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CULTURAL AND MORPHOLOGICAL CHARACTERIZATION OF *Rhizoctonia solani* ISOLATES ASSOCIATED WITH DAMPING OFF OF COTTON GROWN IN BANGLADESH

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ABSTRACT

A total of 50 isolates of *Rhizoctonia solani* was isolated from infected roots and stem base of diseased cotton seedlings collected from different locations throughout Bangladesh. Of them 29 were isolated from samples of Hill cotton. Out of 29 samples of Hill cotton seedlings 16 were collected from 16 locations of Bandarban district, 10 from 10 locations of Rangamati district and other three from 3 locations of Gazipur district. Rest of 21 isolates were associated with samples of 4 American cotton varieties namely CB-3, CB-5 and CB-9, CB-10 grown in the district of Gazipur, Dhaka, Rangpur, Pabna, Kushtia, Meherpur and Natore. The isolates were designated with first letter of district names and first two letters of upzilla names from where the diseased samples were collected along with a serial number. A series of experiments were conducted for characterization of 50 isolates of *R. solani* associated with cotton grown in different locations of Bangladesh. A total of 50 isolates of *R. solani* were found to infect the cotton seedling. Remarkable cultural and morphological variations were found among the isolates. Three types of mycelium and colony structures viz compact, slightly fluffy or fluffy were observed. The colony color was very light brown, light brown, brown and dark brown. Pattern of sclerotia distribution was throughout the plate, at centre, periphery and irregular.

Key words: Cotton, Damping off disease, *Rhizoctonia solani*, Characterization

INTRODUCTION

Cotton is an important industrial as well as cash crop of Bangladesh .The fungus *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*) is a ubiquitous plant pathogen, causing economically-important diseases in a wide range of tropical, sub-tropical and temperate crops. Its pathogenicity is complex. The fungus occurs throughout the world and can damage any part or all of a plant. It is known to cause seed decay, damping off, seedling blight, root rot and crown rot, as well as sorenshine and wirestem, hypocotyles cankers, bud rot, leaf spot, foliage blight and storage rots in different crops (Baker, 1970 and Mubarak, 2003). *Rhizoctonia solani* generally attacks seedlings at ground level (hypocotyls) and grows downwards into the roots. Meristimatic tissues of seedlings are more susceptible to *R. solani* than mature tissues (Bateman and Lumsden, 1995). It causes disease in a broad range of host plants. Pathogenicity studies revealed that individual *R. solani* isolates can be highly pathogenic to one or several plant species, while they are unable to infect others (Richer and Schneider, 1953; Parmeter, 1970; Anderson, 1982; Ogoshi, 1987 and Adams, 1988).

Fenille *et al.* (2002) examined 73 isolates of *R. solani* and observed that 67 were multinucleate and 6 were binucleate. Khandaker (2004) studied 60 isolates of *Rhizoctonia* and found that isolates were markedly variable on their mycelial growth and sclerotial formation. Three types of colony structure were found among the isolates. They were compact, slightly fluffy and fluffy. Off white to dark brown colony colour was observed and sclerotia were formed by most of the isolates. The sclerotia were initiated 5-60 days and the number of multinucleate nucleus ranged 3-12 /cell. Pannecoucque *et al.* (2008) studied 62 isolates of *Rhizoctonia* spp. collected from Belgian cauliflower fields. The majority of the isolates had multinucleate cells and was identified as *R. solani*. *Rhizoctonia solani* is a common soil borne pathogen in Bangladesh causing seedling diseases of various crop including cotton and its

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nature of pathogenicity is complex. Investigation need to carry out to determine the variability of the isolates of *R. solani* present in Bangladesh. Few works have been done regarding this aspect in Bangladesh. Thus the present piece of research was conducted for characterization of the isolates of *R. solani* based on cultural and morphological characters.

MATERIALS AND METHODS

A total of 50 isolates of *R. solani* was isolated from diseased samples of cotton seedlings collected from 50 locations. Pure culture of the isolates were prepared following hyphal tip methods (Tuite, 1969 and Mian, 1995) and subsequently transferred to fresh PDA slants in test tubes. Test tube slants containing pure culture of *R. solani* were stored at 4^0 C. The isolates were designated with abbreviated names of location of collection and a serial number as shown in Table 1. Pure culture of each of the 50 isolates *R. solani* was grown on PDA plates and morphological characters were recorded. The PDA was poured into 9 cm glass Petri dish @ 20 ml/dish. After solidification each plates was inoculated with a 5 mm mycelium block cut from the edge of 3 days old agar culture of individual isolates. The block was placed at the centre of each plate. The inoculated plates were incubated at room temperature (27±2°C). The inoculates plates arranged on the laboratory table following completely randomized design (CRD) with four replications. Colony characters and morphology, and sclerotial characters were recorded by regular observations of the cultures of all isolates.

Colony character: Data on colony structure and colony colour were recorded based on visual observation. The colony was indexed on a 1-3 scale, where 1= compact, 2= slightly fluffy and 3= fluffy mycelial growth. Colony color indexed on a 1-4 scale, where 1= very light brown, 2= light brown, 3= brown and 4= dark brown colony.

Characteristics of sclerotia: Days to initiation of sclerotia was recorded by regular observation of the culture plates. Number of sclerotia developed in Petri dishes of each isolate was counted at 21 days after incubation and average number of sclerotia per dish was computed. Shape and size of sclerotia were also recorded. Sclerotial size was measured by placing the sclerotia on a millimeter scale and 50 sclerotia were measured for size for each plate.

Nucleus number per cell of hyphae: To determine the status of nucleus in individual cells of each isolate, 24 hours old hyphae were stained with safranin O following standard procedures as describe by Bandoni (1979) and Sharma (2002) with slight modifications. The safranin O solution was prepared by mixing 6 ml of 0.75% (w/v) safranin O in 50% (v/v) ethanol, 10 ml of 3% (w/v) KOH, 5 ml of glycerin and 79 ml of distilled water. Each isolate was grown on glass slides in 1.5% (w/v) water agar (WA). After 24 hours of incubation, a drop of freshly prepared safranm O solutions and a drop of 3% KOH solution were placed on the growing hyphae and mixed thoroughly. A cover slip was placed on hyphal branches grown on the slides and stained with safranin O. The slides were observed under a bright-field compound light microscope and nucleus status was recorded. Well stained nuclei appeared as red bodies. The number of nuclei was counted in 100 cells in each isolate.

Mycelial growth rate: After 9 days of incubation when mycelium of some isolates completely covered the plates, radial diameter of colonies was measured. Two measures of radial colony diameter were taken at right angle and average of the two readings was considered as the diameter of the respective isolate.

To determine dry weight of mycelium, all of the isolates were grown on PDA in Petri dishes following the procedures mentioned earlier. A total of 20 ml PDA medium was poured in to a dish and inoculated with 5 mm mycelial blocks cut from the edge of a 3 days old PDA culture of each isolate. The plates were incubated in the laboratory at room temperature of 27±2°C). At the end of the incubation period, the mycelium was separated from the medium by melting the PDA in an oven at 50°C and filtering through pre weighed Whatmann No. 2 filter paper. The filter paper along with mycelium arrested on it was dried in an oven at 70°C for 16 hours. The mycelium weight was computed by subtracting the weight of filter paper from the weight of paper + mycelium.

Table 1. List of isolates of *Rhizoctonia solani* isolated from diseased cotton seedlings collected from 50 locations of Bangladesh

SL. No.	Designation of isolate	Location of sample collection		Variety of cotton
		District	Upzilla	
1	BSA-1	Banderban	Sader	Hill cotton--1
2	BSA-2	Banderban	Sader	Hill cotton--2
3	BTH--3	Banderban	Thanchi	Hill cotton--1
4	BTH--4	Banderban	Thanchi	Hill cotton--1
5	BSA-5	Banderban	Sader	Hill cotton--1
6	BSA--6	Banderban	Sader	Hill cotton--1
7	BRO-7	Banderban	Roanchari	Hill cotton--1
8	BRO-8	Banderban	Roanchari	Hill cotton--1
9	BRU--16	Banderban	Ruma	Hill cotton--2
10	BRU--17	Banderban	Ruma	Hill cotton--1
11	BRU--18	Banderban	Ruma	Hill cotton--1
12	BRU--19	Banderban	Ruma	Hill cotton--1
13	BSA--20	Banderban	Sader	Hill cotton--2
14	BSA--21	Banderban	Sader	Hill cotton--2
15	BSA-45	Banderban	Sader	Hill cotton--1
16	BSA-46	Banderban	Sader	Hill cotton--1
17	RCO--9	Rangamati	Cowkhali	Hill cotton--1
18	RCO--10	Rangamati	Cowkhali	Hill cotton--1
19	RSA--11	Rangamati	Sader	Hill cotton--1
20	RSA--12	Rangamati	Sader	Hill cotton--2
21	RCO--22	Rangamati	Cowkhali	Hill cotton--1
22	RCO--23	Rangamati	Cowkhali	Hill cotton--1
23	RSA--24	Rangamati	Sader	Hill cotton--1
24	RSA--25	Rangamati	Sader	Hill cotton--1
25	RCO--47	Rangamati	Cowkhali	Hill cotton--1
26	RSA--48	Rangamati	Sader	Hill cotton--1
27	GSR--13	Gazipur	Sreepur	Hill cotton--1
28	GSR--14	Gazipur	Sreepur	Hill cotton--1
29	GSR--15	Gazipur	Sreepur	Hill cotton--2
30	GSR--26	Gazipur	Sreepur	CB-3
31	GSR--27	Gazipur	Sreepur	CB-9
32	GSR--28	Gazipur	Sreepur	CB-5
33	GKA--29	Gazipur	Kaliakor	CB-9
34	GKA--30	Gazipur	Kaliakor	CB-9
35	PSA--31	Pabna sader	Sader	CB-9
36	KPR--32	Kustia	Pragpur	CB-9
37	KPR--33	Kustia	Pragpur	CB-9
38	KDO--34	Kustia	Doilatpur	CB-9
39	KDO--35	Kustia	Doilatpur	CB-9
40	NLA--36	Natore	Lalpur	CB-9
41	NLA--37	Natore	Lalpur	CB-3
42	NSA--38	Natore	Sader	CB-9
43	NSA-39	Natore	Sader	CB-5
44	MGA-40	Meherpur	Gangni	CB-5
45	MGA-41	Meherpur	Gangni	CB-9
46	MGA-42	Meherpur	Gangni	CB-5
47	RSA--43	Rangpur	Sader	CB-10
48	RSA--44	Rangpur	Sader	CB-10
49	DDH-49	Dhaka	Dhamrai	CB-9
50	DDH--50	Dhaka	Dhamrai	CB-9

RESULTS AND DISCUSSION

Colony character: The colony of the isolates were colorless when young (Fig. 1A) and became light brown to brown (Fig. 1B) and finally deep black (Fig. 1C) with the advancement of age. Mycelium was consisted of straight runner hyphae (Fig. 2A) and hyphae with monilioid cells (Fig. 2B). Hyphal cells

were found to be multinucleated, branching near the distal septum of cells and constriction of the branch at the point of origin (Fig. 3A). Anastomosis of hyphae was observed in aged colony (Fig. 3B). Among 50 isolates, maximum of 26 had compact mycelium, 8 had slightly fluffy mycelium and rest of 16 had fluffy isolates (Fig. 4A). Colony color was very light brown, light brown, brown and dark brown in 6, 13, and 18 and 13 isolates (Fig. 4B).

Characteristics of sclerotia: Days to sclerotia initiation varied from 4.33 to 12.00 with a mean of 6.83 and standard deviation of ±1.786. The lowest and highest days to sclerotia initiation in plates was found in isolate GSR-15 and PSA-31, respectively. Sclerotial initiation started in 12, 23, 7 and 6 isolates within 4.3 - 5.9, 5.9 - 7.4, 7.4 - 8.9, 8.9 - 10.5 and 10.5 - 12.0 days after incubation, respectively (Fig. 5A). Number of sclerotia per plate ranged 12.0 - 1760 with mean 253.35 and standard deviation of ± 311.656. Isolate RCO-47 produced 1760 sclerotia/plate whereas NLA-37 produced only 12 sclerotia per plate. Per plate sclerotia were less than 100 in 17 isolates, 100-200 in 14, 200-300 in 6, 300-400 in 5 and more than 400 sclerotia were found in another 6 isolates (Fig. 5B). The sclerotia of *R. solani*

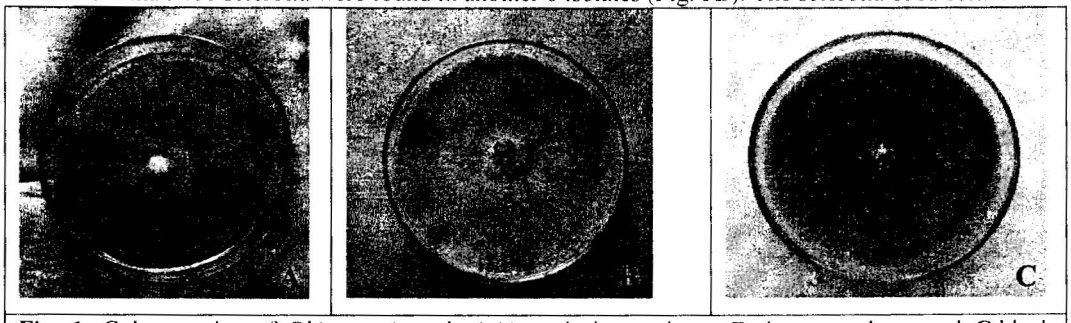


Fig. 1. Colony color of *Rhizoctonia solani* (**A**: colorless colony, **B**: brown colony and **C**:black colony)

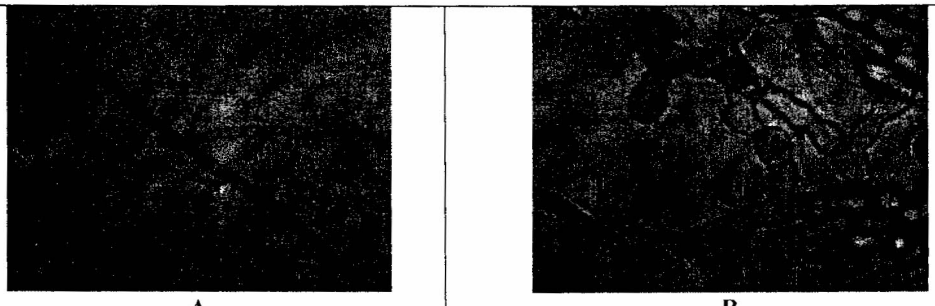


Fig. 2. Mycelium of *Rhizoctonia solani* consisted of straight runner hypha (A) and hyphae with monilioid cells (B)

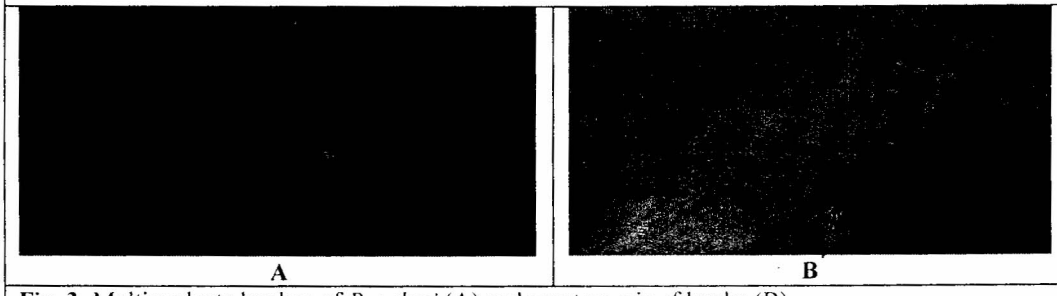


Fig. 3. Multinucleate hyphae of *R. solani* (A) and anastomosis of hypha (B)

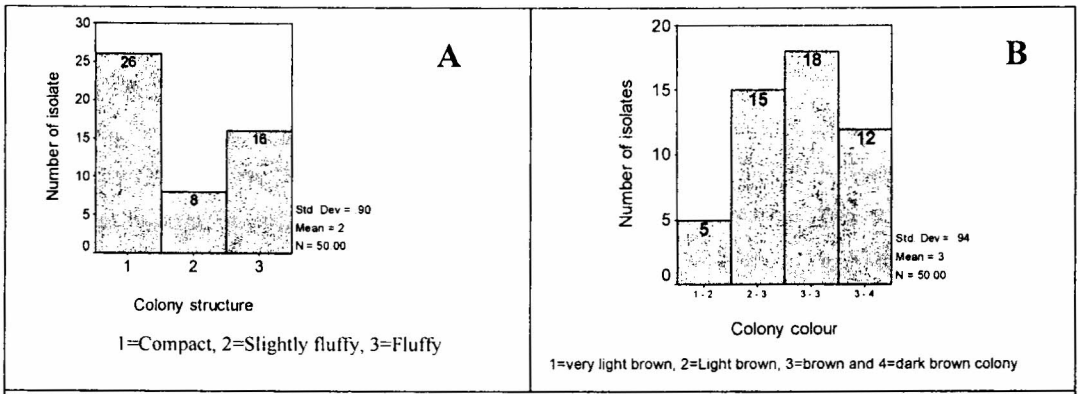


Fig. 4. Variation in colony structure (A) and colony color (B) of 50 isolates of *Rhizoctonia solani*

were very light brown in 6 isolates, light brown in 2 isolates, brown in 21 isolates and dark brown in 21 isolates (Fig. 6A). Out of 50 isolates, sclerotia of 27 were distributed throughout the plates and 15 isolates produced their sclerotia near the periphery of plates. The sclerotia was concentrated at the center of only 4 isolates and the sclerotia were irregularly distributed in Petri dishes of only 2 isolates (Fig. 6B).

Variations in size of sclerotia produced by 50 isolates were remarkable and its range was 1.0 - 2.50 mm, mean 1.67 and standard deviation 0.481 mm. The size of sclerotia was 1.0-1.3 mm in 15 isolates, >1.6-1.9 mm in 14 isolates, 1.9-2.2 mm in 12, >1.30-1.6 in 5 and 2.2-2.5 mm in 3 isolates (Fig. 7).

Nucleus number per cell of hyphae: Number of nuclei per hyphal cell varied 3.8 - 12.5 per cell with a mean of 6.78 nuclei/cell and standard deviation of ± 1.934/cell. The highest number of nuclei per cell was found in isolate RCO-22 and the lowest in KDO-35. Number of nuclei ranged 3.8-5.5, 5.5-7.3, 7.3-9.0, 9.0-10.8 and 10.8-12.5 per cell in 15, 17, 11, 6 and 1 isolates respectively (Fig. 8).

Mycelial growth rate: The rate of radial mycelium growth per day varied from 20.83 to 48.33 mm with a mean of 33.78 mm and standard deviation of ±7.397 mm. The highest growth rate was recorded in RCO-9 and the lowest rate in the isolate GSR-27. The mycelium growth rate was 20.4-26.4, 26.4-32.0, 32.0-37.6, 37.6-43.2, 43.2-48.8 mm in 7, 8, 9, 12 and 14 isolates, respectively (Fig. 9A).

Mycelium dry weight of 50 isolates of *R. solani* varied from 0.61-2.1 g/flask with a mean of 1.36 g and standard deviation of ± 0.353 g/flask. The isolate BSA-5 yielded the maximum mycelium dry weight and the isolate KD-35 yielded the minimum mycelium dry weight. Out of 50 isolates, 4 yielded 0.6-0.9,

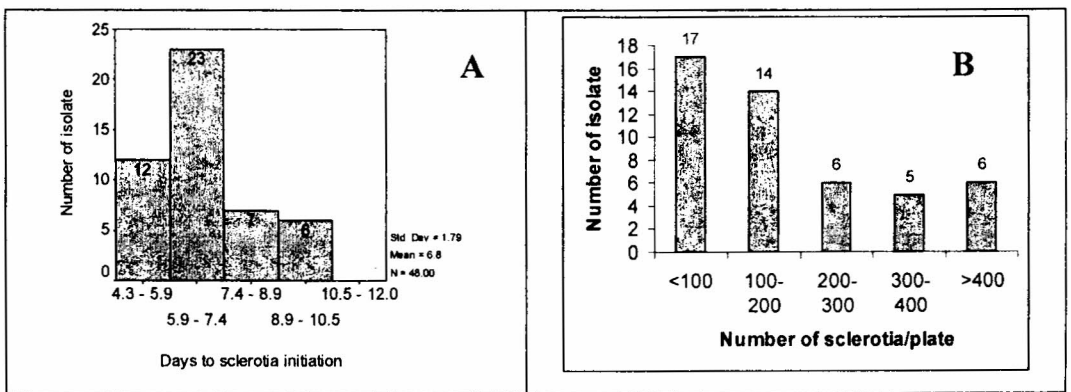


Fig. 5. Variation in days to sclerotium initiation (A) and sclerotium production (B) in 50 isolates of *R. solani* infecting cotton roots

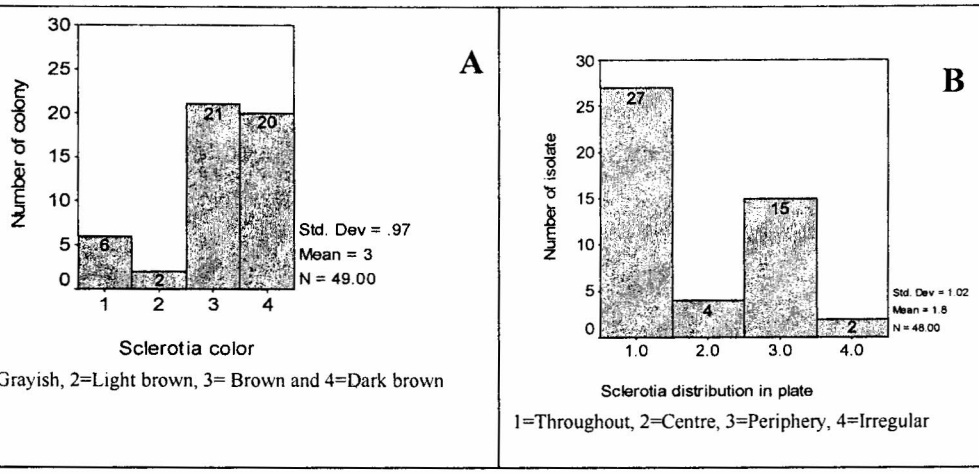


Fig. 6. Variation in color (A) and distribution sclerotia produced (B)by 50 isolates of *R. solani* infecting roots cotton seedling

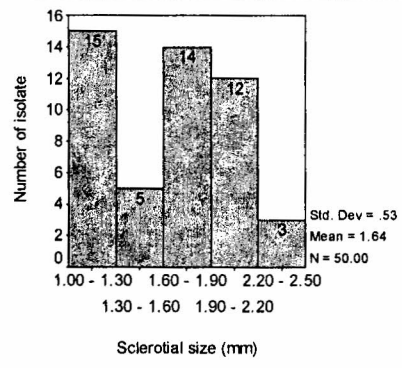


Fig.7. Variation in sclerotia size produced by 50 isolates of *R. solani* infecting roots cotton seedling

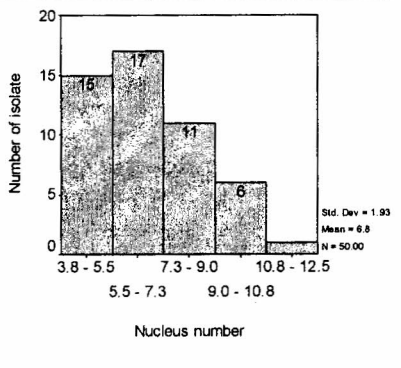
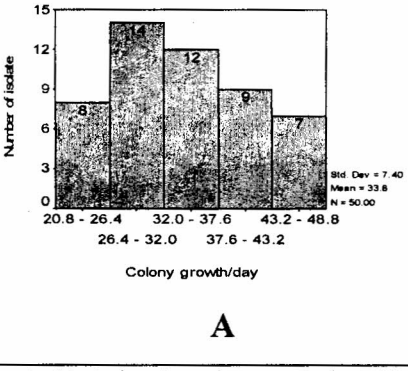


Fig. 8. Variation in number of nucleus per cell of 50 isolates of *R. solani* from cotton roots



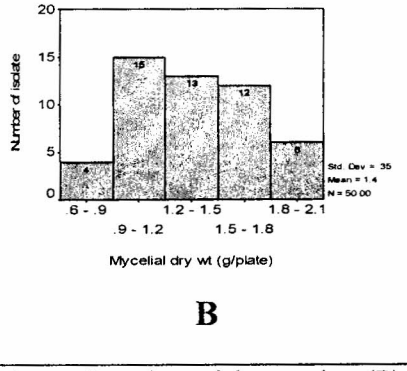


Fig. 9. Variation in colony growth rate per day (A) and mycelium dry weight per plate (B) of 50 isolates of *R. solani* infecting cotton roots

15 yielded 0.9-1.2, 13 yielded 1.2-1.5, 12 yielded 1.5-1.8 and rest of 6 isolates yielded 1.8-2.1 g mycelium dry weight per flask Fig.9B).

Results of the present investigation reveal that there are remarkable variations in colony and cultural characters, mycelium growth, sclerotium characters and number of nucleus/cell of hyphae among 50 isolate of *R. solani* from Bangladesh. Based on those characteristics the isolates were classified into five groups. Investigators from other countries reported similar variations in the isolates of the pathogen isolated from cotton and other crops. Monga and Raj (1994) studied 13 isolates of *R. solani* from cotton plants. On the basis of cultural characters, they divided the isolates into four groups. Nelson *et al.* (1996) collected 98 isolates of *R. solani* from roots and stems of soybean and classified into four anastomosis groups. Seventeen isolates produced few to abundant, white to dark brown or black, small to large sclerotia generally in the middle of the colony (Sharma *et al.*, 2005). Chand and Logan (1983) classify 182 isolates of *R. solani* isolated from tubers of various potato cultivars and found a wide variation in cultural characteristics. They found that the pattern of sclerotial formation being the most stable and well defined character of each isolate and was used to divide them into 6 groups. Among the groups, the mycelial growth of group VI was slower and hyphal diameter was smaller than that of the other groups.

Carling *et al.* (1986) tested 27 plant species against *R. solani* anastomosis group-3 and found characteristic stem lesions on potato and necrotic roots on hairgrass. A superficial discoloration was observed on the roots and crown of several other species. Dark hyphae and selerotia were observed on the crowns of several other species. Carling *et al.* (1994) collected isolates of *R. solani* anastomosis group 11 (AG-11) in Western Australia and Arkansas. Some isolates from each location fruited on 1.5% water agar, 2% V8-juice agar, and soil overlay plates and produced sexual structures typical of *Thanatephorus cucumeris*. Mycelium of isolates of AG-11 growing on potato-dextrose agar was white to light tan when young, but became brown to dark brown with age. Concentric rings of dark and light mycelium were visible in most cultures. Mature sclerotia were tan to light brown and were scattered over the agar surface. Isolates of AG-11 were auxotrophic for thiamine. Fenille *et al.* (2002) worked on seventy three *R. solani* from soybean and observed that nuclear number among *Rhizoctonia* isolates obtained from soyabean seedlings causing hypocotyls rot and foliar blight, sixty seven were multinucleate and six were binucleate. The binucleate cell contained 1.8 to 2.3 nuclei /cell and multinucleate cell contained 8 to 23 nuclei/cell. Pannecoucque *et al.* (2008) studied on sixty-two isolates of *Rhizoctonia* spp. which were collected from Belgian cauliflower fields during 2005 and 2006. The majority of the isolates (60 out of 62) had multinucleate cells and was identified as *R. solani*.

Report from Bangladesh indicates the cultural and morphological variations in isolates of *R. solani* isolated from crops other than cotton. Goswami (2002) studied fifty isolates of *R. solani* from disease samples of different crop fields of Bangladesh and characterized the isolates based on the cultural and morphological characters. The growth rates of different isolates of *R. solani* varied within the range of 2.1-5.2 cm/24 hours. Mycelia of most of the isolates were fluffy, slightly fluffy and compact, colour of the colonies was light brown to blackish brown, production of sclerotia /cm^2 also varied among the isolates from 8.0 to 85.0 and color of the sclerotial in most of the isolates were grayish to brown and few produced creamy to yellowish sclerotia. Distribution of the sclerotia on the plates differed within the isolates and based on sclerotial distribution isolates were grouped into five categories.

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