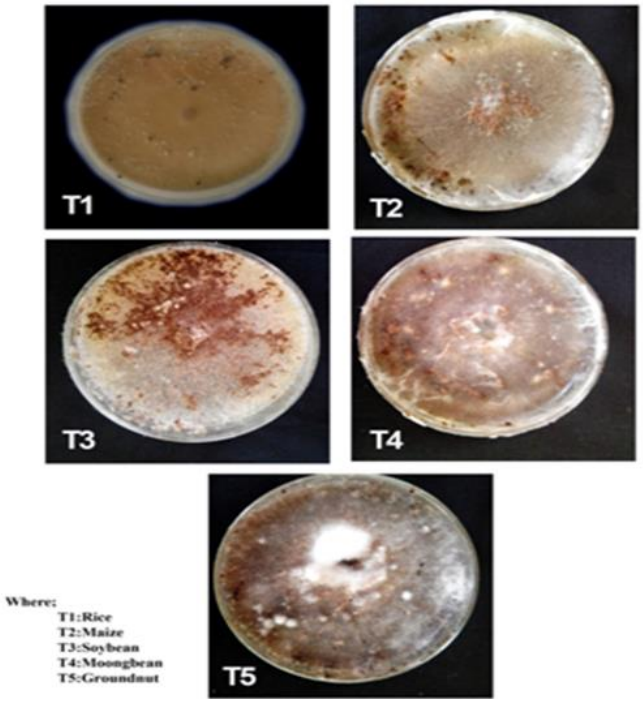

Fig 6: Variation in shape and distribution of sderotia in *Rhizoctonia solani*

References

1. Baker KF. Types of *Rhizoctonia* diseases and their occurrence. In: Parmeter, J. R. Jr. (ed.), *Rhizoctonia solani:* Biology and Pathology. University of California Press, Berkeley, USA, 1970, 125-148.
2. Baker R, Martinson CA. Epidemiology of disease caused by *Rhizoctonia solani*. In Parmeter JR Jr. (ed.) *Rhizoctonia solani*: Biology and Pathology. University of California Press, Berkeley, CA, USA. 1970, 172-188.
3. Basu A, Gupta PKS. Cultural and pathogenic variation in rice isolates of *Rhizoctonia solani* Kuhn. Beitrage-Zur-Tropischen-Landwirts-Chaft-Und-Veterinarmedizin, 1992, 291-297.
4. Dasgupta MK. Rice sheath blight: The challenge continues. In: Singh US, Mukhopadhyay AN, Kumar J, Chaube HS. eds. Plant disease of international importance: Disease of cereals and pulses. Prentice hall, Englewood Cliffs, New Jersey, 1992, 130-150.
5. Dubey SC. Biological management of web blight of groundnut (*Rhizoctonia solani*). J Mycol. Pl. Pathol. 2000; 30(1):89-90.
6. Garibaldi A, Minuto G, Nicoletti R, Gullino ML. First report of a blight caused by *Rhizoctonia solani* on Anubias heterophylla in Italy. Plant Dis. 2003; 87(8):1005.
7. Gautam SR. Studies on bayleton and bayton, *in vitro* stability, systemecity and response in soybean [*Glycine max* (L.) Merrill]. Ph.D Thesis, G.B.P.U.A.T., Pantnagar, 1981, 159.
8. Godoy-Lutz G, Arias J, Steadman JR, Eskridge KM. Role of natural seed infection by the web blight pathogen in common bean seed damage, seedling emergence, and early disease development, Plant Dis. 1996; 80:887-890.
9. Goswami BK, Bhuiyan KA, Mian IH. Morphological and pathogenic variations in the isolates of *Rhizoctonia solani* in Bangladesh. Bangladesh J Agril. Res. 2010; 35(3):375-380.
10. Hepperly FR, Mignucci JS, Sinclair JB, Smith RS. *Rhizoctonia* web blight of soybean in Fuerto Rico. Plant Dis. 1982; 66:256-257.
11. Holcomb GE, Carling DE. First report of web blight caused by *Rhizoctonia solani* on *Catharanthus roseus* in Louisiana. Plant Dis. 2002; 86(11):1272.
12. Lal M, Kandhar J. Cultural and morphological variability in *Rhizoctonia solani* isolates causing sheath blight of rice. J Mycol Pl Pathol. 2009; 39(1):77-81.
13. Maier, Stafferdt EE. Cultural variability of selected isolates of *Rhizoctonia solani* and *Thielaviopois basicola* and the variability in their pathogenicity to acala and pima cotton, respectively. Plant Disease Reptr. 1960; 44:956-961.
14. Ogoshi A. The genus Rhizoctonia. In. Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Eds. Sneh B, Jabaji-Hare S, Neate S, Dijst G. Kluwer Academic Publishers, The Netherlands, 1996, 1-9.
15. Saksena HK, Dwivedi RP. Web blight of black gram caused by *Thanatephorus cucumeris*. Indian J Farms Sci. 1973; 1:58-61.
16. Sartorato A, Nechet KL, Halfeld VBA. Genetic diversity of *Rhizoctonia solani* isolates collected from Cowpea in the State of Roraima. Fitopatologia Brasileira. 2006; 31(3):297-301.
17. Shailbala, Tripathi HS. Current status of research on web blight disease of urd bean. Agric. Rev. 2007; 28(1):1-9.
18. Sharma D, Thrimurty VS. Effect of age of mycelium and sclerotia in sheath blight of rice(*Oryza sativa* L.) disease development. Plant Archives. 2004; 4(2):363-365.
19. Singh SK, Satyanarayana K, Reddy APK. Studies on morphology, growth habit, hyphal anastomosis, and virulence pattern of five isolates of sheath blight pathogen of rice. Indian Phytopathology. 1990; 43(3):368-371.
20. Singh A, Malhotra SK. Pathological and physiological studies on some root diseases of soybean. Bhartiya Krishi Anusahdhan Patrika. 1994; 9:113-116.
21. Singh BR, Sing M, Yadav MD, Dingar SM. Yield loss in mungbean due to Cercospora leaf spot, yellow mosaic, Science and culture, C. S. Azad University. Agric. & Tech. Kanpur, India. 1982; 48(12):435-436.
22. Staffeldt EE, Maier CR. Cultural and pathogenic variability of *R. solani* isolates from cotton growing areas of New Mexico. Plant Disease Reptr. 1959; 43:1063-1066.
23. Tiwari A, Khare MN. Variability among isolates of *R. solani* infecting mung bean. Indian Phytopath. 1998; 51(4):334-337.