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Effect of Different Media and pH on Mycelial Growth of *Sclerotium oryzae* Causing Sheath Rot of Rice

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ABSTRACT

Sclerotium oryzae causing sheath rot of rice, is one of the most important pathogen causing heavy crop losses in all the major rice growing areas of the world, including Bangladesh. The growth and reproduction of this soil borne pathogen depends on growing media and abiotic factors. Therefore, to evaluate the effect of different culture media and pH on mycelial radial growth of *S. oryzae,* the experiment was conducted in the Plant Pathology Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh. Performance of different media, carbon source and nitrogen source on radial mycelial growth was differed significantly. The maximum mycelial radial growth was observed on rice stem agar medium (70.40 mm) and minimum mycelial radial growth was recorded on cornmeal agar medium (50.60 mm). Considering carbon source, the maximum radial mycelial growth was found in glucose (66.40 mm) amended medium and no statistical differences were observed among sucrose, starch and Czapek dox agar containing medium. Based on nitrogen source, maximum radial mycelial growth was found in peptone medium (69.40 mm) but no statistical differences were exposed between Potassium nitrate (KNO_3) and Sodium nitrate ($NaNO_3$). pH- 6 played a vital role in radial mycelial growth (73.75 mm) of *S. oryzae*.

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INTRODUCTION

Rice (*Oryzae sativa* L.) is one of the important staple food crops for more than 60% of the world's population. Over 90% of the rice produced in the world is consumed in the Asian countries. India is the number one rice producing country in the southwest Asia with an area of 43.5 million hectares where the annual production is 893.1 million metric tons, and it ranks second to China in production in the world perspective (CMIE Report, 2010). It is also the staple food of Bangladesh, but yield of this crop is comparatively lower than that of the neighboring countries. At present the total annual rice production of our country is approximately 34.71 million metric tons and the average yield is around 2.88 mt ha^{-1} (BBS, 2016) while the world's total production is 497.8 million metric tons (FAO, 2016).

Among the major yield limiting factors diseases are most important. Therefore, minimizing the losses caused by disease is the most important approach to increase productivity. Sheath rot disease of rice caused by *Sclerotium oryzae* (Perfect state - *Magnaporthe salvinii* Krause and Webster, conidial state - *Nakataea sigmoidea* Cav.) has been one of the major limiting factors in rice production (Bedi, 1953; Ghose et al., 1960). The disease was recorded in all rice growing region worldwide (Ou, 1972 and 1985). The pathogen causes 5–80% losses in grain yield in different parts of the world (Al Heeti and El - Bahadli, 1982; Li et al., 1984; Ou, 1985; Cother and Nicol, 1999).

A survey was conducted during 1979 - '81 in different region of Bangladesh where 20 rice diseases and sheath rot was recorded (Miah et al., 1985).

In the recent time sheath rot disease was recorded on Kalijira rice at the ground level of tillers and 57% tillers were infected due to this disease. Frequency of association of *S. oryzae* was 54% (Shamsi et al., 2010).

Symptoms are usually seen at the later growth stages. Necrotic lesions begin on the outer leaf sheath near the water line, these spread gradually to the inner sheaths and the stem base. At maturity lodging may occur and sclerotia found within the tissues (Ou, 1985).

The sclerotium is nearly spherical, smooth, black, shiny and measuring 200 to 350 µ in diameter, visible to the naked eyes as black dots. The sclerotial stage of fungus (*S. oryzae*) is commonly found on paddy and some other wild grasses (Pandey, 2012). The mycelial growth and sclerotia formation of this fungus is influenced by many factors including pH, temperature, individual nutrients and volatile compounds like ethanol and C:N ratio (Devi *et al*. 1999; Linderman and Gillbert, 1973).

These pathogens exhibit variation in their morphological, biological, immunological characteristics and pathogenicity in different environmental conditions. The growth of *Sclerotium rolfssi* favoured mostly by peptone as nitrogen sources and then by KNO_3 (Azhar *et al*., 2003). The main purpose of this research was to estimate the effect of different culture media and pH on some physiological parameters and mycelial radial growth of *S. oryzae*.

MATERIALS AND METHODS

Collection of Disease Sample

Typical symptom of sheath rot disease was collected from

greenhouse of Plant Breeding and Biotechnology Laboratory, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh.

Isolation, Purification, Preservation and Identification of Fungus

The fungus was isolated following standard procedures of Dhingra and Sinclair (1985). To obtain pure culture of *S. oryzae*, a hyphal tip from water agar was transferred aseptically to PDA petridish by using a sterile fine needle and then incubated at a temperature of 25±2 ^{0}C for 52 hours. Advanced hyphae were transferred aseptically into the test tube slant containing PDA with pH 6.5 and incubated at room temperature (25±2 ^{0}C) for 52 hours. After incubation, these slants were carefully checked for contamination and then preserved at 4 ^{0}C in a refrigerator for further use. Fungal isolates were identified based on the characteristics of hyphae and sclerotia (Lilly and Barnett, 1951).

Pathogenicity Test of *S. oryzae*

Pathogenicity test was done on excised rice stem with sheath. Healthy stem of rice were cut into pieces (about 1 inch) and surface sterilized with 70% ethanol for 10 seconds. After that cut pieces were placed on sterilized blotting paper for the removal of excess ethanol present in the stem. Stem pieces were injured softly by flame sterilized pointed needles and then placed onto sterilized water agar. Advanced hyphae were cut from 30 hours old pure cultures aseptically with the help of cork borer and then placed at three injured places (both ends and centre) onto the stems (Dhingra and Sinclair, 1985). Then typical sheath rot symptoms were observed on sheath and re-isolation was done following the protocol of Dhingra and Sinclair, 1985.

Preparation of Different Growth Media

The various media composition (g L^{-1}) shown below were tested.

Potato dextrose agar (PDA)

PDA was prepared following the standard procedure (Anonymous, 1968). Potato 200 g, dextrose 20 g, agar 15 g, distilled water 1L.

Rice stem agar and Betel vine stem agar

20 g rice stem / betel vine stem was taken and pasted with the help of mortar-pestle by adding 5 ml distilled water for preparing the paste. After that 995 ml distilled water was added slowly in to the paste. It was then sieved in to a beaker; 20 g dextrose and 15 g agar added with it.

Cornmeal agar

20 g cornmeal extract, 20 g dextrose, 15 g agar, 1L distilled water.

Preparation of Different Carbon and Nitrogen Sources Media

Czapek dox agar medium

2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 30 g Sucrose and 15 g agar, 1L distilled water.

Carbon sources

Glucose (13.5 g L^{-1}), Sucrose (12.5 g L^{-1}) and Starch (12.5 g L^{-1}) were tried individually as a constitute of carbon source in Czapek dox Agar medium.

Nitrogen sources

Three carbon compounds viz; Peptone (2.5 g L^{-1}), Sodium nitrate (8.5 g L^{-1}) and potassium nitrate (10 g L^{-1}) were tried individually as a constitute of nitrogen source in Czapek dox agar medium.

Preparation of Media at Different pH

Five different pH levels namely 5.0, 6.0, 7.0, 8.0 and 9.0 were taken as treatments. Hundred (100) ml PDA was taken in 250 ml conical flask. Different pH were adjusted by adding either 0.1N HCl or 0.1N NaOH and measured by pH meter. All the media were sterilized at 121^{0}C temperature, 15 PSI for 15 min. After cooling, the media were poured in to 90 mm petridishes.

Inoculation and Incubation

All these treatments were replicated into five plates. Advanced hyphae of 3 days old culture was used for inoculation. A 5 mm block of the mycelium was cut with flame sterilized cork borer (5 mm). The mycelial blocks were taken from the edge of the colony. Each mycelial block was placed upside down at the centre of each petridish. All these operation were done under aseptic condition. The inoculated petridishes were kept in the growth chamber at (25±2 ^{0}C) until the mycelia touch the edge of petridishes.

Measurement of the Mycelial Growth of the Fungus

Mycelial growth of the isolates was measured by averaging the two dimensions (90 mm petridish) taken for each colony after six days of inoculation for different growth media, carbon source, nitrogen source and three days for pH.

Experimental Design and Data Analysis

The experiment was laid out under completely randomized design (CRD) with three replications. The data were analyzed statistically using STAR (statistical tools for agricultural research) program, version-02, IRRI, Los Baños, Philippines computer program.

RESULTS

Pathogenicity of *S. oryzae*

After performing a pathogenicity test typical sheath rot symptoms were found on stem (Figure 1).

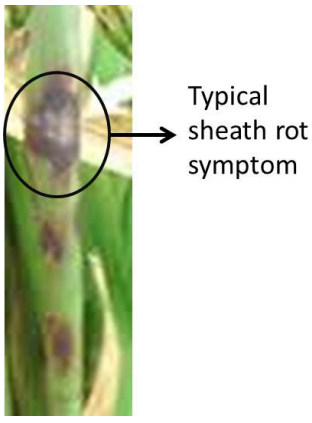

Figure 1. Sheath rot symptoms on rice stem

Effect of Different Culture Media on Mycelial Growth of *S. oryza*e

In the present investigation, growth of the pathogen *S. oryzae* was significantly different ($P \leq 0.01$) in different culture media. The most effective medium for the growth of the fungus was rice stem agar which showed 70.40 mm diameter colony growth of the *S. oryzae* at four days after incubation and the lowest colony growth was recorded in cornmeal agar medium (50.60 mm) (Figure 2). However, radial mycelial growth on PDA (57.00 mm) and betel vine stem agar medium (58.00 mm) was statistically similar.

Colony Characteristics of *S. oryzae* on Different Media

Colony characteristics of *S. oryzae* on different culture media presented in Table 1. Some color variations were found in upper and lower surface. The upper surface color of rice stem agar was pink but lower surface was pinkish, in PDA medium upper surface color was whitish while the lower surface color was reddish. However grayish color was noticed in both surfaces of betel vine stem agar medium and both whitish color in cornmeal agar medium. Irregular colony margin was found in rice stem agar and betel vine stem agar medium while regular margin existed in PDA and cornmeal agar medium. Loose colony texture was found in rice stem agar, betel vine stem agar and cornmeal agar medium but velvety and compact colony texture was observed in PDA medium. In case of hyphal thickness, rice stem agar, betel vine stem agar and cornmeal agar medium had thin hyphal thickness but thick hyphal growth was noticed in PDA medium.

Effect of Different Carbon Sources on Mycelial Growth of *S. oryzae*

In Czapek dox agar medium carbon component was replaced with glucose, sucrose and starch. The results indicated that all the carbon sources vary significantly at $P \leq 0.01$ in their effect on the colony growth of the *S. oryzae* (Figure 3). However, glucose was found to be the best carbon sources and showed maximum colony growth (66.40 mm).

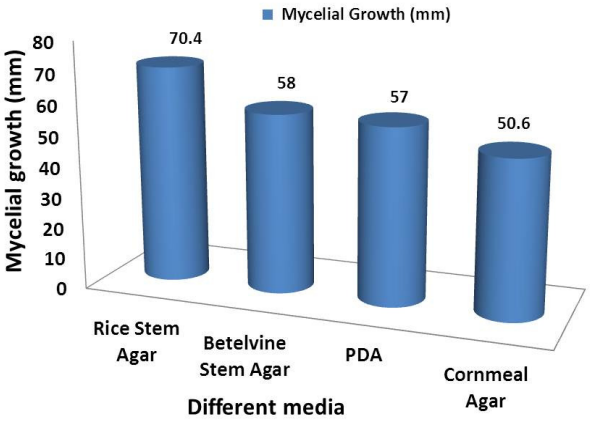

Figure 2. Effect of different media on mycelial growth

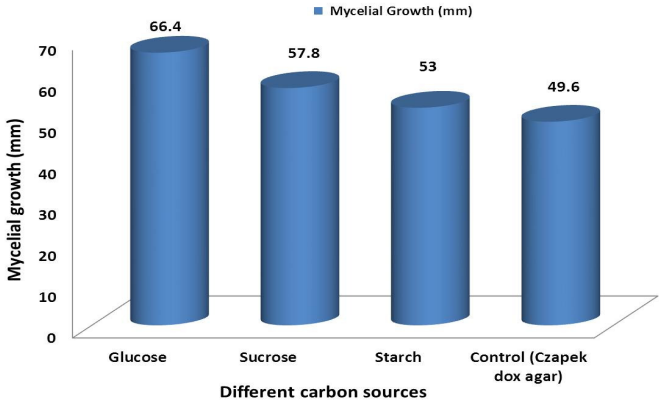

Figure 3. Effect of different carbon sources on mycelial growth

Table 1. Colony characteristics of *S. oryzae* on different media

Growth media	Surface	Color	Margin	Texture	Hyphal Thickness
Rice Stem Agar Medium	Upper	Pink	Irregular	Loose	Thin
	Lower	Pinkish			
PDA Medium	Upper	Whitish	Regular	Velvety and	Thick
	Lower	Reddish		compact	
Betel vine Stem Agar Medium	Upper	Grayish	Irregular	Loose	Thin
	Lower	Grayish			
Cornmeal Agar Medium	Upper	Whitish	Regular	Loose	Thin
	Lower	Whitish			

Effect of Different Nitrogen Sources on Mycelial Growth of *S. oryzae*

The Nitrogen sources showed significant effect ($P \leq 0.01$) on colony growth of *S. oryzae*. Maximum growth of the pathogen (69.40 mm in diameter) was obtained with Peptone used as nitrogen source in the media. However, there was no statistical difference in mycelial growth of *S. oryzae* between KNO_3 (50.60 mm) and $NaNO_3$ (48.80 mm) containing media. Organic nitrogen was responsible for vigorous mycelial growth of *S. oryzae* (Figure 4).

Effect of Different pH Level on Mycelial Growth of *S. oryzae*

S. oryzae showed significant variations ($P \leq 0.01$) on different pH level. From regression equation (Figure 5), a significant negative linear relationship was observed between the different pH level and mycelial growth. However, when the pH level rises up to 9 radial mycelial growth decreased. The maximum mycelial growth was recorded at pH 6.0 (73.75 mm) which was statistically similar with pH 5.0 (70.75 mm). The lowest value was found at pH 9.0 (45.75 mm). However pH 7.0 and pH 8.0 was statistically closely related to each other.

DISCUSSION

In this experiment the most effective supporting medium for the growth of the fungus was rice stem agar. Kumar et al. (2003a) multiplied *S. oryzae* on a 2:1 mixture of rice and rice husk, typha (*Typha angustata*) pieces, rice stem pieces of susceptible cultivar PR 106 and rice grain husk + sand medium. In another experiment Kumar et al. (2003b) multiplied *S. oryzae* inoculum on rice grain husk + sand medium produced maximum disease (62%) under pot conditions. Shamsi et al. (2010) indicated that *S. oryzae* frequently forms sclerotia on PDA medium. Misra and Mohammad (1964) recorded good growth and sclerotial production of the fungus when multiplied on sterilized soil–oats or sand–maize medium (soil 95 g, crushed oats or maize 5 g) and potato dextrose agar. Amin (1976) recorded good growth of *S. oryzae* on peptone-sucrose-agar medium. The study indicated that more growing media could be tested to identify the most suitable medium for the growth of *S. oryzae*.

Glucose was found to be the best carbon sources and showed maximum colony growth (66.40 mm). But different results were obtained by Naz *et al.* (2012). She reported that sucrose was the most appropriate sources of carbon for obtaining maximum colony growth of the pathogen. The fungus may utilize certain simple form of complex carbon compounds into simple, which may be readily metabolized (Bais *et al*., 1970). On the other hand, monosaccharides i.e., glucose was reported to be the most suitable carbon supplement for colony growth, biomass production and sporulation of *C. heterostrophus (*Kumar and Rani, 2009).

The organic nitrogen was responsible for vigorous mycelial growth of *S. oryzae*. Evans and Black (1981) also found greater dry weight in organic nitrogen sources than inorganic ones while studied the effect of 23 organic and 3 inorganic nitrogen sources on growth, sporulation and polyphenol oxidase activity in *Bipolaris maydis.* Similar observations were made by Azhar *et al.* (2003) for the mycelial study of *S. rolfsii.* Various researchers have studied the effect of C : N on fungal growth (Merida and Roria, 1994; Elson *et al.,* 1997; Engelkes *et al*., 1998).

The maximum mycelial growth was recorded at pH 6.0. Azhar *et al.* (2003) also observed the tested pH levels (5 to 8) were found equally suitable for growth of *S. rolfsii*. Farooq *et al.* (2005) also studied the most suitable pH level for growth of *Fusarium oxysporum* f. sp. *ciceri.* was 7.0 and 6.0. The study has been supported by Mahen *et al*. (1995) who noticed that the growth occurs over a wide range of pH (1.4 - 8.8).

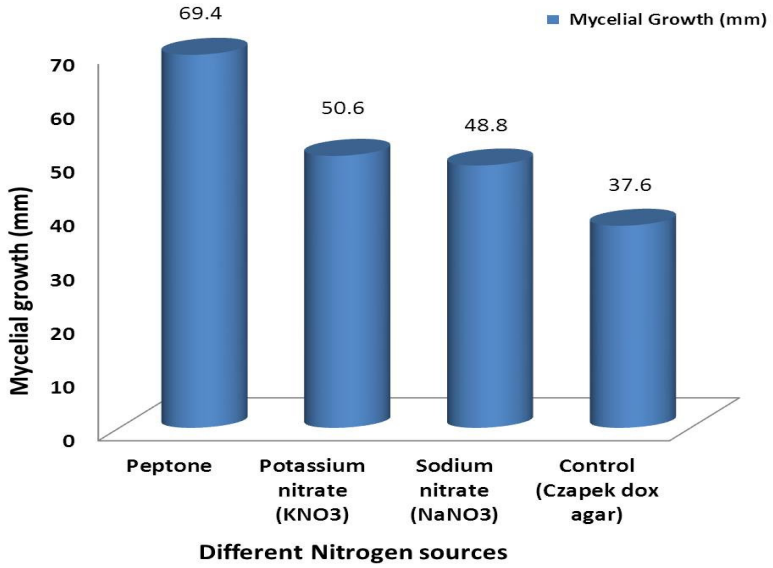

Figure 4. Effect of different nitrogen sources on mycelial growth

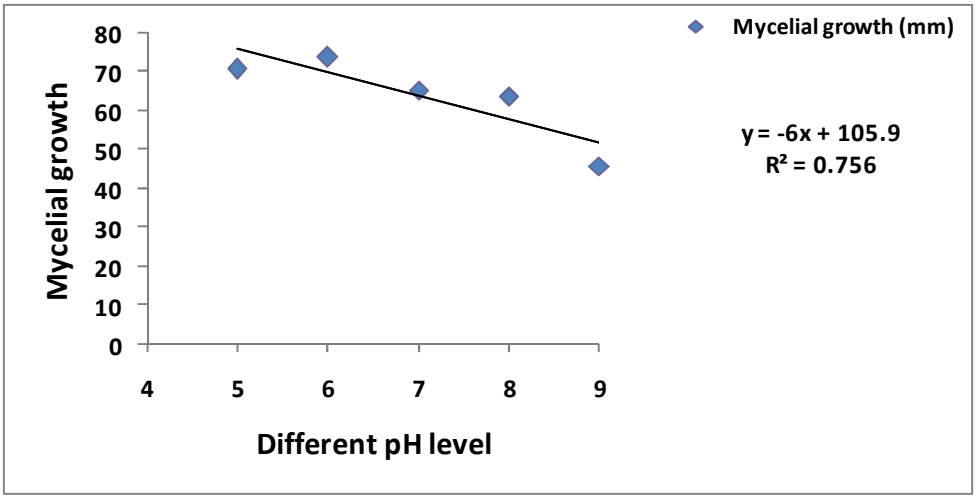

Figure 5. Functional relationship between different level of pH and radial mycelial growth of *S. oryzae*

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