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Mycelial growth inhibition of *Rhizoctonia* by indigenous medicinal plant extract

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Abstract

Pure cultures of four different isolates were established from the infected rice sheath collected different areas of Mymensingh District on the basis of different morphological characteristics including shape, colour (upper side and reverse side), sclerotial shape, sclerotial colour and compactness. The efficacy of six plant extracts viz. Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Shetodron (*Leucas aspera*), Mahogoni leaf (*Swietenia mahagoni*), Mahogoni seed(*Swietenia mahagoni*) and Bishkatali (*Polygonum hydropiper*) was investigated against different the four isolates of *Rhizoctonia* sp. The radial mycelial growth of the fungus from 2-7 days was significantly differed among the different treatments. At 4 day after culture colony diameter was reached maximum (4.00 cm) in PDA plates of non-treated control treatment. Average colony diameter of *Rhizoctonia* sp. from 2-7 days after culture ranged from 0 cm to 4.00 cm. The highest colony diameter (4.00 cm) was recorded in control at 4 days after culture. At 4 days after culture, highest percent inhibition of radial mycelial growth over contril was found for Isolate 1 by Garlic (97.50%), Neem (96.75%), and Biskatali (94.25%); for Isolate 2 by Bishkatali (93.25%); for Isolate 3 by Bishkatali (95.75%), Mahogoni seed (85.75%) and Garlic (85.00%), and for Isolate 4 by Bishkatali (95.25%) and Shetodron (89.25%). Among the plant extracts, Bishkatali was highly effective to suppress mycelial growth of all isolates of *Rhizoctonia* sp.

Key words: Rice, sheath blight, *Rhizoctonia*, mycelial growth, plant extract

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Introduction

Rice (*Oryza sativa*) is one of the leading food crops and the most widely cultivated cereal crop in the world. Bangladesh is one of the major rice producing countries (IRRI, 2015) in the world. Based on production of milled rice in 2016-2017, Bangladesh was the fourth leading country with the production volume of 34581 million metric tons behind the China (Mainland), India and Indonesia (Statisticia, 2015). The average yield of the year 2013 of rice was 4.3755 ton/ha, which was much lower than the yield in Japan (6.728 ton/ha), China (6.7249 ton/ha), Vietnam (5.573 ton/ha), Brazil (5.006 ton/ha) and Indonesia (5.152 ton/ha) as mentioned by FAOSTAT (2015).

There are several constrains of rice production and its low yield in Bangladesh. Among these disease, sheath blight is considered as the major one. More than 60 diseases attack the rice crop every year among which sheath blight ,bacterial leaf blight, stem rot and blast are considered important diseases at various parts of rice growing areas of the world (Latif *et al.* 2011). Sheath blight of rice occurs in all rice producing areas of the world (Ou, 1985; Savary *et al.* 2006) and may

cause up to a 50% decrease in the rice yield under favorable conditions around the world (Zheng *et al.* 2013). Sheath blight caused 14-17% yield loss in different varieties during Aus, Aman and Boro seasons in Bangladesh (Shahjahan *et al.* 1986). Sheath blight caused by *Rhizoctonia solani, Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae, Rhizoctonia zeae* and *Sclerotium hydrophilum* distributed worldwide and cause yield loss in rice growing countries is a major disease (Oniki *et al*.1985). It spreads in epidemic form in Bangladesh (Shahjahan *et al.* 1986).

Application of fungicides is the major measure for controlling sheath blight of rice for over three decades in Asian countries (Zhang *et al.* 2013). However, extensive and continuous use of a single chemical may lead to undesirable effects such as residual toxicity and environmental pollution, and also increases the risk of resistance development (Brent and Hollomon, 1998). Therefore, it is necessary to develop environmentally friendly, low residual and effective alternative methods for the management of the disease. Screening of natural products of plant origin, either directly as crude preparations or as pure compounds for plant disease control, is getting much attention due to easy biodegradability, low toxicity and minimum residues in the agro-ecosystem (Aye and Matsumoto, 2011).

During recent years, use of plant extracts for controlling plant diseases is gaining importance due to their antifungal and antibacterial properties. Few plant extracts are reported to inhibit the germination of the fungal spores and mycelial growth (Ahmed *et al.*, 2013). Therefore, the present study was undertaken to study the different isolates of *Rhizoctonia* sp. isolated from rice plants of Mymensingh district, and to evaluate the efficacy of some plant extracts against *Rhizoctonia* sp. in terms of mycelial growth.

Materials and Methods

The experiment was conducted at the Microbiology and Biocontrol Laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. The experiments were carried out during July/2014 to September/2015.

***Isolation and Identification of pathogen*:** Pathogen, *Rhizoctonia* sp. was isolated from infected tillers of rice sheath in a sterilized moist chamber. Specimens having typical symptom of sheath blight of rice were collected from the rice field of the BAU farm and different villages of Mymensingh Sadar. Rice stem having blighted sheath was cut into 10 cm long pieces and placed in sterilized table tissue papers moistened by sterilized distilled water. The pieces of infected sheath were placed on the wet tissue and were kept in humid chamber by maintaining moist condition. After observing white sclerotial growth, sclerotia were transferred to PDA media and pure culture was established. The fungal isolates were indentified observing the morphological and microscopic characteristics described by Liner and Carling, (1994) and Telmadarrehei *et al*. (2011). The pure cultures of different isolates of *Rhizoctonia* sp. are presented in Fig. 1. Morphological characters include colony colour, colony growth (shape), compactness etc.

***Treatments*:** Seven treatments were checked in PDA media as 10% solution of plant extracts. These are: Treatment 1 = Control (Non-treated), Treatment 2 = Garlic (*Allium sativum*) clove extract, Treatment 3 = Neem (*Azadirachta indica*) leaf extract, Treatment 4 = Shetodron (*Leucas aspera*) leaf extract, Treatment 5 = Mahagoni (*Swietenia mahagoni*) leaf extract, Treatment 6 = Mahagoni seed extract and Treatment 7= Bishkatali (*Polygonum hydropiper*) leaf extract. Preparation of PDA containing plant extracts was done in two steps. In first step, clean peeled slice potato tubers of 200 g were boiled in 500 ml of water. Water was sieved from boiled potato slices, and 20g dextrose and 20 g agar were added to it. In second step, 100g plant parts were used for preparing 500 ml extract by cutting and blending in sterilized distilled water. Finally two solutions were mixed to get one liter 10% solution of plant extract in PDA. The content was

autoclaved and was poured into petridishes for subsequent study.

***Inhibition of mycelia growth of Rhizoctonia sp.*:** Growth inhabition was recorded by the modification of food poisoning technique of Monjil *et al.*(2013). Inocula of *Rhizoctonia* sp. was transferred by cutting block (1.10 cm in diameter) from a full growth culture by a block cutter. Radial mycelia growth was measured daily by a measuring scale. Effect of plant extract was calculated as percent growth inhibition using the following formula reported by Satish *et al. (*2007); Dubey *et al.* (2009).

Growth inhibition **(%)** = $\frac{C-T}{C} \times 100$

Where **C** = Growth in the untreated portion
T = Growth in the treated portion

***Design of experiment and Data analysis*:** Laboratory experiment was done using Completely Randomized Design (CRD) with three replications. The data on different parameters were statistically analyzed by using analysis of variance (ANOVA) technique to find out the level of significance (Gomez and Gomez, 1984). The effect of the treatments was compared by Duncan's Multiple Range Test (DMRT). The collected data were analyzed using a statistically computer package (MSTATC).

Results and Discussion

***Morphological study of Rhizoctonia sp. grown in PDA media*:** Morphological study of four isolates of *Rhizoctonia* sp. isolated from rice sheath grown in PDA media is presented in Table 1 and Figure 1. Morphological characteristics including shape, colour (upper side and reverse side), sclerotial shape, sclerotial colour and compactness were studied for the four isolates. Isolate 1 produce regular shaped compact mycelia with off-white upper side and yellowish-white reverse side. Sclerotia were smaller and round, white in young stage and grey in mature stage. Isolate 2 produced regular shaped slightly loose mycelia with creamy upper side and off-white reverse side. Sclerotia were bigger and round, white in young stage and grey in mature stage. Isolate 3 produced regular shaped loose mycelia with creamy upper side and off-white reverse side. Sclerotia were bigger and round, white in young stage and black in mature stage. Isolate 4 produced regular shaped loose mycelia with grey upper side and blackish-grey reverse side. Sclerotia were bigger and round, white in young stage and black in mature stage. Morphological study of four isolates are shown in Figure 1. These findings are consistent with data obtained from previous studies of Debbarma and Dutta (2015) reported morphological variability were found in different isolates of *Rhizoctonia* sp. including colony size, colony growth, colour and sclerotia formation (ring at periphery, peripheral or scattered) and texture (smooth or rough). Shan *et al*. (2002) studied cultural morphology and variation, which was found among different isolates including colony colour, growth rate, shape and colony appearance. Moni *et al*. (2015) observed significant variation among different isolates of *Rhizoctonia* sp. in sclerotial size, shape and distribution.

Rhizoctonia sp. causes sheath blight of rice can be controlled by using different kinds of control agents including chemical, bio-agents and plant extracts. Chemical control of disease was quite popular for reducing crop losses. But at present use of chemical for the control of disease is being discouraged for health hazard and environmental pollution (Ahmed *et al.* 2013, Aye and Matsumoto 2011). It is necessary to consider non-toxic and eco-friendly approaches. Several researchers have reported on the fungicidal and bactericidal effects of plant extracts on specific plant pathogens (Dubey *et al*., 2009; Joseph *et al*., 2008). Sehajpal *et al.* (2009) studied the effect of plant extracts against *Rhizoctonia* sp. causing sheath blight of rice. In the present experiment efficacy of different plant extracts against *Rhizoctonia* sp. was evaluated.

***Evaluation of some plant extracts on the radial mycelial growth of Rhizoctonia sp.*:** Evaluation of some plant extracts on radial growth of *Rhizoctonia* sp.

at 1-7 days after culture is presented in Figs. 2 (A, B, C & D). In case of Isolate 1, significant differences of radial mycelial growth of the *Rhizoctonia* sp. were observed in culture after 2-7 days of growth. Colony diameter at two days after culture ranged from 0.03 to 0.57 cm. The lowest colony diameter (cm) was recorded in Biskatali (0.03) and Garlic (0.03) followed by Shetodron (0.07) and Neem (0.10). Statistically similar results were observed in Garlic and Biskatali. The highest colony diameter (cm) was recorded in Mahogoni leaf (0.57).

Table 1. Morphological study of *Rhizoctonia* sp. grown in PDA media

Isolate	Shape	Colour		Sclerotial shape	Sclerotial colour	Compactness
		Upper side	Reverse side			
Isolate 1	Regular	Off white	Yellowish white	Small and round	White in young stage and grey in mature stage	Compact
Isolate 2	Regular	Creamy	Off white	Bigger and round	White in young stage and grey in mature stage	Slightly loose
Isolate 3	Regular	Creamy	Off white	Bigger and round	White in young stage and black in mature stage	Loose
Isolate 4	Regular	Grey	Blackish Grey	Bigger and round	White in young stage and blackish in mature stage	Loose

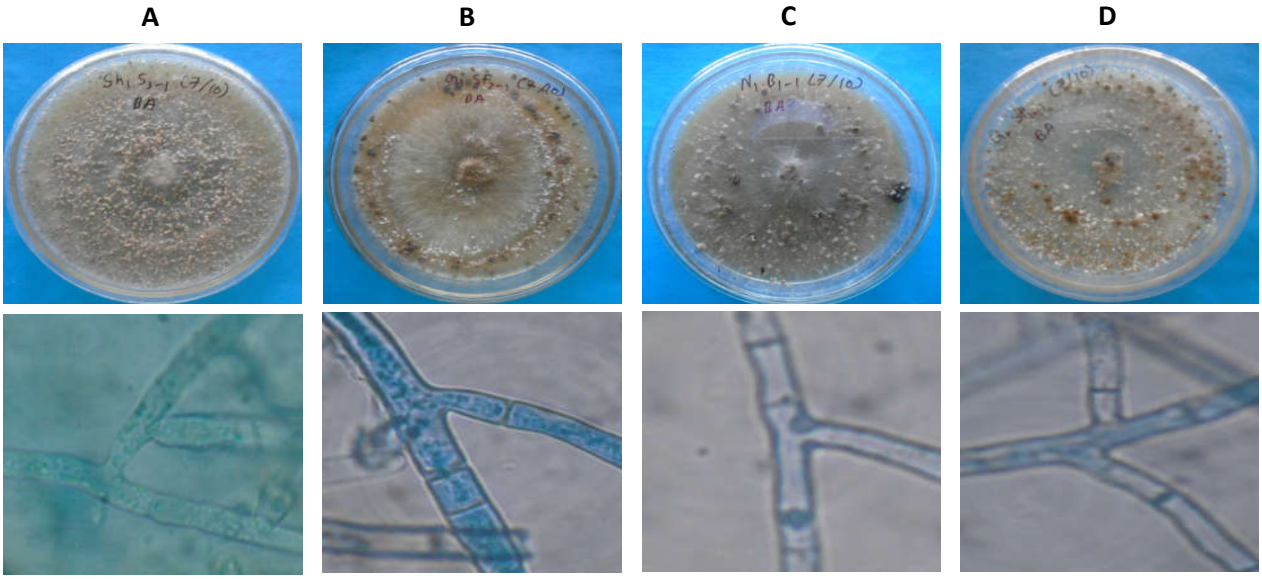

Figure 1. Morphological characters of *Rhizoctonia* sp., A, Isolate 1; B, Isolate 2; C, Isolate 3 and D, Isolate 4. Upper panel showing the mycelial growth and sclerotia production on PDA, lower panel showing mycelial structures under a compound microscope (100×

Three days after culture average colony diameter (cm) ranged from 0.03 to 3.23 cm. The lowest colony diameter (cm) was recorded in Garlic (0.03) followed by Bishkatali (0.07). The highest colony diameter (cm) was recorded in Mahogoni leaf (3.23). Four days after culture average colony diameter ranged from 0.10 cm to 4.00 cm. The lowest colony diameter (cm) was recorded in Garlic (0.10) followed by Neem (0.13) and

Bishkatali (0.23). The highest colony diameter (cm) was recorded in control and Mahogoni leaf (4.00, full).Control PDA plates became full due to mycelium growth. Up to seven days similar trends of mycelial growth were recorded. At seven days after culture, the lowest colony diameter (cm) was recorded in Neem (0.37) followed by Garlic (0.4) Shetodron (0.5) and Bishkatali (0.73). The highest colony diameter (cm) was recorded in control, Mahogoni leaf and Mahogoni seed. In case of Isolate 2, colony diameter at two days after culture ranged from 0.07 to 1.8 cm. The lowest colony diameter (cm) was recorded in Biskatali (0.07) followed Garlic (0.2), Shetodron (0.23) and Neem (0.63). The highest colony diameter (cm) was recorded in Mahogoni leaf and Mahogoni seed (1.8). From 3-7 days after culture, lowest micelial growth was observed Biskatali extracts followed by Garlic. On the other hand highest mycelial growth was observed in Mehogoni leaf and Control treatments. In case of Isolate 3, colony diameter at two days after culture ranged from 0.07 to 0.77 cm. The lowest colony diameter (cm) was recorded in Biskatali (0.07) followed by Garlic (0.1). The highest colony diameter (cm) was recorded in control (0.77). Three days after culture average colony diameter (cm) ranged from 0.10 to 3.03 cm. The lowest colony diameter (cm) was recorded in Bishkatali (0.1) followed by Garlic (0.4) and Mahogoni seed (0.4). The highest colony diameter (cm) was recorded in Mahogoni leaf (3.03). Similar trends of mycelial growth were observed up to 7 days study. Mycelial growth reached full plate (4.00 cm) at 4 day after culture in Control and Mehogoni leaf treatments. In case of Isolate 4, colony diameter at two days after culture ranged from 0.07 to 1.43 cm. The lowest colony diameter (cm) was recorded in Garlic (0.07) followed by Mahogoni seed (0.17), Shetodron (0.17) and Biskatali (0.17). The highest colony diameter (cm) was recorded in Mahogoni leaf (1.43). From 3-7 days, similar trends of mycelial growth was observed, where lowest mycelial growth was found in Biskatali treatment followed by Shetodron. At seven days after culture highest Colony diameter (cm) reached to full plate (4.00 cm) recorded in Control, Mahogoni leaf, Mahogoni seed, Neem and Garlic treatment at six days after culture. The findings are consistent with data obtained from previous studies of Sehajpal *et al.* (2009), who reported the effect of several plant extracts against *Rhizoctonia* sp. causing sheath blight of rice. Ashrafuzzaman and Khan, (1992) reported that extract of Allamanda (*Allamanda cathertica*), Meheadi (*Lawsonia alba*) and Duranta (*Duranta plumeiri*) inhibited mycelial growth and sclerotial formation of *Rhizoctonia solani* effectively. Plodpai *et al.* (2013) reported the anti-*Rhizoctonia* activity by *Desmos chinensis* extracts and its mechanism of action. Ahmed *et al.* (2013) reported that extract of Garlic, Allamanda, Neem, Chirata and Bishkatali inhibited mycelial growth and sclerotial formation of *Rhizoctonia.* Reddy *et al.* (2002) reported that efficacy of plant extracts and chemicals on the mycelial growth and sclerotial production of *Rhizoctonia* sp. the pathogen of sheath blight of rice. The findings are consistent with data obtained from previous studies of Islam (2014) reported that Durba, Bishkatali, Thankuni, Nishinda and Tulsi reduce the mycelial growth and sclerotia production of *Rhizoctonia* sp. The extracts of *Allium sativum*, *Cymbopogon nardus* and *Nigella saliva* showed better result in controlling the mycelia growth and selerotial formations of *Rhizoctonia* and its pathogenecity to rice plants.

Inhibition of radial mycelial growth over control: Inhibition of radial mycelial growth of *Rhizoctonia* sp. over control at 4th days after culturing was calculated and presented in Table 2. In case of Isolate 1, percent inhibition of mycelial growth in the PDA containing Garlic extract (T_2) was 97.50%, Neem (T_3) was 96.75, Bishkatali (T_7) was 94.25% and Shetodron (T_4) was 93.25%. Mahogoni leaf (T_5) and Mahogoni seed (T_6) extracts was not effective for inhibiting mycelial growth of *Rhizoctonia* sp. In Isolate 2 highest inhibition of mycelial growth of over control at 4th days after culturing was recorded in the PDA containing Bishkatali extract (T_7=93.25%) followed by

T_3 (53.25%). Mahogoni leaf (T_5), Mahogoni seed (T_6) and Shetodron (T_4) extracts exerted little or no inhibition of mycelial growth over control. In case of Isolate 3, highest percent inhibition of mycelial growth was recorded in the PDA containing Bishkatali extract (T_7=95.75%) followed by Mahogoni seed (T_6=85.75%) and Garlic (T_2=85.00%). Mahogoni leaf (T_5), Neem (T_3) and Shetodron (T_4) extracts were not effective for inhibiting mycelial growth of *Rhizoctonia* sp.

Table 2. Inhibition of radial mycelial growth over control

Treatments	Colony diameter (cm) at 4 days after culture				Percent (%) inhibition over control			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 1	Isolate 2	Isolate 3	Isolate 4
T_1	4.00	4.00	4.00	4.00	-	-	-	-
T_2	0.10	3.50	0.60	2.57	97.5	12.50	85.00	35.75
T_3	0.13	1.87	4.00	3.30	96.75	53.25	0.00	17.50
T_4	0.27	3.53	3.40	0.43	93.25	11.75	15.00	89.25
T_5	4.00	4.00	4.00	3.90	0.00	0.00	0.00	2.50
T_6	3.67	3.43	0.57	3.70	8.25	14.25	85.75	7.50
T_7	0.23	0.27	0.17	0.19	94.25	93.25	95.75	95.25

T_1= Control, T_2 =Garlic, T_3 = Neem, T_4 = Shetodron, T_5 = Mahogoni leaf, T_6= Mahogoni seed and T_7 = Bishkata

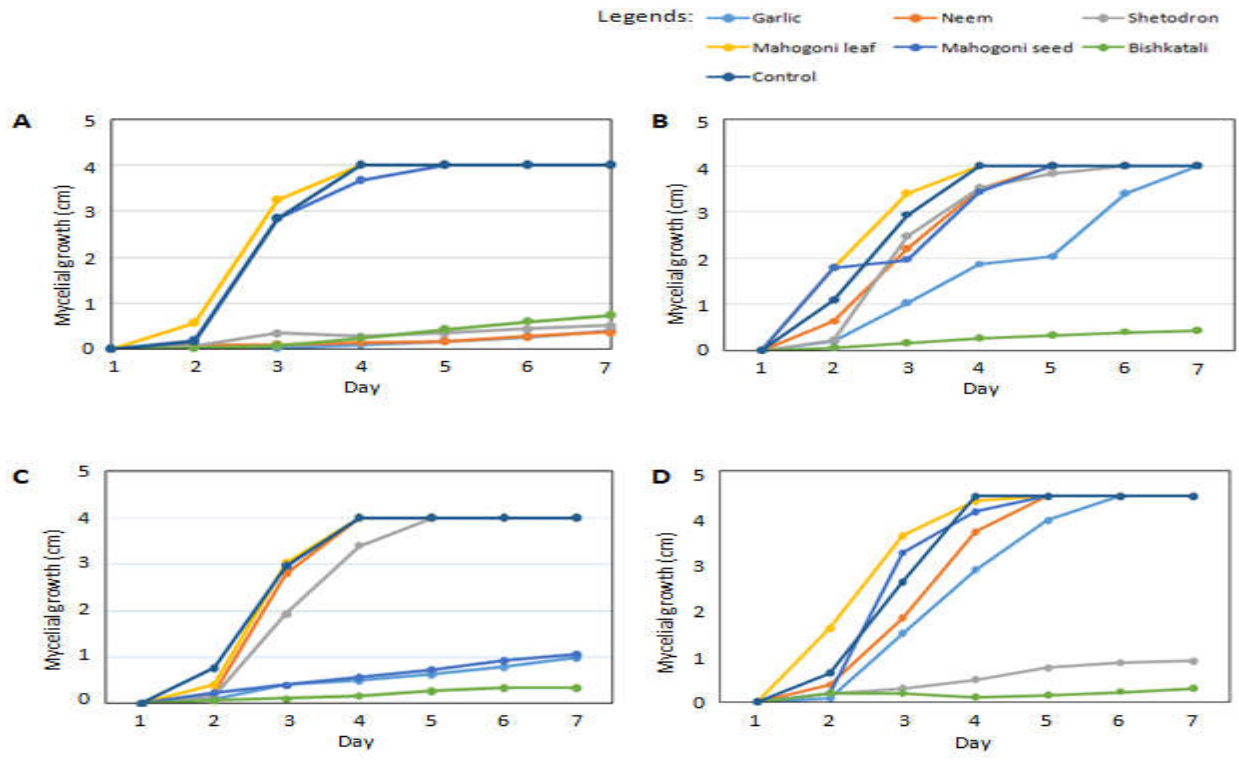

Figure 2. Inhibition of sclerotia production of *Rhizoctonia* sp. over control of Isolate-4 at 10 days of culturing, A, Isolate 1; B, Isolate 2; C, Isolate 3 and D, Isolate 4.

In Isolate 4, highest percent inhibition of mycelial growth was recorded in the PDA containing Bishkatali extract (T_7=95.25%) followed by Shetodron (T_4=89.25%). Mahogoni leaf (T_5), Mahogoni seed (T_6) and Neem (T_3) extracts were not effective for inhibiting mycelial growth of *Rhizoctonia* sp.

Plant extracts are being explored as safer alternative to synthetic chemicals for crop protection against diseases. Using plant extracts as bio-pesticide offers grower unique benefits such as generally herbal products does not have longer residual toxicity. Most of the product shows wide window of crop safety and resistance to these compounds is not developed as quickly as with synthetic fungicide due to its multiple mode of action. However, these plant extracts may therefore reduce the use of chemical fungicides.

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