Role of nutrient sources and photoperiodic conditions on colony morphology, growth and sclerotial development in *Rhizoctonia solani*

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Colony morphology, growth and formation of sclerotia in *Rhizoctonia solani* was studied under different photoperiodic conditions on different carbon and nitrogen sources. The growth of mycelia and sclerotia were found to be significantly affected by duration of light and carbon/ nitrogen ratio in the media. Mycelial growth was found to be fastest under nitrogen rich condition with oat meal as source of carbon and nitrogen. Whereas formation of sclerotia was highest on carbon rich conditions with high carbon nitrogen ratio on potato and dextrose as sources of nutrients. Colony morphology was loose when oat meal was the carbon/nitrogen source but with potato dextrose and malt extract as sources of carbon/nitrogen it was compact. Under carbon and nitrogen deficient condition colony growth was dispersed. Among different photoperiods highest growth of colony and fastest development of sclerotia was observed under alternating light and dark conditions on various nutrient sources. Continuous dark condition showed less colony growth and produced even lesser number of sclerotia than continuous light condition.

Key words: Carbon, nitrogen, colony growth, mycelia, nutrient source, photoperiods, *Rhizoctonia solani*, sclerotia

INTRODUCTION

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris*), a wide spread necrotrophic fungal pathogen, causes devastating diseases in a wide range of plants (Basu *et al*. 2016). Mycelial growth and development of sclerotia in many fungi are greatly affected by different biotic and abiotic factors (Ritchie *et al.* 2009; Dutta *et al*. 2012; Mondal *et al*. 2019; Koley *et al*. 2019).

Light and nutrient sources are two important criteria among abiotic factors that have a huge impact on mycelial growth of *Sclerotinia minor* and *Hyaloperonospora arabidopsidis* (Osato *et al*. 2016, Telli *et al*. 2020). According to some earlier reports, different colours of light have been found to increase formation of sclerotia in fungi like *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* (Quadri and Fatima 2017). On the contrary, there are some reports of blue light suppressing the formation of sclerotia in *Botrytis cinerea* and *Verticillium albo-atrum* (Mishra *et al*. 2014). Dutta *et al*. (2012) have reported that different intensities of light can affect development of sclerotia in *R. solani*. In a previous study from our lab we have showed the effect of different light intensity on branching pattern in hyphae and germination of sclerotia of *R. solani* (Koley *et al*. 2019).

In earlier studies, status of nutrient sources and different culture conditions have been found to affect growth of *Myrothecium roridum(Iqbal et al. 2021), Metarhizium anisopliae (Rangel et al. 2008), Cordyceps militaris (Shih et al. 2007), Rhizopus stolonifer (Amiri et al. 2011), Hypholoma fasciculare* (Harold *et al*. 2005). In another study from our lab it was shown that availability of nutrients enhanced germination of sclerotia and also continuous light increased number of sclerotia of *R. solani* (Mandal *et al*. 2019). But there is no report on impact of carbon and nitrogen sources and different photoperiodic conditions on diameter of colony, colony morphology and formation of sclerotia of *R. solani*. In this present study attempts

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have been made to analyze the role of carbon and nitrogen contents from different sources on growth and morphology of *R. solani* colony under different photoperiodic conditions.

MATERIALS AND METHODS

Fungal material

Rhizoctonia solani Kühn AG1-1A isolate (obtained from Rice Research Station, Chinsurah, West Bengal, India) was used in the experiment. This fungus was grown in potato dextrose agar (PDA) medium at a temperature of 30 ± 2^0C. Mature sclerotia obtained from a fully grown PDA plate were used for further study.

Experimental conditions

Harvested sclerotia were inoculated on four growth media with different sources and content of carbon and nitrogen viz. high carbon and low nitrogen with potato and dextrose as source of carbon and nitrogen (potato dextrose agar), high carbon and high nitrogen with malt as source of carbon and nitrogen (malt extract agar), low carbon and high nitrogen with oat meal as source of carbon and nitrogen (oat meal agar) and carbon and nitrogen deficient (agar) and grown under three different photoperiods viz. 24 hours continuous dark period (24DD), 24 hours continuous light period (24LL), and alternating 12 hours light and 12 hours dark periods (12/12LD).

Study of growth of R. solani colony

For this study, this fungus was grown up to 9 days under different photoperiodic conditions on different nutrient sources. Growth of *R. solani* colony on the plates was observed and analyzed periodically in terms of diameter of colony from germinated sclerotia.

Analysis of formation of sclerotia under different photoperiodic conditions and on different carbon and nitrogen sources

R. solani was grown on different carbon and nitrogen sources under different photoperiodic conditions viz. 24DD, 24LL and 12/12LD at room temperature for 9 days. Formation of sclerotia in those plates was observed and number of sclerotia formed was documented.

RESULTS AND DISCUSSION

Differential growth of R. solani colony on different sources of carbon and nitrogen under different photoperiods

In the present study, we have observed the role of different photoperiodic conditions on colony morphology and growth of *R. solani* on different sources and amount of carbon and nitrogen.

Growth of *R. solani* colony was observed under different photoperiodic conditions viz. 24DD, 24LL, 12/12LD at 2 days, 4 days and 9 days post inoculation (dpi). At 2 dpi, highest colony growth was found when grown under alternating light dark periods (diameter of colony 3.75cm). Whereas, lesser colony growth was observed under continuous light conditions (diameter of colony 3.3cm) and continuous dark conditions (diameter of colony 2.3cm) at 2dpi. At 5dpi, diameter of colony was 9cm under all photoperiodic conditions. Thus our study revealed that alternating light and dark periods were found to be most suitable for growth of *R. solani* colony as alternating light dark conditions significantly increased colony growth than continuous dark and light conditions. Koley *et al.* (2019) have shown that light intensity significantly affected branching of hyphal of *R. solani*.

We found that nitrogen rich and lower carbon conditions had maximum impact on growth of colony. Growth of *R. solani* was found to be highest (diameter of colony 3.9cm) when oat meal was used as source of carbon and nitrogen having high nitrogen content and low carbon content (Fig.1). Whereas, diameter of colony was 3.8cm, and 2.6cm (Table 1) under lower nitrogen and higher carbon content with potato dextrose (Fig.2) and malt extract as sources of carbon and nitrogen respectively (Fig. 3). Under nitrogen and carbon deficient condition growth was lowest (diameter of colony 2.1cm) (Fig. 4). So, nitrogen rich conditions were found to be more suitable for growth of *R. solani*. Growth of fungal colony observed in different carbon and nitrogen sources as follows: oat meal as source > potato dextrose as source > malt extract as source > carbon and nitrogen deficient condition.Itoo and Reshi (2013) reported that differential growth of ectomycorrhizal fungal species was observed different carbon and nitrogen sources and concentrations. They found

Table 1 Diameter of *Rhizoctonia solani* colony at 2 days post inoculation on different contents of carbon and nitrogen from different sources under different photoperiodic conditions viz. 24 hours continuous dark (24DD), 24 hours continuous light (24LL) and 12 hours of alternate light-dark (12/12LD) periods.

Carbon and Nitrogen Content and Sources	Diameter of colony (cm) under different photoperiodic conditions		
	Continuous Dark 24DD	Continuous Light 24LL	Alternate Light-Dark 12/12LD
High Carbon Low Nitrogen (Potato dextrose)	6.4cm	7.8cm	8.8cm
High Carbon High Nitrogen (Malt Extract)	3.2cm	6.2cm	6.2cm
Low Carbon High Nitrogen (Oat meal)	6.2cm	8.4cm	9cm
Carbon Nitrogen Deficient	2.4cm	4.2cm	6.2cm

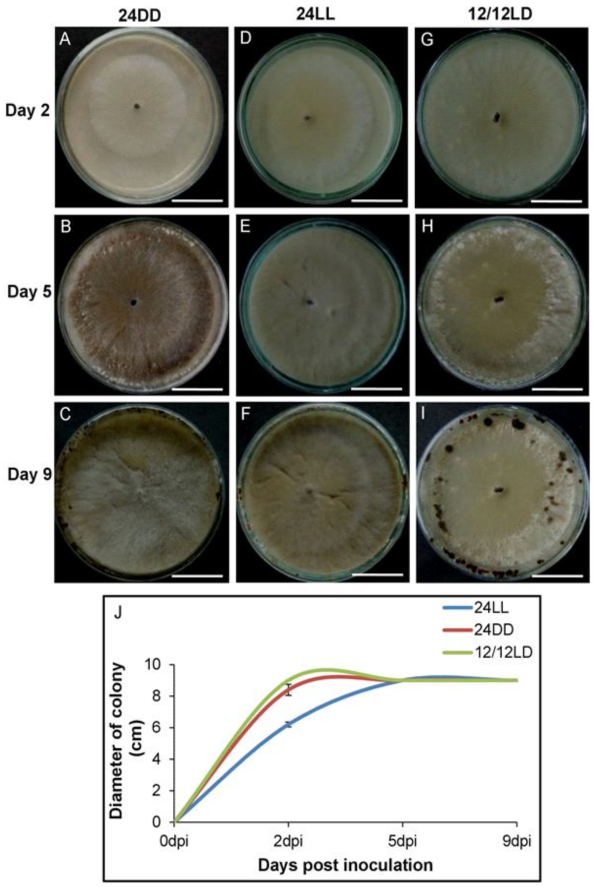

Fig. 1: Colony morphology of *Rhizoctonia solani* and formation of sclerotia on low carbon low nitrogen condition with oat meal as nutrient sources under different photoperiodic conditions.(A-C) Under 24 hours continuous dark periods (24DD), (D-F) under 24 hours continuous light periods (24LL), (G-I) under alternating 12 hours light and 12 hours dark periods (12/12LD), (J) graphical representation of diameter of colony under 24DD, 24LL and 12/12LD conditions. Bar = 3cm.

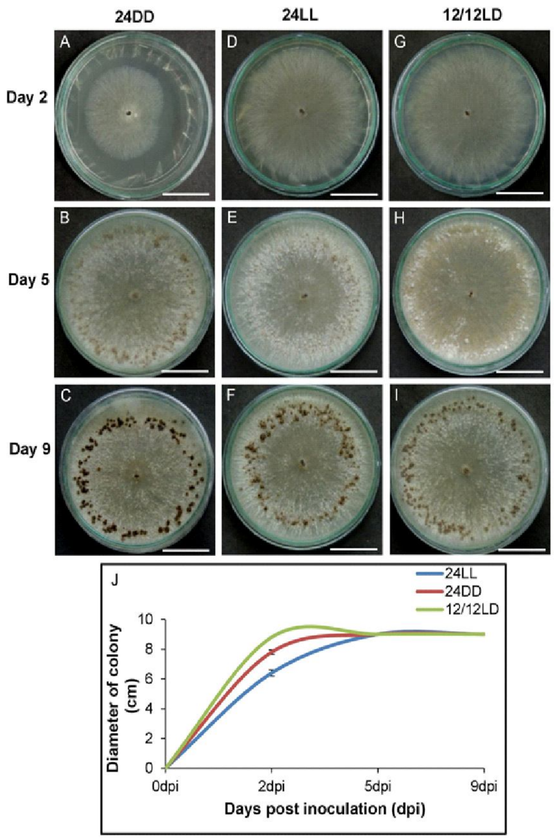

Fig. 2: Colony morphology of *Rhizoctonia solani* and formation of sclerotia on high carbon low nitrogen condition with potato dextrose as nutrient sources under different photoperiodic conditions, at 2nd, 5th and 9th day post inoculation (A-C) Under 24 hours continuous dark periods (24DD), (D-F) under 24 hours continuous light periods (24LL), (G-I) under alternate 12 hours of light and dark periods (12/12LD), (J) graphical representation of diameter of colony under 24DD, 24LL and 12/12LD conditions. Bar = 3cm.

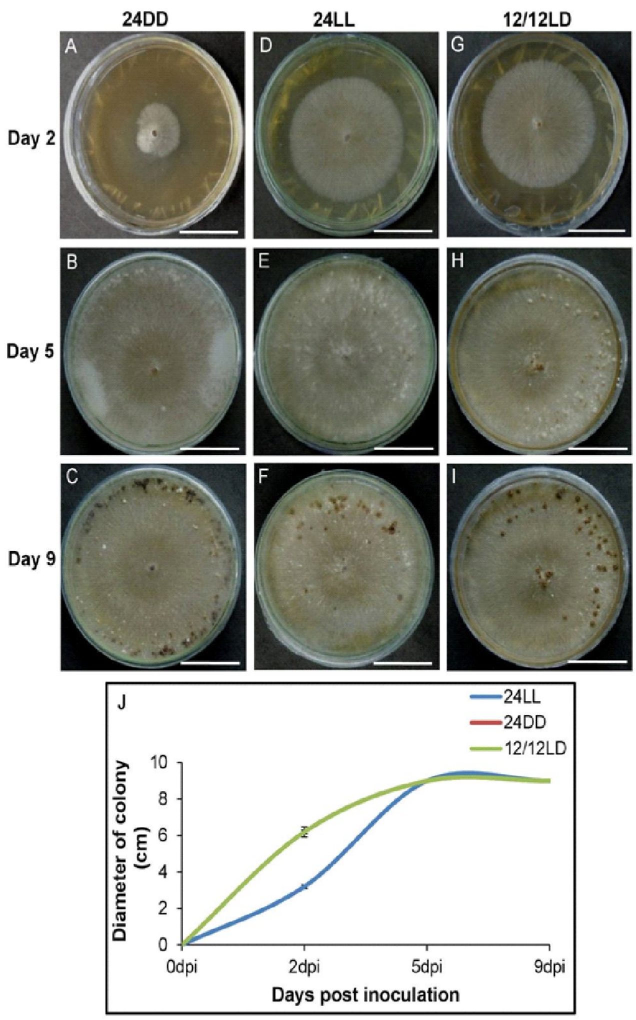

Fig. 3: Colony morphology of *Rhizoctonia solani* and formation of sclerotia on high carbon high nitrogen condition with malt extract as nutrient sources under different photoperiodic conditions.(A-C) Under 24 hours continuous dark periods (24DD), (D-F) under 24 hours continuous light periods (24LL), (G-I) under alternating 12 hours light and 12 hours dark periods (12/12LD), (J) graphical representation of diameter of colony under 24DD, 24LL and 12/12LD conditions. Bar = 3cm.

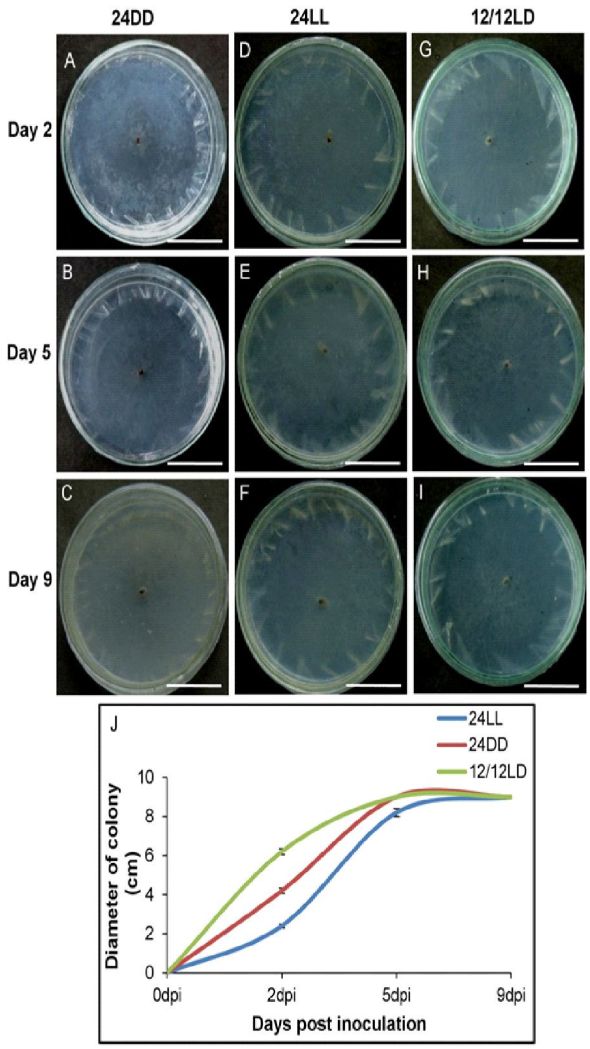

Fig. 4: Colony morphology of *Rhizoctonia solani* and formation of sclerotia on carbon and nitrogen deficient conditions under different photoperiods (A-C) Under 24 hours continuous dark periods (24DD), (D-F) under 24 hours continuous light periods (24LL), (G-I) under alternating 12 hours light and 12 hours dark periods (12/12LD), (J) graphical representation of diameter of colony under 24DD, 24LL and 12/12LD conditions. Bar = 3cm.

huge differences in utilization of different carbon and nitrogen sources for growth which is corroborating our findings.

Effect of different sources of carbon and nitrogen and different photoperiods on the morphology of *R. solani* colony

When carbon and nitrogen source was oat meal, the morphology of fungal colony was loose and white aerial hyphae were observed at the edges of colony (Fig. 1). But morphology of colony was flat, compact and less aerial hyphae were observed when potato dextrose and malt were used

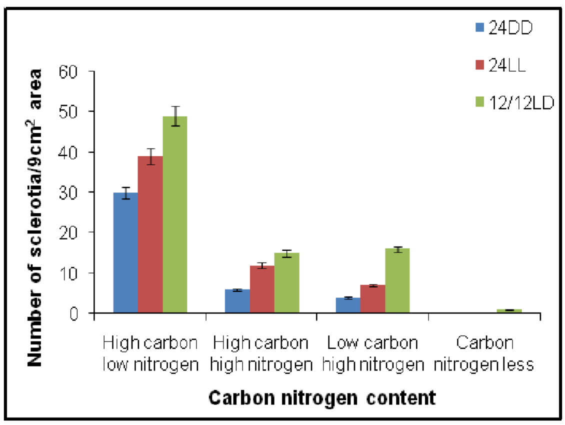

Fig. 5: Number of sclerotia formed under different photoperiodic conditions on different carbon and nitrogen contents. Explanation of legend: 24 DD- 24 hours dark; 24 LL-24 hours light; 12/12 LD-12 hours light alternating with 12 hours dark.

as sources of carbon and nitrogen respectively (Figs. 2& 3). In carbon and nitrogen deficient conditions, growth of colony was dispersed and flat (Fig. 4).

Carbon rich conditions increased formation of sclerotia under different photoperiods

Formation of sclerotia was observed under different photoperiodic conditions on different nutrients. Development of sclerotia was found to be lowest when grown under continuous dark conditions.

We have also studied how different sources of carbon and nitrogen of growth media affected formation of sclerotia. Sclerotia formation started at 4 dpi on lower carbon conditions with malt extract or oat meal as sources of carbon and nitrogen (Figs. 1 & 3). Formation of sclerotia started after 3 dpi when potato and dextrose was used as nutrient sources having higher carbon and lower nitrogen content (Fig. 2). In carbon and nitrogen deficient condition, sclerotia formation was found to start at 9 dpi (Fig. 4).

The number of sclerotia was also found to be maximum (118) when carbon content was highest with potato and dextrose as sources of carbon whereas in carbon and nitrogen deficient condition, number of sclerotia produced was least (i.e. 3 in number) (Figs. 2 & 4). When malt extract and oat meal was used as source of carbon and nitrogen number sclerotia formed was 11 and 9 respectively. In growing conditions with highest nitrogen content and oat meal as source of carbon and nitrogen, no sclerotia were observed on surface of media and most of the sclerotia were found to be formed on the upper layer of the colony (Fig. 1). Sclerotia were formed in concentric rings when carbon content of media was higher with malt or potato-dextrose used as sources of carbon and nitrogen. In carbon and nitrogen deficient condition, sclerotia were formed only under alternate light-dark periods (Fig. 4-I). Sclerotia formation was found to increase (1.3 and 2 times) under alternating light-dark conditions (12/12LD) compared to both continuous light and continuous dark condition (Fig. 5). Thus our study revealed that alternating light and dark photoperiod (12/12LD)was favorable for development of sclerotia. As per earlier reports, light and nutrient status of media affects sclerotia formation in different fungi (Liu and Guo, 2009; Mishra *et al*. 2012; Koley *et al*. 2019). The report of Ritchie *et al*. (2009) on nutrient rich media producing more sclerotia compared to nutrient poor media corroborates with our findings.

In conclusion, our present study revealed that nitrogen rich condition supported colony growth and carbon rich conditions were more suitable for formation of sclerotia. Whereas, alternating light dark conditions enhanced both growth of colony and sclerotia formation compared to continuous dark and light conditions.

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