EFFECT OF CULTURE MEDIUM, TEMPERATURE AND PHOTOPERIOD ON MYCELIAL GROWTH AND SPORULATION OF *MAGNAPORTHE ORYZAE*

M. B. Rishad[1], A. Sultana[2], S. Chakraborty[3], S. S. Silvi[4] and M. A. R. Khokon[5*]

[1]Md. Bodruzzaman Rishad, MS student, [2]Afruja Sultana, Assistant Professor, [3]Shila Chakraborty, Research Assistant, [4]Sabmira Showkat Silvi, [5]Md. Atiqur Rahman Khokon, Professor, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202

* Corresponding author, e-mail: atiq.ppath@bau.edu.bd

ABSTRACT

Rishad, M. B., Sultana, A., Chakraborty, S., Silvi, S. S. and Khokon, M. A. R. 2021. Effect of culture medium, temperature and photoperiod on mycelial growth and sporulation of *Magnaporthe oryzae*. Bangladesh J. Plant Pathol. 37(1&2):1-6

Various physical and nutritional factors influence the mycelium growth and sporulation of plant pathogenic fungi including *Magnaporthe oryzae*, the causal agent of rice blast disease. Three independent experiments were conducted to find out the effects of two culture media (corn meal (CMA) and Oat meal agar (OMA)), three temperature regimes (22, 25 and 29C) and three light conditions (continuous, light/darkness and continuous darkness) during incubation on mycelium growth and sporulation of the fungus. Data on mycelium growth were recorded at 3, 6, 9, 12 and 15 days after incubation (DAI) and sporulation at 5, 10 and 15 DAI. It was found that colony growth and sporulation increased gradually with the progress of incubation period showing maximum colony diameter and spore production at final stage of data collection (15 DAI) in all experiments. At 15 DAI, mycelium growth was higher on OMA (45.66 mm) than (39.33 mm) CMA but sporulation was higher on CMA ($5.55x10^6$/ml) than OMA ($4.25x10^6$/ml). Irrespective of culture media, maximum mycelium growth and sporulation was recorded at 25C followed by 22 and 29C. Irrespective of culture media, maximum mycelium growth and sporulation was recorded at 25C followed by 22 and 29C. At 15 DAI, the colony diameter was 41.66, 39.33 and 36.25 mm at 25C 22C and 29C, respectively. On CMA, and the mycelium diameter was 44.70, 41.25 and 44.33 at 25, 22 and 29C, respectively on OMA. The maximum mycelium growth was recorded under continuous light followed by light alternate with darkness, and continuous darkness. On the other hand, the sporulation was maximal under light alternate with darkness followed by continuous light and continuous darkness producing 1.85-4.83, 2.26-5.66 and 1.05-4.58 ($\times 10^6$ spore)/ml respectively on CMA. Findings of the research reveal that OMA medium is better than CMA for mycelium growth whereas CMA is more suitable for sporulation than OMA. Both mycelium growth and sporulation on two media are maximal at25 followed by 29 and 22C. Continuous light is the best condition for mycelium growth but light alternate with darkness is most suitable for sporulation of *M. oryzae*.

Key words: *Magnaporthe oryzae,* culture media, temperature regimes, photoperiod, mycelium growth, sporulation

INTRODUCTION

Rice is a staple food for most of the people of the world including Bangladesh. According to FAO (FAO 2009) the production of rice must be doubled by 2050 to cope up with the increasing food demand in ever increasing mouth. Rice blast caused by the ascomycete *Magnaporthe oryzae* is one of the top listed 10 fungal diseases that threaten world food security (Dean2012). It is the biggest constraint of rice production that directly decreases grain yields and indirectly increases the cost of production (Skamnioti and Gurr 2009). Rice blast is liable for yield losses of about 10 to 30% annually (Wilson and Talbot2009). In ambient conditions, this disease can destroy whole rice plants within 15 to 20 days and can cause total yield loss of up to 100% (Musiime *et al*. 2005). Rice blast is also a common and destructive disease of rice in Bangladesh and can cause up to 65.0% yield loss in conducive condition (Khatun *et al*. 2021).

Soltani *et al.* (2013) evaluated potato dextrose agar (PDA), potato carrots agar (PCA) and water agar (WA) for vegetative growth and sporulation of *P. oryzae*. They used three light regimens, i.e. continuous light, 16.8 hr light/darkness, and continuous darkness and incubated at 26C. The PDA was noted as the best medium for *Pyricularia oryzae* vegetative growth,

regardless of light condition. The fungus could sporulate when light was provided either continuously or at intervals showing maximum sporulation at a combination of 16.8 hr light/ darkness intervals.

Rajput *et al.* (2017) found that temperature has a significant influence on growth and sporulation of rice leaf blast pathogen (*M. oryzae*) on agar medium. Both growth and sporulation were increased up to a temperature (27C) and declined further in response to increased or decreased with temperature (32C and 22C). In an *in-vitro* study, the highest mean mycelial growth of the fungus *P. oryzae* was recorded on Host Extract Agar (4.08 cm) followed by Oat meal agar (3.83 cm) and least mean mycelium growth on Richard's agar media (3.21cm). In general, the Potato Dextrose Agar was found more appropriate for cultural and morphological study of rice blast fungus (Manjunatha and Krishnappa 2019).

Available reports revealed that conidia production of *M. oryzae* was favored by the natural media than synthetic media and photoperiod also greatly influenced conidia production(Lee *et al*. 2006, Perelló *et al*. 2017).Nevertheless, the impact of all these factors on *M. oryzae* isolates needs further investigation to maximize conidia production for artificial inoculation in various studies. Quick and suitable methods of culturing and production of conidia are essential to produce inocula of *M. oryzae* to expedite further studies related to development of resistant varieties and other management tactics against rice blast. With this view in mind the present study was undertaken to determine suitable medium, temperature level and photoperiod for maximum mycelium growth and sporulation of rice blast fungus, *M. oryzae*.

MATERIALS AND METHODS

Three independent experiments were conducted under laboratory conditions to find out effect of two media, three temperature regimes and three photoperiods. An isolate of *M. oryzae* were selected for those experiment.

Isolation and purification of rice blast pathogen

Magnaporthe oryzae was isolated by picking up a single conidium following the method described by Jia (2009) with some modifications. Blast infected rice panicles were collected from the fields, cut into small pieces and surface sterilized with 10% chlorox. Sterilized panicle necks were placed on moist filter paper in glass petridishes at three pieces per dish covered with a lid and incubated for 48 hr. Incubated neck pieces were checked regularly under a stereo-binocular microscope to observe the colony growth of *M. oryzae*. After sporulation, few conidia were picked up by gentle touching of colony under a stereo microscope with tip of sterilized needle previously dipped in liquid water agar. Conidia on the needle tip were subsequently transferred onto PDA plates and spread with a glass rod. Plates were incubated for 2 days at 25C. Under a stereo microscope, a single germinated conidium was marked with a needle by making a stretch around it from the back and then agar block containing the germinated conidium was cut and transferred to another PDA plate for mycelium growth. Necessary multiplication of the pathogen was done on PDA.

Effect of culture media, temperature and photoperiod on mycelium growth and sporulation

Effects of two culture media *viz.* oat meal agar (OMA) and corn meal agar (CMA), three temperature regimes *viz.* 22, 25 and 29C and three photoperiods *viz*.24 hr. continuous light, 12 hr light alternate with12 hr dark, and 24 hr. continuous dark on mycelia growth and sporulation of *M. oryzae* were evaluated. The ingredients of OMA were oat meal 60g and agar 12.5 g and those of CMA were corn meal 50.0g and agar 15.0 g per liter medium. Both of those were used as basic medium to find out effect of temperature as well as photoperiod. After autoclaving the media were poured into 90 mm glass petridishes at 20 ml dish. After solidification of media, plates were inoculated with 5 mm mycelium discs cut from 10 days old pure PDA culture of the fungus. The discs were placed upside down at the center of each plate at one disc per plate. Except study with temperature levels, all plates were incubated at 25±1C.

Data record

Radial mycelium growth of *M. oryzae* recorded at 3, 6, 9, 12 and 15 days and spore production was determined at 5, 10 and 15 days after incubation (DAI)of test plates. Two measurements of diameter of fungal colonies in plates were recorded crosswise at right angles and means of two measurements was considered as colony diameter (Ahmed *et al*. 2003). Spore production was measured using a Haemocytometer following the formulae, spores/ml = (n) x 10^4, where: n = the average spore count per square of the four corner squares counted.

RESULTS AND DISCUSSION

Effect of culture media on growth and sporulation

of *M. oryzae*

The radial mycelium growth of *M. oryzae* increased gradually with the progress of incubation period showing comparatively higher growth on OMA than CMA plate. The maximum colony diameter of 45.66 mm was observed on OMA and 39.33 mm on CMA at 15 days after incubation (DAI). Differences in the parameter on two media were significant at 9, 12 and 15 DAI (Fig. 1). On the contrary, the production of conidia was higher on CMA plate than OMA. Spore production was also increased gradually with the progress of incubation period showing the highest amount of $5.55\times10^6$and 4.25×10^6conidia/ml on CMA and OMA, respectively at 15 DAI. The differences in conidia production on two media was significant at 10 DAI when the amount of sporulation was 3.66×10^6 and 2.65×10^6conidia/mlon CMA and OMA plate, respectively (Fig. 2).

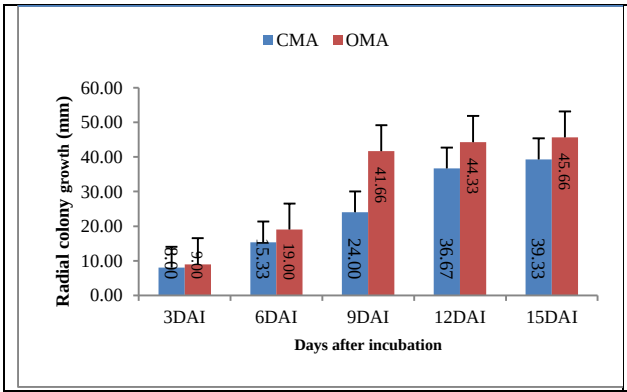

Figure 1. Effect of culture media on mycelium growth of *Magnaporthe oryzae*

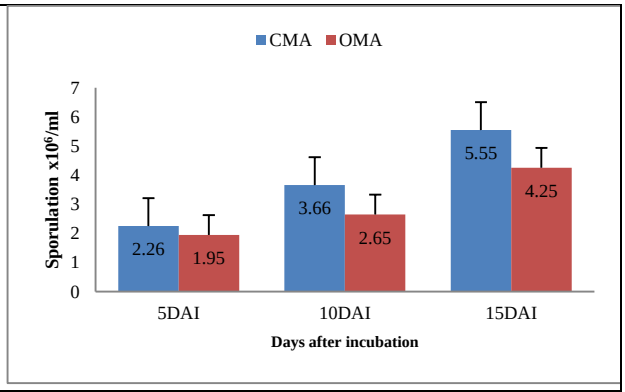

Figure 2. Effect of culture media on sporulation of *Magnaporthe oryzae*

Effect of temperature regime on growth and sporulation of *M. oryzae*

On both CMA and OMA, the radial mycelium growth as well as sporulation of rice blast fungus was the maximum at 25C followed by 22 and 29C and both parameters increased gradually with the progress of days after incubation. The radial colony diameter ranged 6.66-9.19, 14.12-16.66, 23.33-26.66, 33.66-37.33 and 36.25-41.33mm on CMA, and 7.33-10.13, 12.00-19.66, 36.33-43.15, 40.18-44.70 and 41.25-44.70mm on OMA at 22, 25 and 29C, respectively during 3-15DAI. At every stage of data collection, mycelium growth of the fungus on both media at 22 and 29C was statistically similar but significantly lower compared to 25C with some exceptions, where growth at 3 and 9 DAI on CMA and at 3 DAI on OMA was statistically similar. In case of OMA, mycelium growth reached the peak at 12 DAI at 22 and 25C (44.33 and 44.70 mm, respectively) which remained constant after further increase in incubation period (Table1).

Sporulation ranged 0.65-1.48, 2.20-3.38 and 3.03-4.35/ml on CMA and 0.45-1.10, 0.85-2.05 and 2.25-3.35/ml on OMA at 22, 25 and 29C and at 5, 10 and 15 DAI, respectively. Most of the time of data recording, sporulation was significantly higher at 25C compared to 22 and 29C, where sporulation was statistically similar (Table 2).

Table 1. Effect of temperature on radial mycelium growth of *M. oryzae* on corn meal agar and oat meal agar recorded at different days after incubation (DAI)

Temperature regime (C)	Mycelium growth on CMA (mm)					Mycelium growth on OMA (mm)				
	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI
22	8.12 ab	15.66 a	24.33 b	36.66 a	39.33 a	9.23 ab	19.19 a	42.27 a	44.33 a	44.33 a
25	9.19 a	16.66 a	26.66 a	37.33 a	41.66 a	10.13 a	19.66 a	43.15 a	44.70 a	44.70 a
29	6.66 b	14.12 b	23.33 b	33.66 b	36.25 b	7.33 b	12.00 b	36.33 b	40.18 b	41.25 b
CV (%)	11.17	4.82	4.03	2.27	3.50	10.04	5.23	3.50	2.06	1.76

Means within the same column with a common letter do not differ significantly(P=0.05).

Table 2. Effect of temperature on sporulation of *M. oryzae* on corn meal agar and oat meal agar recorded at different

days after incubation (DAI)

Treatment	Sporulation (×10^{6}spore/ml) in Corn meal agar			Sporulation (×10^6 spore/ml) in Oat meal agar		
	5 DAI	10 DAI	15 DAI	5 DAI	10 DAI	15 DAI
22C	1.05 b	2.75 b	3.55 b	0.75 b	1.53 b	2.83 b
25C	1.48 a	3.38 a	4.35 a	1.10 a	2.05 a	3.35 a
29C	0.65 c	2.20 b	3.03 b	0.45 b	0.85 c	2.25 b
CV(%)	5.78	3.38	1.69	13.04	4.16	2.13

Means within the same column with a common letter do not differ significantly (P=0.05).

Effect of photoperiod on growth and sporulation of *M. oryzae*

The maximum mycelium growth on two media was recorded under continuous (24 hr) light followed by light alternate with darkness, and continuous (24 hr) darkness at all stage of data collection. The mycelium growth of *M. oryzae* ranged 9.12-41.66, 8.54-39.33 and 6.66-36.13on CMA, and 10.15-, 9.27-44.33 and 7.33-43.17 44.70 mm on OMA under continuous light, light alternate with darkness and continuous darkness, respectively during 3-15 DAI. (Table 3).

The range of sporulation was 1.85-4.83, 2.26-5.66 and 1.05-4.58 (×10^6 spore)/ml on CMA, and 1.55-3.83, 2.10-4.35 and 0.55-3.13 (×10^6 spore)/ml on OMA under continuous light, light alternate with darkness and continuous darkness during 5-15 DAI. The sporulation was maximal under light alternate with light followed by continuous light and continuous darkness. Every time of data collection differences in sporulation fewer than three different light conditions were significant (Table 4).

Table 3. Effect of light and darkness on radial mycelium growth of *M. oryzae* on corn meal agar and oat meal agar at different days after incubation (DAI)

Treatment	Mycelium growth (mm) in Corn meal agar					Mycelium growth (mm) in Oat meal agar				
	3DAI	6 DAI	9 DAI	12 DAI	15 DAI	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI
Continuous Light	9.12a	16.66 a	26.33 a	38.16 a	41.66 a	10.15 a	19.66 a	43.57 a	44.70 a	44.70a
Light/Dark	8.54a	15.57 ab	25.23 ab	34.66 ab	39.33ab	9.27 ab	19.15 a	41.66a	44.33a	44.33a
Continuous Dark	6.66a	14.38 b	24.12 b	33.33b	36.13 b	7.33 b	12.47 b	36.26 b	39.32 b	43.17a
CV (%)	11.17	5.71	2.64	3.65	4.18	10.04	5.23	3.88	2.08	3.13

Means within the same column with a common letter do not differ significantly (P=0.05).

Table 4. Effect of photoperiod on Sporulation of *M. oryzae* in corn meal agar and oat meal agar at different days after incubation (DAI)

Treatment	Sporulation (×10^6 spore/ml) in Corn meal agar			Sporulation (×10^6 spore/mL) in Oat meal agar		
	5 DAI	10 DAI	15 DAI	5 DAI	10 DAI	15 DAI
Light	1.85 b	3.15 b	4.83 b	1.55 b	2.55 b	3.83 b
Light/Dark	2.26 a	3.63 a	5.55 a	2.10 a	2.75 a	4.35 a
Dark	1.05 c	2.55 c	4.58 c	0.55 c	2.03 c	3.13 c
CV (%)	3.39	1.98	1.54	7.15	2.5	2.96

Means within the same column with a common letter do not differ significantly (P=0.05).

In the present study it was found that CMA yielded higher radial colony diameter of *M. oryzae* than OMA

but higher sporulation was recorded on OMA than CMA. Best temperature regime for maximum vegetative growth as well as sporulation of the fungus on CMA or OMA was 25Cfollowed by 22 and 29C. Irrespective of culture media, higher mycelium growth was observed under continuous light followed by light alternate with darkness. The lowest growth was recorded from continuous darkness. The maximum sporulation was found under light alternate with darkness followed by continuous light. Findings of the present research work reveal that OMA is better than CMA to support mycelium growth of *M. oryzae*. On the contrary, CMA is superior to OMA for sporulation of the pathogen. Incubation under alternate light and darkness and at 25C temperature is suitable for growth and sporulation of the pathogen.

The findings of the present study are in agreement with the findings of many researchers. Hajano *et al.* (2013) observed that *M. oryzae* grew well on OMA. Similarly, Chandra (2011) obtained highest colony growth on oat meal agar. Yamanaka and Yamaguichi (1985) reported oat meal decoction agar as promising for spore production of *M. oryzae*. Soltani *et al.* (2013) found that PDAwas the best medium for *P. oryzae* vegetative growth. Regardless of light condition RPCA can be used as the best culture medium for *P. oryzae* in order to obtain a high number of conidia.

In the present experiment, temperature showed a considerable influence on growth and sporulation of *M. oryzae* showing 25C as the best for growth and sporulation of the pathogen. Rajput *et al. (*2017) also observed higher growth and sporulation at 25C. In this study, maximum radial mycelium growth was obtained under continuous light whereas maximum sporulation was obtained under light alternate with dark. Soltani *et al.* (2013) reported similar findings that combination of 16/8 hr light/ darkness intervals and adding rice materials to culture media could induce *P. oryzae* for a better sporulation. Yamanaka and Yamaguichi (1985) found that *M. oryzae* produced maximum mycelium growth when incubated culture was exposed under three days continuous dark, four days light and seventh days onwards 16 hour light and 8 hour darkness.

Considering the findings culture medium OMA, incubation period 15 days at 25C under alternate light and darkness conditions may be recommended to produce conidia for inoculum of *M. oryzae*.

ACKNOWLEDGEMENT

The author(s) acknowledged the Ministry of Education (MoE), The peoples' Republic of Bangladesh for funding the research project vide Project no. 201

LITERATURE CITED

Ahmed, F., Hossain, I. and Aminuzzaman, F.M. 2003. Effect of different pathotypes of *Bipolaris sorokiniana* on leaf blight severity and yield contributing characters of wheat cv kanchan inoculating at maximum severity stage. *Pak. J. Biol.Sci*6:693-696.

Chandra, S.P.M. 2011. Culture media influence on vegetative growth and *in vitro* conidia production of *Magnaporthe oryzae*. *J. Cell Pl. Sci.*2(3): 9-11.

Dean, R. 2012. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Pl.Pathol*. 13: 804–804.

FAO. 2009. FAO's director-general on how to feed the world in 2050. Insights from an Expert Meeting at FAO. 1: 1–35.

Hajano, J.U.D., Lodhi, A.M., Khanzad, M.A., Rajput, M.A. and Shah, G.S. 2013. Influence of abiotic factors on the vegetative growth and sporulation of *Magnaporthe oryzae* Couch. *Pak. J. Phytopatho.*25(1): 65-70.

Jia, Y. 2009. A user-friendly method to isolate and single spore the fungi *Magnaporthe oryzae* and *Magnaporthe grisea* obtained from diseased field samples. *Pl. Heal. Prog.*10. URL: https://doi.org/10.1094/PHP-2009-1215-01-BR

Khatun, M. T., Nessa, B., Salam, M. U. and Kabir, M. S. 2021. Strategy for Rice Disease

Management in Bangladesh. *Bangladesh Rice J.* 25 (1): 23-36. doi.org/10.3329/brj.v25i1.55177

Lee, K., Singh, P., Chung, W.C., Ash, J., Kim, T.S. and Hang, L.2006. Light regulation of asexual development in the rice blast fungus *Magnaporthe oryzae*. Fung. Gen. Biol.43:694–706.

Manjunatha, B., and Krishnappa, M. 2019. Effect of Different Culture Media on the Growth and Development of *Pyricularia oryzae* from Different Rice Growing Regions of Karnataka.*Int.J.Curr.Microbiol.App.Sci.* 8(06): 579-587.

Musiime, O., Tenywa, M.M., Majaliwa, M.J.G., Lufafa, A., Nanfumba, D., Wasige, J.E., Woomer, P.L. and Kyondha, M. 2005. Constraints to rice production in Bugiri District. *African Crop Science Conference Proceedings*7:1495–1499.

Perelló, A.E., Martinez, I., Sanabria, A., Altamirano, R. and Sibole, J.V. 2017. Pathogenicity of isolates of *Magnaporthe* spp. from wheat and grasses infecting seedlings and mature wheat plants in Argentina. *Pl. Pathol.*66:1149–61.

Rajput, L.S., Sharma, T., Madhusudhan, P. and Sinha, P. 2017. Effect of Temperature on Growth and Sporulation of Rice Leaf Blast Pathogen *Magnaporthe oryzae.Int. J. Curr. Microbiol. App. Sci.6*(3): 394-401.

Skamnioti, P. and Gurr, S.J. 2009. Against the grain: safeguarding rice from rice blast disease. *Tren.Biotech*.27(3): 141–50.

Soltani, J., Mahdieh, S. and Moghaddam, H. 2013. An investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae. Prog. Biol. Sci.*3(2): 135-143.

Wilson, R.A. and Talbot, N.J. 2009. Under pressure: Investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev. Microbiol*.7:185.

Yamanaka, S. and Yamaguchi, T. 1985. Rice blast disease, Yokendo, Tokyo.