

Original Research Article

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Pathogenic Variability of Isolates of *Rhizoctonia solani* of Different Agricultural Crops

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ABSTRACT

Rhizoctonia solani is an ubiquitous soil borne plant pathogen causes versatile diseases in agricultural crops. Due to the attack of the pathogen, plant show symptom like damping off, wire stem, stem rot, collar rot, web blight, charcoal rot, black scurf, sheath blight and banded leaf and sheath blight etc in different host crops. In the present study we isolated different isolates of *R. solani* from different host plant of different locations. The association of pathogenic isolates was confirmed by Koch postulate which was conducted on hypocotyl by mixing the soil with culture having *R. solani* and seeds were sown for germination. Fifteen days after the germination of seeds, seedlings showed symptoms, whereas controlled seedlings remained healthy. Further, pathogenicity test was also conducted on leaves by inoculating freshly cultured mycelial disc (5 mm) on healthy plants of different hosts, non-inoculated plants served as control. Few inoculated plants developed symptoms, whereas controlled plants remained healthy. We also evaluated cross infectivity of different isolates in different crops plant through standard method and found that isolates of rice (RS-1 and RS-2) and setaria (RS-4) causes infection to all the tested host plants like maize, green gram, cabbage and potato being highest score 5. But few isolates of *R. solani*, RS-3, RS-5, RS-5, RS-7, RS-8, RS-9, RS-10 could not cause infection to all the host plant tested except few. Long pepper (RS-6) could not cause any infection on maize and green gram and showed score 0.

Keywords

Pathogenicity, Host, *Rhizoctoniasolani*

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Introduction

Rhizoctonia solani (Teleomorph: *Thanatephorus* spp.) is soil borne plant pathogenic fungus, which is ubiquitous in nature with a wide host range and worldwide distribution causing diseases ranging from field to horticultural crops (Sneh et al., 1996). *R. solani* frequently exists as thread-like growth on plants or in culture, and is

considered as a soil borne pathogen. The infection caused by *R. solanica* attack the crop in any stage. viz., from seedling to harvesting stage. It is best known to cause various plant diseases such as collar rot (Dutta and Das, 2002), root rot, damping off, wire stem, stem rot (Dutta and Das, 1999), bare patch of cereals, root rot of sugar beet, belly rot of cucumber, sheath blight of rice (Das et al., 1997), black scurf of potatoes.

Rhizoctonia can be found across all areas of the world (environmental conditions permitting) where its host crops are located.

R. solani shows high variability in its cultural characters and pathogenic behavior. The variability is so high that it is difficult to classify different isolates into groups. Isolates of multinucleate *R. solani* are genetically diverse in their cultural, morphological and physiological characteristics as well as in their pathogenic range of host plants (Kuninga *et al.*, 1997; Gonzalez *et al.*, 2001 and 2006; Sharon *et al.*, 2006 and 2008). But literature related to the isolates of north eastern states of India, their variability of infection is rare. Searching literature also showed that cross infectivity test of *Rhizoctonia* isolates of different host of the region has not been done.

With this background, the present study was conducted to isolate the different isolates of *R. solani* from different crop host and studied variability on pathogenic ability through cross infectivity tests.

Materials and Methods

The experiment was conducted in the net house of Mycology Research Section, Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam.

Collection of sample

Diseased samples of different crops showing typical symptom infected by *R. solani* were collected from Assam (Instructional Cum Research farm and Experimental farm, Department of Horticulture, AAU, Jorhat) in air tight zip plastic bag. Collected samples were brought to the laboratory of Mycology Research Section, AAU, Jorhat, Assam.

Isolation and purification of putative organism

To isolate the fungi, collected diseased samples were surface sterilized with 4.0 percent sodium hypochlorite (NaOCl) solution and then rinsed twice with double distilled sterile water. In sterile Petriplates, the diseased specimens were crushed and a small portion (size: 1-2mm) of infected part with small healthy parts were transferred to culture plate containing PDA under aseptic condition. Inoculated plates were incubated at $25\pm 1^{\circ}\text{C}$ for 5-7 days.

Plates were observed regularly for growth and development of associated micro-organisms. After five 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 method.

Maintenance of culture

Isolated and purified cultures were maintained by transferring periodically in fresh PDA medium and storing in refrigerator at 4°C for further studies.

Preparation pot mixture and planting

Inoculum for infestation of soil was prepared by mixing seven (7) days old culture of *R. solani* on Potato Dextrose Agar (PDA) media (100 ml) in 100 ml of sterile water and pots were filled with the infested soil. Suspension of fungal propagules were thoroughly mixed with soil @ 4.0 per cent on dry weight equivalent.

The infested soil was kept moist for three (3) days before planting. Ten seeds were sown in each of three (3) replicate pots of infested soil.

Pathogenicity test

Pathogenicity test of the isolated, purified and characterized isolates of *R. solani* were conducted between crops. Inoculum of the pathogen more particularly mycelium were inoculated on both hypocotyls and leaves of the targeted plants. Each plant was grown individually in pre-sterilized soil in earthen and plastic pots and arranged in a completely randomized block design in a greenhouse.

Pathogenicity test on hypocotyls

Pathogenicity of ten (10) collected isolates of *R. solani* were carried out in pots filled with sterilized soil. Healthy seedlings of potato, rice, green gram, cabbage and maize were selected for inoculation 1 week after seeding. Inoculum for infestation of soil was prepared by mixing seven (7) days old culture on Potato Dextrose Agar (PDA) media (100 ml) in 100 ml of sterile water. Suspension of fungal propagules were thoroughly mixed with soil @ 4 per cent on dry weight equivalent. The infested soil was kept moist for three (3) days before planting. Ten seeds were sown in each of three (3) replicate pots of infested soil. Seedlings grown in sterilized

soil without inoculum served as control. Disease incidence was recorded after fifteen (15) days of inoculation. Disease severity was recorded on six(6) point scale given by Zhou *et al.*, 2009.

0: Seedling well- developed, no visible lesions on lower stem and roots
 1: Seedling growing well with a few small lesions on lower stem and roots, total infected area <25 per cent.

2: Growth of the seedling is retarded, moderate necrosis or small lesions scattered over the lower stem and roots, total infected area 25-50 per cent.

3: Seedling growth is hampered, large lesions on the lower stem and roots, total area 50-75 per cent.

4: Seedling growth is seriously hampered, girdling lesions on the lower stem and roots, total infected area > 75 per cent.

5: Death of plant. Treatment combinations followed to test the pathogenicity of ten different isolates of *R. solani* on hypocotyls of five different crops were as follows

T₁: RS-1 + Green gram	T₂₁: RS-6 + Green gram
T₂: RS-1 + Maize	T₂₂: RS-6+ Maize
T₃: RS-1 + Cabbage	T₂₃: RS-6 + Cabbage
T₄: RS-1 + Potato	T₂₄: RS-6 + Potato
T₅: RS-2 + Green gram	T₂₅: RS-7 + Green gram
T₆: RS-2 + Maize	T₂₆: RS-7 + Maize
T₇: RS-2 + Cabbage	T₂₇: RS-7 + Cabbage
T₈: RS-2 + Potato	T₂₈: RS-7 + Potato
T₉: RS-3 + Green gram	T₂₉: RS-8 + Green gram
T₁₀: RS-3 + Maize	T₃₀: RS-8 + Maize
T₁₁: RS-3 + Cabbage	T₃₁: RS-8 + Cabbage

T₁₂: RS-3 + Potato	T₃₂: RS-8 + Potato
T₁₃: RS-4 + Green gram	T₃₃: RS-9 + Green gram
T₁₄: RS-4 + Maize	T₃₄: RS-9 + Maize
T₁₅: RS-4 + Cabbage	T₃₅: RS-9 + Cabbage
T₁₆: RS-4 + Potato	T₃₆: RS-9 + Potato
T₁₇: RS-5 + Green gram	T₃₇: RS-10 + Green gram
T₁₈: RS-5 + Maize	T₃₈: RS-10 + Maize
T₁₉: RS-5 + Cabbage	T₃₉: RS10 + Cabbage
T₂₀: RS-5 + Potato	T₄₀: RS-10+ Potato

Pathogenicity test on leaves

Pathogenicity of *R. solani* was determined by inoculating the leaves of 30 days old seedlings growing in previously steamed soil. Inoculation was done by placing a mycelial agar disc (6 mm dia.) in the center of each leaf. After inoculation, plants were covered with a plastic bag for 24 hr. PDA agar disc (6 mm dia.) without fungus was served as control. For each isolates three plants per pot were inoculated and replicated thrice. Five days after inoculation, the severity of leaf infection was rated on six point scale as mentioned above.

Treatment combinations followed to test the pathogenicity of ten (10) different isolates of *R. solani* on leaves of five (5) different crops were same as pathogenicity test on hypocotyls as mentioned above.

Results and Discussion

Cultural characteristics of *R. solani*

Colony colour, growth pattern and radial growth of ten different isolates of *R. solani* were observed. Colony colour, growth pattern and radial growth showed great diversity in all the isolates. Based on colony pigmentation, isolates were classified as

white, grey, yellow and orange. Radial growth (diameter) of *R. solani* was recorded after 72 hrs of incubation. Based on radial growth pattern, all the *Rhizoctonia* isolates were classified into three groups - abundant, moderate, and scarce.

Morphological characteristics of different isolates of *R. solani*

For morphological characters, branching pattern, septa, width of hypha and nuclei number of *R. solani* were studied on Compound microscope. Compound microscope studies revealed that all the isolates of *R. solani* characteristically having hyphal branching at right angle, septate with lateral branches constricted at the junction. Hyphal width of all the isolates of *R. solani* ranged from 5.6 µm to 7.2 µm.

Pathogenicity test of different isolates of *R. solani*

Pathogenicity test on hypocotyls

Results of cross inoculation of inoculum of all the isolates of *R. solani* (Plate 1 and 2) showed positive reaction to the hypocotyl of potato with severity score 4 (seedling growth is hampered, large lesions on the lower stem and roots). Two isolate (RS-1 and RS-2) cause

infection on all the five tested crops (rice, maize, green gram, cabbage and potato). Highest disease severity score of 5 was recorded by RS-1, RS-2, RS-3, RS-4, RS-5, RS-6, RS-9 and RS-10 on cabbage (Plate 3b, Table 1). All the infected seedlings were found dead after 15 days of its germination. Isolate RS-3, RS-5, RS-6, RS-9 and RS-10 could not able cause infection on hypocotyls of maize showing disease severity score of 0 i.e., seedling well developed, no visible lesions on lower roots developed (Plate 3b). Similarly, RS-6 to RS-10 did not cause

infection on green gram but RS-1, RS-2, RS-3, RS-4 and RS-5 cause severe infection with disease severity score of 5 causing total death of the seedlings (Plate 3a). All the ten isolates of *R. solani* cause infection on hypocotyl of potato with disease severity score of 4. Whereas in case of rice all the ten isolates though cause hypocotyls infection but with variable disease score. RS-1, RS-2, RS-3, RS-4, RS-7 and RS-8 cause disease severity score of 4, isolate RS-5 and RS-6 cause disease score of 3 and RS-9 and RS-10 cause disease severity score of 1 (Table 1).

Table1 Reaction and disease severity of different isolates of *Rhizoctonia solani* on hypocotyl of different hosts

Sources	Isolates	Rice	Maize	Green gram	Cabbage	Potato
Rice(Sheath)	RS-1	+ve (4)*	+ve (4)	+ve (5)	+ve (5)	+ve (4)
Rice(Leaf)	RS-2	+ve (4)	+ve (4)	+ve (5)	+ve (5)	+ve (4)
Cowpea(Collar)	RS-3	+ve (4)	-ve (0)	+ve (5)	+ve (5)	+ve (4)
Setaria	RS-4	+ve (4)	+ve (4)	+ve (5)	+ve (5)	+ve (4)
Greengram	RS-5	+ve (3)	-ve (0)	+ve (5)	+ve (5)	+ve (4)
Long pepper	RS-6	+ve (3)	-ve (0)	-ve (0)	+ve (5)	+ve (4)
Maize(Leaf)	RS-7	+ve (4)	+ve (4)	-ve (0)	-ve (0)	+ve (4)
Maize-T	RS-8	+ve (4)	+ve (4)	-ve (0)	-ve (0)	+ve (4)
Cauliflower-T	RS-9	+ve (1)	-ve (0)	-ve (0)	+ve (5)	+ve (5)
Cabbage-T	RS-10	+ve (1)	-ve (0)	-ve (0)	+ve (5)	+ve (4)

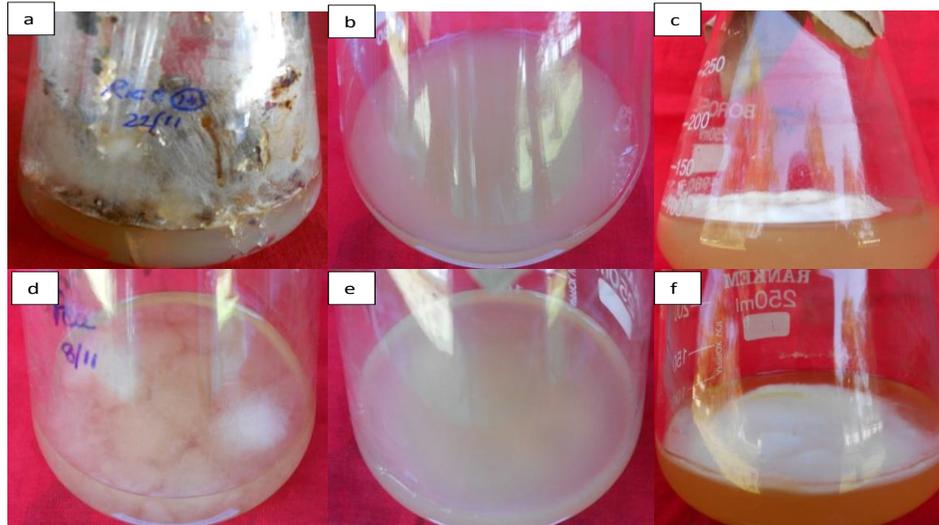
*Data are mean of three replications and data in the parentheses are disease severity score. (+ve = Infection occurred, -ve = No infection observed)

Table.2 Reaction and disease severity of different isolates of *Rhizoctonia solani* on leaves of different hosts

Source	Isolates	Rice	Maize	Green gram	Cabbage	Potato
Rice-J	RS-1	+ve(4)*	+ve (3)	+ve (3)	+ve (5)	-ve (0)
Rice-T	RS-2	+ve (4)	+ve (3)	+ve (3)	+ve (5)	-ve (0)
Cowpea(Collar)	RS-3	+ve (3)	+ve (2)	+ve (2)	+ve (5)	-ve (0)
Setaria	RS-4	+ve (3)	-ve (0)	+ve (3)	+ve (5)	+ve (3)
Greengram	RS-5	+ve (4)	-ve (0)	+ve (5)	+ve (5)	+ve (3)
Long pepper	RS-6	+ve (0)	-ve (0)	+ve (3)	+ve (5)	+ve (3)
Maize-J	RS-7	+ve (4)	+ve (4)	+ve (3)	+ve (5)	-ve (0)
Maize-T	RS-8	+ve (4)	+ve (4)	+ve (3)	+ve (5)	-ve (0)
Cauliflower-T	RS-9	-ve (0)	-ve (0)	+ve (3)	+ve (5)	-ve (0)
Cabbage-T	RS-10	-ve (0)	-ve (0)	+ve (3)	+ve (5)	-ve (0)

*Data are mean of three replications and data in the parentheses are disease severity score (+ve = Infection occurred, -ve = No infection observed)

Plate.1 (a-f) Preparation of inoculum of different isolates of *R.solani*



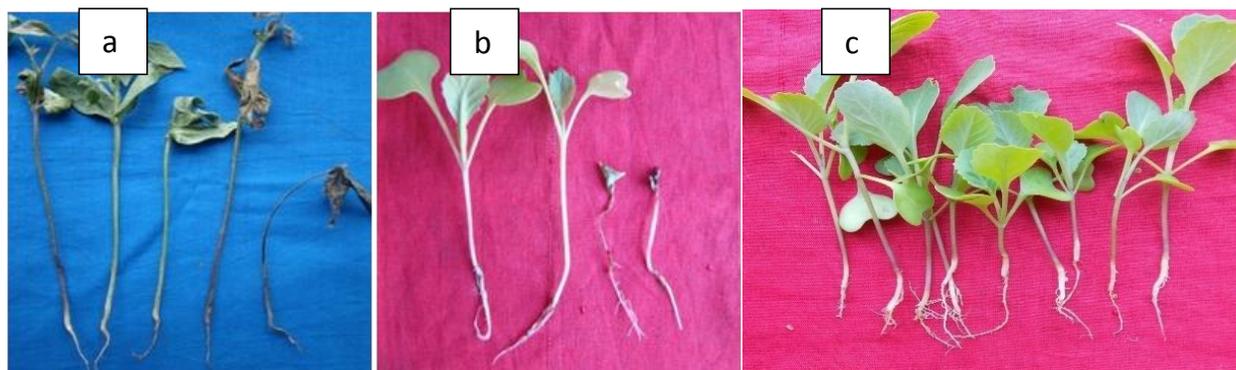
(a: RS-1, b: RS-2, c:RS-3, d:RS-4, e:RS-5, f:RS-6)

Plate. 2(a-e) General view of pot experiment for cross inoculation with different isolates of *R. solani* on different host



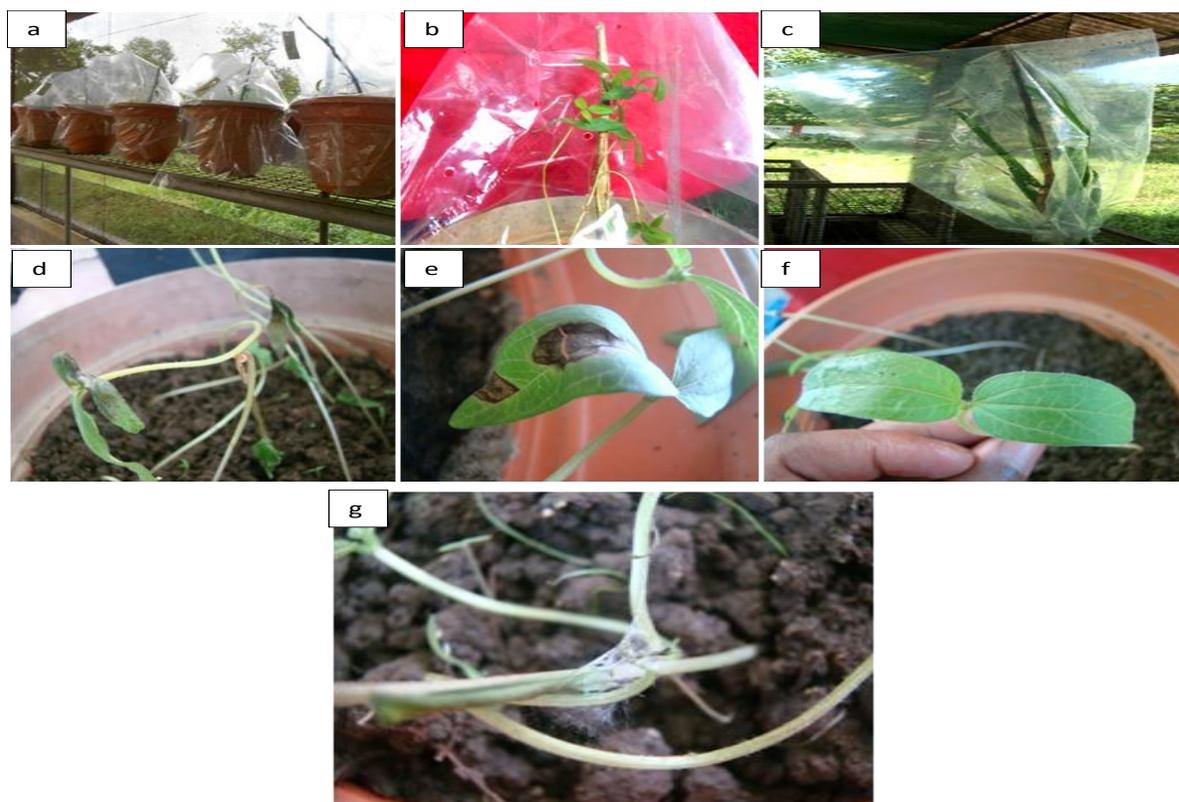
a: Green gram b: Green gram, c: Potato, d: Maize, e: Cabbage

Plate.3(a-c) Cross inoculation of different isolates of *R. solani* on



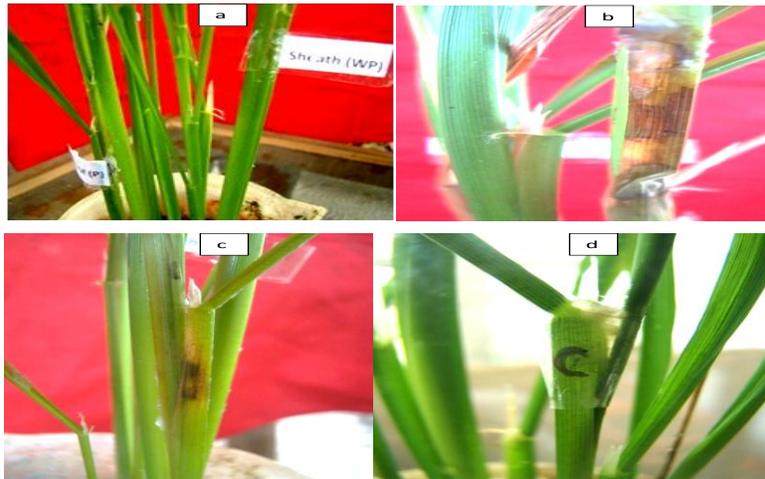
a. Green gram b. Cabbage showing the symptom c. Healthy cabbage seedling in control

Plate.4 (a-h) Cross inoculation of different isolates of *R. solani* on different crops



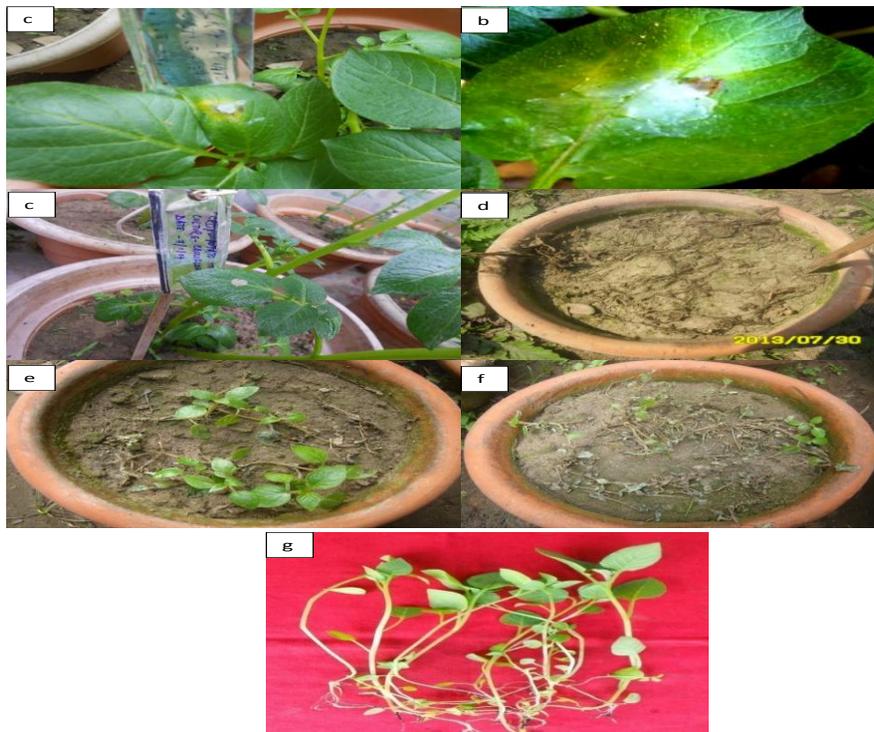
- a. General view of inoculated plant
- b. Closer view of inoculated plant covered with polybag for maintenance of humidity
- c. Inoculated leaves of rice covered with polybag for maintenance of moisture
- d. Infection on green gram leaf by RS-1
- e. Healthy green gram leaf after inoculation with RS-8
- f. Infection on green gam leaf by RS-3
- g. Infection on green gram leaf by RS-4

Plate.5 (a-d) Reactions of different isolates of *R. solani* on inoculation to rice seedlings



- a: Inoculated (and marked) leaf and sheath of rice hill
- b: Rice seedling showing symptom of infection of RS-8
- c: Infection of RS-5 on rice
- d: Control (no infection)

Plate.6 (a-g) Reactions of different isolates of *R. solani* on inoculation to potato leaves and seedlings



- (a: Pathogenicity test of RS-9 on potato, b: Infection of RS-4 on potato leaves, c: Infection of RS-5 on potato leaves, d: Infection of RS-6 on potato leaves, e: Infection of RS-8 on potato on hypocotyl, f: Infection of RS-9 on potato on hypocotyl, g: Healthy potato plant in control)

Pathogenicity test on leaves

Results of cross inoculation of all the isolates of *R. solani* showed different reaction on the leaves of five different crops (rice, maize, green gram, cabbage and potato). Disease severity score varied from 0 to 5. Highest disease severity score of 5 was recorded on cabbage for all the 10 isolates of *R. solani*. All the infected seedling were found dead after 15 days of its germination. Rice isolate RS-1 caused infection to all the tested crops (Plate 4d) except potato (Table 2). Maximum disease severity score of 5 was recorded when RS-1 tested against the leaves of cabbage whereas disease severity score 4 was recorded on rice leaves and disease severity score 3 was recorded for maize and green gram (Plate 4f). All the 10 isolates of *R. solani* caused infection on leaves of maize and cabbage. Isolates RS-1, RS-2, RS-3, RS-7 and RS-8 cause infection on all the tested crops except potato leaves. Isolates RS-4, RS-5, RS-6, RS-9 and RS-10 did not cause infection on maize. RS-9 and RS-10 did not cause infection on rice (Table 2). Isolate RS-8 did not cause infection on green gram showing healthy after its inoculation (Plate 4e), whereas isolate RS-3 caused typical symptom of *Rhizoctonia* infection on green gram (Plate 4f). RS-4 caused severe infection on inoculation to green gram which also developed aerial mycelium (Plate 4g). Isolate RS-8 on inoculation to rice developed typical symptom of *Rhizoctonia* infection (Plate 5a). Similarly, RS-5 and RS-7 also caused infection with disease severity score of 4 (Plate 5c and d). No infection was recorded in the control pot (Plate 5e). Results on pathogenicity of potato showed that RS-9 did not cause any infection of potato leaves with disease severity score 0 (Plate 6a) whereas, RS-4, RS-5 and RS-6 caused infection on potato leaves with disease severity score of 3 (Plate 6c, d and e). On the other hand, RS-7, RS-8 and RS-9 on inoculation to soil cause infection on

hypocotyls of potato with disease severity score of 4, 4 and 5 respectively (Plate 6g, e and f).

Cultural variability

All the isolates of *R. solani* showed great diversity in terms of colony colour, growth pattern and radial growth. Based on colony pigmentation isolates were assigned to four (4) groups as white, grey, yellow and orange (Debbarma and Dutta, 2015). Six isolates of *R. solani* while studying variability was observed 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 (Lal and Kandhari, 2009). On the basis of growth pattern, the isolates were categorized into three groups: abundant, moderate and slight. Similarly, Burpee *et al.*, (1980) had also grouped the growth pattern of *R. solani* in to same three groups, *viz.*, abundant, moderate and scarce.

Morphological variability

Compound microscope studies revealed that all the 10 isolates of *R. solani* characteristically having hyphal branching at right angle, septate with lateral branches constricted at the junction. A similar report was also given by Lal and Kandhari (2009) while studying on the variability of 25 isolates of *R. solani*. In their compound microscope studies they found that all the 25 isolates of *R. solani* under study was 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. Hyphal width of all the 10 isolates of *R. solani* ranged from 5.6 μm to 7.2 μm . Based on the texture, sclerotia was classified in two groups *viz.*, smooth and rough. Out of 24 isolates under study, 12 isolates were grouped in smooth category, and

remaining 12 isolates in rough category (Hoa, 1994).

Pathogenicity test

Results of cross inoculation of all the ten (10) isolates of *R. solani* showed positive reaction to the hypocotyl of potato with severity score 4 (seedling growth is hampered, large lesions on the lower stem and roots). Highest disease severity score of 5 was recorded by RS-1 to RS-6 and RS-9 and RS-10 was on cabbage. All the infected seedling were found dead after 15 days of its germination. RS-3, RS-5, RS-6, RS-9 and RS-10 could not able cause infection on maize showing disease severity score of 0 i.e., seedling well developed, no visible lesions on lower roots developed. Similarly, RS-6 to RS-10 did not cause infection on green gram but RS-1 to RS-5 cause severe infection with severity score of 5 causing total death of the seedlings. Similarly, results of cross inoculation of all the 10 isolates of *R. solani* showed different reaction on the leaves of five different crops (rice, maize, green gram, cabbage and potato). Disease severity score varied from 0 to 5. Highest disease severity score of 5 was recorded on cabbage for all the 10 isolates of *R. solani*. All the infected seedling were found dead after 15 days of its germination. Rice isolate RS-1 caused infection to all the tested crops except potato. RS-4, RS-5, RS-6, RS-9 and RS-10 did not cause infection on maize. Similarly, RS-1 to RS-3 and RS-7 to RS-10 did not showed infection on potato leaves. Few reports in the literature have shown the virulence of AG-4 isolates on roots and aerial parts of plants, but it was low to moderate in latter tissues (Olaya *et al.*, 1994). However, in another study, AG-4 failed to cause foliar symptoms but proved highly virulent on hypocotyls or roots of dry bean and soybean (Muyolo *et al.*, 1993). Disease severity was lowest in rice seedlings inoculated with isolates R3 and R5, and

highest in those inoculated with isolates R9 and R11 (Basu *et al.*, 2004). Dath (1985) also reported that rice isolates of *R. solani* with larger sclerotia were more virulent. Li *et al.*, (1998) have also described the pathogenicity of AG-4 isolates on sheaths of maize. In an study Dutta *et al.*, (2015) reported the pathogenicity

In conclusion a great diversity in the genus *Rhizoctonia* has been observed culturally, morphologically and genetically along with the wide host range of the genus. *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. Therefore, knowledge of the specific *Rhizoctonia* spp. and anastomosis groups and their pathogenicity is important. In the present studies pathogenic variability of different isolates has been observed. All the isolates did not found have the ability of infects all the tested host. Even at disease score variability has been observed. So, we suggests, further studies on anastomosis classification of multinucleate and binucleate species of *Rhizoctonia*.

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