J.Mycopathol.Res. 62(4) : 735-739, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online) 10.57023/JMycR.62.4.2024.735

Differential colony growth and morphology of *Colletotrichum capsici* under different culture conditions and photoperiods

SUVANWITA LAHIRI, MOUMITA CHATTOPADHYAY, SREEJA NAG AND SUREKHA KUNDU*
Molecular and Applied Mycology and Pathology Laboratory, Department of Botany, University of Calcutta

Received : 21.06.2024 Accepted : 31.08.2024 Published : 30.12.2024

Growth of fungi is affected by carbon and nitrogen availability in nutrient sources, as well as exposure to light, which provides environmental cues to the fungi. The morphology of fungal colonies is also known to be affected by these factors. This study aims to investigate the impact of carbon and nitrogen sources on colony growth and morphology of *Colletotrichum capsici* under different photoperiods. High carbon and low nitrogen levels provided by potato extract and dextrose caused enhanced fungal growth *in vitro* under continuous light exposure and continuous dark photoperiod. However, slower growth was observed when *C. capsici* was grown under alternate light-dark regime. Exposure to light caused the colony to have a thick elevated appearance with a pale yellow colour whereas the dark grown colony appeared white. The growth rate was slowest under continuous dark regime in oat meal extract which is a nitrogen rich nutrient source, whereas both continuous light and alternate light-dark photoperiods showed better growth. Interestingly, continuous dark photoperiod caused higher growth in malt extract having high contents of both carbon and nitrogen in contrast to growth under continuous light and alternate light-dark regimes. The colonies on both oat extract and malt extract were thin, compact and pink in colour under all photoperiods.

Keywords : Carbon, *Colletotrichum capsici*, colony growth, morphology, nitrogen, nutrient source, photoperiod

INTRODUCTION

Selection of suitable culture conditions is crucial for studying fungal growth. Earlier studies have shown that hyphal growth of microfungi is affected by several physical and chemical factors (Devi et al., 2018). Macroelements like C, O, H, N, S and P are required for the synthesis of the four major biomolecules while Ca, Mg and Fe are important ions used for serving important cellular functions whereas microelements like Mn, Zn, CO, Mo, Ni and Cu act as cofactors for various enzymes (Basu *et al*. 2015).

Fungi has been shown to possess an adaptable system which helps the organism to adjust to its changing environment and maintain homeostasis (Blechert *et al*. 2023). One of the chief factors that affect fungal growth is availability of carbon and nitrogen in nutrient sources. Conventionally, fungi are grown in natural and/or synthetic media containing high amounts of carbohydrates, nitrogen at pH 5-6 at temperatures ranging between 15-37 ºC (Basu *et al.* 2015). Natural media derived from commonly available materials like potato dextrose, corn meal etc. are easy to prepare and also economical (Basu *et al*. 2015).

Besides carbon and nitrogen availabilities, another fundamental environmental factor that influences fungal growth is light regimes. Investigations on *Botrytis cinerea* have documented how light regulates its morphogenesis and conidiation (Schumacher, 2017; Brandhoff *et al*. 2017). Its role in regulating morphology of fungal colonies and production of reproductive structures has been reported in *Neurospora crassa*, *Aspergillus nidulans*, *Phycomyces blakesleeanus* (Yu *et al*. 2018). Light has been shown to negatively affect mating and haploid fruit body formation in the pathogenic fungus *Cryptococcus neoformans* (Idnurm *et al*. 2015). Previous researches from our lab have shown that radial growth of *Rhizoctonia solani* as well as germination of sclerotia was notably

*Correspondence: surekha_kundu@yahoo.com

affected by altered light regimes along with variations in carbon and nitrogen availability in nutrient sources (Saha *et al*. 2022).

Colletotrichum capsici is a destructive phytopathogenic fungus that causes anthracnose in chilli resulting in devastating agricultural losses worldwide (Mishra *et al.* 2018). Inspite of being an economically important phytopathogen, there is a lack of detailed research on the effect of nutrient sources in conjunction with different photoperiodic conditions on the mycelial growth of *C. capsici*. Thus, in this present study we wanted to study how nutrient sources and photoperiods impact the morphology and radial growth of *C. capsici* colony.

MATERIALS AND METHODS

Fungal material

Pure culture of *Colletotrichum capsici* (Syd.) Butler & Bisby was obtained from MTCC (strain 9691).

Culture and maintenance of fungus

The original fungal sample of *C. capsici* was sub-cultured on potato dextrose agar (PDA) medium and incubated at 28^0C in incubator. The culture was maintained by sub-culturing under similar conditions and kept in 4^0C for storage.

Experimental set up

C. capsici was grown in three nutrient media with varying carbon and nitrogen contents viz. agar supplemented with potato and dextrose having low nitrogen and high carbon (potato dextrose agar, PDA), agar supplemented with malt extract having high nitrogen and high carbon (malt extract agar, MEA) and agar supplemented with oat extract having high nitrogen and low carbon (oat meal extract, OMA). The fungal cultures were subjected to three different photoperiodic regimes viz. 12 hrs light and 12 hrs dark periods (12/12LD), 24 hrs dark period (24DD) (with very brief exposure to light under 5 minutes for photography) and 24 hrs continuous light period (24LL).

Timing of onset of growth from inoculum

Mycelial discs (3 mm size) were taken as inoculum from the growing edges of 7-day old *C. capsici* culture and placed on each type of medium and incubated under the chosen photoperiods i.e. 24LL, 12/12LD and 24DD for 2 hours post inoculation (hpi), 3hpi and 5hpi. Hyphal initiation from the mycelial disc was observed under a compound microscope (Zeiss Winkel 161971) and the average length of the growing hyphae was measured with ocular micrometer and stage micrometer.

Measurement of C. capsici colony growth

Growth of *C. capsici* under different growth conditions was determined by measuring the colony diameter up to 7days. Data was recorded and photographs were taken at 0, 3, 5 and 7 days post inoculation (dpi). Colony diameter was measured and graphically expressed.

Statistical Analysis

The analysis of all experimental data was done according to our previous publication (Chowdhury *et al*. 2017). All analyses were performed as three independent experiments with at least three replicates in each and the values were represented as the mean ± SD.

RESULTS AND DISCUSSION

Time of commencement of growth of colony

The growth of *C. capsici* hyphae initiated early at 2 hpi when potato extract with dextrose containing high carbon and low nitrogen was used under both 24LL and 24DD photoperiods (with very brief exposure to light, under 5 minutes for photography) (Fig.1 A,B). At 2 hpi the average hyphal length was 54µm under 24LL and 45.1 µm under 24DD conditions, which was significantly higher than other nutrient sources. Nitrogen-rich oat meal extract delayed the commencement of hyphal growth upto 3hpi under both 24LL and 24DD photoperiods (average hyphal length at 3hpi was 30.1 µm and 47.8 µm respectively). When malt extract rich in both carbon and nitrogen, was used, 24DD induced early growth at 2hpi (29.1 µm) in contrast to 24LL lacking new growth (Fig.1 A,B).

Rapid hyphal extension in filamentous fungi is mediated by transport of growth promoting cell

wall modifying enzymes, membranes and proteins that enable hyphal growth on solid substratum (Steinberg *et al*. 2017). Blechert *et al*. (2023) showed that inorganic nitrogen sources and high concentrations of amino acids inhibit growth of *Trichophyton rubrum*. Similar inhibition was observed in our study where nitrogen-rich oat meal extract delayed the growth of *C. capsici* colony significantly. Limited nitrogen availability triggers rapid mycelial extension and increases fungal biomass production in soil fungi whereas high amounts of nitrogen deterred growth (Camenzind *et al*. 2020). This is in agreement

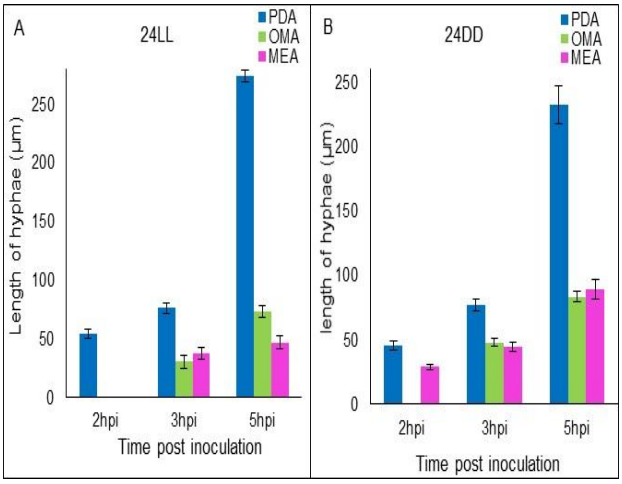

Fig. 1. Comparison of commencement of hyphal growth of *Colletotrichum capsici* under different culture conditions during the initial hours. (A) Rate of growth under continuous light regime (24LL). (B) Rate of growth under dark regime (24DD) with very brief exposure to light. Bars represent mean ± SD of three independent experiments with three replicates.

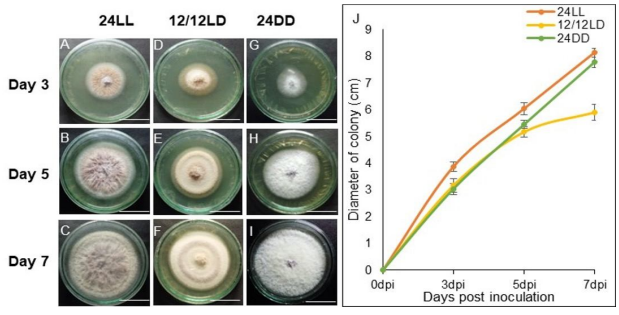

Fig.2. Morphology of *Colletotrichum capsici* colony on high carbon and low nitrogen nutrient source from potato and dextrose (PDA Media), under different photoperiodic conditions. (A-C) under continuous light (24LL), (D-F) under alternate light-dark cycle (12/12LD), (G-I) under dark cycle (24DD). (J) Comparison of growth under different light regimes showing similar rates upto 5dpi; subsequently significantly lesser growth under 12/12LD from 5dpi till the end of experiment. Bars represent mean ± SD of three independent experiments with three replicates. Scale = 5cm.

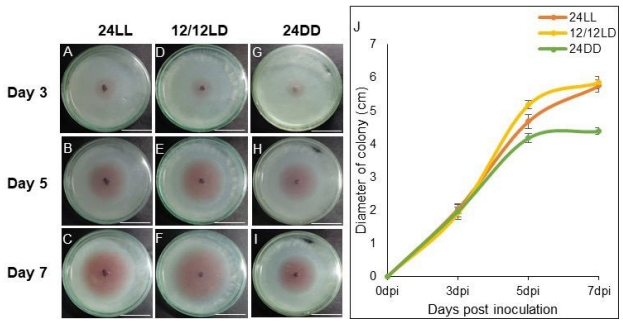

Fig. 3. Morphology of *Colletotrichum capsici* colony on low carbon and high nitrogen nutrient source from Oatmeal (OMA Media), under different photoperiodic conditions. (A-C) under continuous light (24LL), (D-F) under alternate light-dark cycle (12/12LD), (G-I) under dark cycle (24DD). (J) Comparison of growth under different light regimes showing almost similar growth rate up to 3dpi; subsequently showing growth was plateaued out for 24DD at 5dpi in contrast to the other two experimental sets. Bars represent mean ± SD of three independent experiments with three replicates. Scale = 5cm.

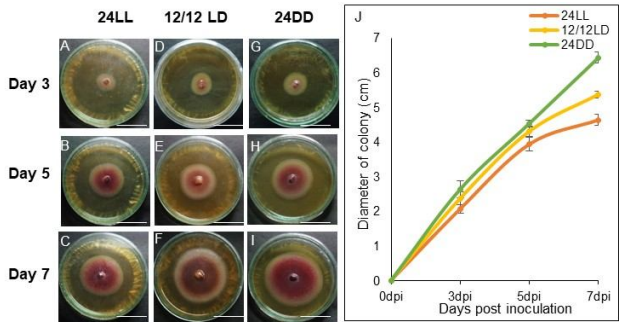

Fig. 4: Morphology of *Colletotrichum capsici* colony on high carbon and high nitrogen nutrient source from Malt extract (MEA Media), under different photoperiodic conditions. (A-C) under continuous light (24LL), (D-F) under alternate light-dark cycle (12/12LD), (G-I) under dark cycle (24DD). (J) Comparison of growth under different light regime showing similar rate of growth up to 5dpi; subsequently showing significant difference amongst the experimental sets at 7dpi. Bars represent mean ± SD of three independent experiments with three replicates. Scale= 5cm.

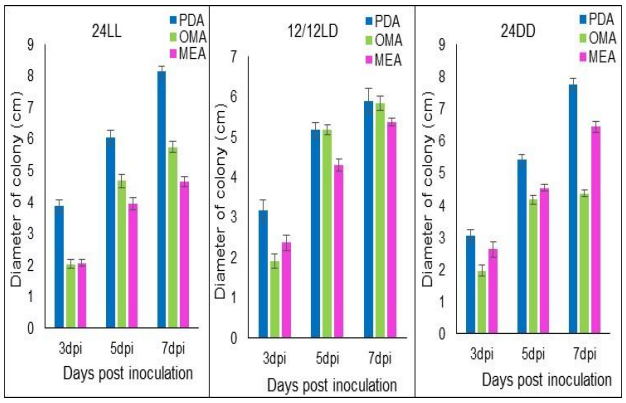

Fig.5. Comparison of growth of *Colletotrichum capsici* under different culture conditions. (A) Rate of growth under continuous light regime. (B) Rate of growth under alternate light-dark cycle. (C) Rate of growth under dark regime. Bars represent mean ± SD of three independent experiments with three replicates.

with our observations where potato dextrose containing high carbon and low nitrogen supported the fastest hyphal elongation, followed by malt extract which had high carbon and nitrogen contents while nitrogen-rich oat meal showed the most delayed response under the chosen photoperiods.

Morphology of C. capsici colony is influenced by nutrient sources and photoperiodic conditions

In this study, when potato dextrose was used as nutrient source, the colony showed an overall thick, elevated and cottony texture. It had pale yellow colour and thick feathery texture when exposed to 24LL but the dark grown 24DD colony appeared white. Interestingly, the colony subjected to 12/12LD photoperiod showed alternate concentric rings that corresponded to the alternate light and dark cycles (Fig.2 A-I). When *C. capsici* was grown on oat meal under the chosen photoperiods, the colonies were thin, flat, light pink in colour at the centre, while the edges having younger hyphae appeared white (Fig. 3 A-I). Similar morphology was observed on malt extract which produced flat, thin and dark pink coloured colonies with entire, translucent white margins (Fig. 4 A-I).

Fungi can be characterized based on their colony characteristics and growth rate along with morphology of mycelia, conidia and appressoria and the findings were shown to conform to the phylogenetic analyses by Liu *et al*. (2016). When *T. rubrum* was grown on potato and dextrose, it enhanced pigment formation and conidia development (Basu *et al*. 2015). The morphology of fungal colonies *in vitro* vary significantly depending upon the carbon and nitrogen availability. Our findings are in agreement with the previous studies stating that distinct morphological differences are produced due to variation in carbon and nitrogen contents in the growth medium in several types of fungi (Devi *et al*. 2018; Moura *et al*. 2020; Gwon *et al*. 2022).

Influence of different ratios of carbon, nitrogen and photoperiodic conditions on growth of C. capsici

The growth of *C. capsici* was maximum on potato extract and dextrose having higher carbon and lower nitrogen content (Fig. 2 A-I and 5 A-C). Under 24LL, at 3dpi the colony diameter was 3.9cm which was significantly greater than the colonies grown under 12/12LD (3.1cm) and 24DD (3.0cm). However, after 5dpi, the rate of growth under 12/12LD failed to increase significantly (Fig.2F). Significantly smaller colonies were produced on carbon-deficit and nitrogen-rich oat meal under all photoperiods (Fig.3 A-I and 5 A-C). At 3 dpi all three cultures grew slowly having colony diameters 1.9 cm -2.0 cm (Fig. 3 A, D, G). Interestingly, *C. capsici* colony plateaued out after 5dpi under 24DD in contrast to the other experimental set ups (Fig. 3 H, I, J). Conversely, after 5dpi, 24DD showed maximum mycelial growth on malt extract (Fig. 4 A-I and 5 A-C). Although at 3dpi, all three cultures showed similar rates of growth (2.0cm, 2.4cm and 2.6cm for 24LL, 12/12LD and 24DD respectively), growth was slow for 12/12LD and 24LL even after 5dpi (Fig. 4 A-I, J). It is noteworthy to mention that under 12/12LD conditions, the differences between different nutrient conditions were minimal (Fig.5B). Fungi depend largely on the availability of carbon, nitrogen, phosphorus, sulphur etc. in its surroundings (Blechert *et al*. 2023). Higher radial growth of *Metarhizium anisopliae* colony and highest virulence of its conidia was shown to be induced by C:N ratio of 35:1 while other ratios reduced radial growth and virulence (Shah et al., 2005). Studies have also shown that variation in nutrient sources cause differential growth of *Fusarium* (Westphal *et al*. 2021) and *Magnaporthe oryzae triticum* (Ashrafi *et al*. 2021). Filamentous fungi are capable of sensing visible light to regulate hyphal growth, reproduction, mitigation of stress and maintenance of circadian clock (Schumacher, 2017). Exposure to light has been shown to regulate production of secondary metabolites in some *Fusarium* species (Westphal *et al*. 2021). Similarly, mycelial development is affected by light in *Aspergillus nidulans* and *Trichoderma atroviride* (Yu et al., 2019). The differential effect of exposure to different photoperiodic regimes in our study is in accordance with studies on *Botrytis sp.*, (Brandhoff *et al*. 2017; Schumacher. 2017), *R. solani* (Koley *et al*. 2019; Mandal *et al*. 2019; Saha *et al*. 2022).

CONCLUSION

From our present study, it may be concluded that high carbon: nitrogen ratio under continuous light is the most favourable condition for the most proliferative growth of *C. capsici in vitro*. It was also observed that growth of *C. capsici* was least favoured by potato and dextrose extract under 12/ 12LD, oat meal extract under 24DD and malt extract under 24LL. Lastly, alternate light-dark regime showed the minimal distinction among the three nutrient sources. Comprehensive information on the two chief parameters affecting growth *in vitro* may serve as a useful tool for further studies to determine the underlying mechanisms pertaining to pathogenicity of *C. capsici*. Further research in this field is required to conclusively interpret the relationship between the culture conditions of the fungus and its virulence and extended viability.

ACKNOWLEDGEMENT

UGC-CAS, DBT-Builder and DST-PURSE provided funding and infrastructure. SL acknowledges SVMCM and MC acknowledges UGC for fellowships.

DECLARATION

Conflict of Interest. The authors declare no conflict of interest.

REFERENCES

Ashrafi, M.L., Aminuzzaman, F.M., Rehena, M.K., Tanni, J.F. and Ahmed, M. (2021) Characterization of *Magnaporthe oryzae triticum* on Different Culture Media. *Open Access Library J.* **8**: 1-15.

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M., & Khurana, S. (2015). Evolution of bacterial and fungal growth media. Bioinformation **11**: 182–184.

Blechert, O., Xiong, S., Chen, J., Brand, A., & Zhan, P. 2023. Nutritional requirements of the human pathogenic fungus, Trichophyton rubrum, and nutritional immunity of the human skin as barrier against colonization. Fungal Biology Reviews, 45, 100330.

Brandhoff, B., Simon, A., Dornieden, A., Schumacher, J. 2017. Regulation of conidiation in Botrytis cinerea involves the light-responsive transcriptional regulators BcLTF3 and BcREG1. *Current Genet.* **63**: 931–949.

Camenzind, T., Lehmann, A., Ahland, J., Rumpel, S., Rillig, M.C. 2020. Trait-based approaches reveal fungal adaptations to nutrient-limiting conditions. *Environ. Microbiol*. **22**: 3548-3560.

Chaudhuri, T., Biswas, A., Panja, B. 2021. Cultural and morphological characterisation of Colletotrichum fragrans of dracaena using various nutrient sources along with fungicide sensitivity tests. *Inter. J. Chem. Stud*. **9**: 3359–3362.

Devi, K. S., Misra, D. K., Saha, J. K., Devi, P. S., Sinha, B. 2018. Screening of suitable culture media for growth, cultural and morphological characters of Pycnidia forming fungi. *Inter. J. Curr. Microbiol. Appl. Sci.* **7**: 4207–4214.

Gwon, J., Park, H., Eom, A. 2022. Effect of Temperature, pH, and Media on the Mycelial Growth of *Tuber koreanum*. *Mycobiology*, **50**: 238–243.

Idnurm A., Heitman, J. 2005. Light controls growth and development via a conserved pathway in the fungal kingdom. *PLoS Biol* **3**: e95

Koley, P., Mondal, M., Saha, A., Kundu, S. 2019. Effect of light intensity on mycelial growth and hyphal branching of *Rhizoctonia solani* Kühn. in culture. *Res. J. Life Sci. Bioinform. Pharmaceut. Chem. Sci*. **5**: 972-981.

Liu, F., Tang, G., Zheng, X. et al. 2016. Molecular and phenotypic characterization of Colletotrichum species associated with anthracnose disease in peppers from Sichuan Province, China. *Sci. Rep*. **6**: 32761.

Majumdar, N., Mandal, N. C. 2019. Influence of culture media on mycelial growth, sporulation and spore size of quiescent pathogen *Colletotrichum gloeosporioides* isolated from banana. *The Pharma Innov. J.* **8**: 385–390.

Mandal, M., Koley, P., Saha, A., Kundu, S. 2019. Effect of Culture Conditions on Formation and Germination of Sclerotia of *Rhizoctonia solani*. *Int. J. Adv. Sci. Res. Manag*. **4**: 325-329.

Meletiadis, J., Meis, J.F., Mouton, J.W., Verweij, P.E. 2001. Analysis of growth characteristics of filamentous fungi in different nutrient media. *J. Clin. Microbiol.* **39**: 478-84.

Mishra, R., Rout, E., Joshi, R. K. 2018. Identification of Resistant Sources Against Anthracnose Disease Caused by *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* in *Capsicum annuum* L. *Proc. Nat. Acad. Sci. Ind. Section B: Biologic. Sci.* **89**: 517–524.

Moura, R. D., De Castro, L M., Culik, M. P., Fernandes, A.R., Fernandes, P. M. B., Ventura, J. A. 2020. Culture medium for improved production of conidia for identification and systematic studies of *Fusarium* pathogens. *J. Microbiolog. Methods* **173**: 105915.

Saha, A., Brahmachari, S., Deb, C., Thakur, M., Das, A., Lahiri, S., Kundu, S. 2022. Role of nutrient sources and photoperiodic conditions on colony morphology, growth and sclerotial development in *Rhizoctonia solani*. *J. Mycopathol. Res*. **60**: 207-212.

Schumacher, J. 2017. How light affects the life of *Botrytis*. *Fung. Genet. Biol.* **106** : 26–41.

Shah, F. A., Wang, C., Butt, T. M. 2005. Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae*. *FEMS Microbiol. Letts*. **251**: 259–266.

Steinberg, G., Peñalva, M. A., Riquelme, M., Wösten, H. A., Harris, S. D. 2017. Cell biology of hyphal growth. *Microbiol. Spectrum* **5**:10.1128.

Westphal, K. R., Heidelbach, S., Zeuner, E. J., Riisgaard-Jensen, M., Nielsen, M. E., Vestergaard, S. Z., Bekker, N. S., Skovmark, J., Olesen, C. K., Thomsen, K. H., Niebling, S. K., Sørensen, J. L., Søndergaard, T. E. 2021. The effects of different potato dextrose agar media on secondary metabolite production in *Fusarium*. *Inter. J. Food Microbiol.* **347**: 109171.

Yu, Z., Fischer, R. 2018. Light sensing and responses in fungi. *Nature Rev. Microbiol.* **17**: 25–36.