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Research Article

Determination of Ideal Culture Media for Optimal Mycelia Growth of Rice Blast Pathogen (*MagnaportheOryzae*)

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Abstract

The study was carried out to evaluate the optimal culture medium for mycelia growth of strains ofrice blast pathogen (*Magnaportheoryzae)* in the laboratory. The blast infested leaves, stem and panicle collected from farmers' field in three Local Government Areas (Gbako, Katcha and Lavun LGAs) of Niger State, Nigeria. Samples were cultured using Potato Dextrose Agar (PDA). The incubation was done at ambient temperature of 28 ± 2^0C and the associated fungi were identified. Pathogenicity test was done in the screen house to authenticate the causative organism of rice blast. Growth rate of *M. oryzae* was then assessed in four different media namely: Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Malt Extract Agar (MEA) and Rice Agar (RA). A total of ten strains of *M. oryzae* were isolated and given hypothetical names MOR01 to MOR10. The result of pathogenicity test confirmed the isolates to be causative organism of the rice blast. All the media test supported the growth of *M. oryzae*. However, at the end of the evaluation, MOR10 showed more affinity for PDA with highest mycelia radial growth of 80mm in diameter. MOR07 had the least mycelia radial growth of 70mm. In PCA, MOR09 had the highest mycelia radial growth of 70mm, while the radial growth of MOR03, MOR05 and MOR06 were the highest (65mm each) in MEA. Therefore, for the optimal production of *M. oryzae*, PDA followed by PCA are the recommended optimal culture media.

Keywords: Rice blast, *Magnaportheoryzae*, Agar Media, Mycelia, Pathogenicity

Introduction

Magnaporthe oryzae (syn *Pyricularia oryzae*), the rice blast pathogen is a filamentous, haploid heterothallic fungus belonging to the family Magnaporthaceae (Gilbert *et al.,* 2004). Rice blast is an infectious fungal disease which is distributed worldwide and prevailing in more than 85 countries of the world (Scardaci *et al.,* 1997; Jamal-U-deen *et al.,* 2012). It is one of the most serious and widespread constraint of rice cultivation in West Africa (Akator *et al.,* 2013). The fungus *M. oryzae* attacks all stages of the crop and symptoms appear on leaves, nodes, neck and panicle (Seebold *et al.,*2004; Ghazanfar *et al.*, 2009). Heavy yield losses caused by blast pathogen have been reported in many rice growing countries like India (75%), Phillipines (50%) and Nigeria (40%) (Ghazanfar *et al.,* 2009). It is estimated that about 14-18% yield reduction was caused by this disease worldwide (Mew and Gonzales, 2002; Jamal-U-deen, 2012; Hajano *et al.,* 2013). Blast occurs mostly in rainfed upland rice ecology, however, water deficiency or prolonged drought predisposes the lowland rice ecology to severe infection by blast. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi (Kumara and Rawal, 2008). Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and

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sporulation on media are important biological characteristics (St-Germain and Summerbell, 1996). A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Kumara and Rawal, 2008; Kuhn and Ghannoum, 2003). Many workers have reported that vegetative growth and production of fruiting structures in fungi is greatly influenced by culture media, light intervals and temperature (Quroshi and Meah, 1991; Alam *et al.*, 2001; Jayaswal *et al.*, 2003; Xiao and Sitton, 2004; Englander *et al.*, 2006; Khanzada *et al.*, 2006; Sharma and Pandey, 2010; Goyal *et al.*, 2011). The present study is conducted to isolate different strains of *Magnoporthe oryzae* from leaves, stems and panicles of rice plants infected with rice blast disease and determine the optimal culture media for growth.

Materials and Methods

Collection of samples:

The blast infested leaves, stems and panicles of rice plants were collected from five farmer's-field located in Gbako (Latitude 9°40'00"N and Longitude 6°03'33"E), Katcha (Latitude 9°02'60.00"N and Longitude 6°08'60.00E), and Lavun (Latitude 9°20'00"N and Longitude 5°60'00"E) Local Governments Areas of Niger State, Nigeria.

Preparation of media:

Potato Dextrose Agar (PDA)

Thirty nine (39) gram of PDA (Hi-media) was suspended in 1000ml distilled water and heated to dissolve the powder completely, the medium was sterilized by autoclaving at 121°C for 15minutes.(Aremu *et al.*, 2013)

Malt Extract Agar (MEA)

Fifty (50) gram of synthetic Sigma-Aldrich Malt Extract Agar (MEA) was suspended in 1000ml distilled water and heated to dissolve the powder completely; the medium was sterilized by autoclaving at 121°C for 15minutes (Borjesson*et al.*, 1990).

Potato Carrot Agar (PCA)

Forty gram carrots and forty (40) gram Irish potatoes were separately washed and peeled, chopped and boiled each in one litre of water each for 5minutes, filtered off, sterilized for 60minutes at 121°C. Two hundred and fifty (250) ml potatoes extract and 250ml carrot extract were added to 500ml distilled water, 15g of agar was added and autoclaved at 121°C for 15min. (Adebola and Amadi, 2012)

Rice Agar

Ten (10) gram of polished rice was boiled in750ml of distilled water for 30minutes and the water was decanted and the volume was made up to 1litre. Twenty (20) gram of agar was also added and autoclaved at 121°C for 30minutes (Maheshwari 2002).

Fungal isolation and identification

Collected blast infested rice leaf, stem and panicle samples were sterilized in mercuric chloride (0.01%) and 5 discs taken from the periphery of necrotic region were placed on Potato Dextrose Agar (PDA), to which streptomycin ($1mlL^{-1}$) has been added and incubated at 28±2°C for 3 days. A Single conidium was picked up with a sterile needle under microscopic observation, transferred individually to PDA plates and incubated at ambient temperature (Gomathinayagam *et al.*, 2013). The monoculture was prepared and stored on PDA slants at 4°C. Subculture was made at regular intervals. The fungal isolates were identified using the fungal family of the world mycological monograph (Cannon and Kirk, 2007; Adebola and Amadi, 2012) under microscopic observation.

Inoculum production

Inoculum was prepared on rice using a modified method of Adebola and Amadi, (2012). Five mycelia plugs (5mm) of 3-day- old culture of each isolates were transferred to a potato broth medium (200g/l) that has been autoclaved twice for 60mins. Each of the suspension was left on shaker for 5days at 28 ± 2°C and 95rpm. Ten polythene bags were separately filled with 300g of rice and 70ml distilled water each. They were autoclaved for three consecutive days and cooled to room temperature. Thirty (30ml) of the

cultures was poured into the bags containing the rice and sealed. The bags were opened after 48hrs and the contents were spread on trays and incubated further for 3days at 28 ± 2°C for initiation of spore production. Finally, they were dried for 6days. Suspension of each was prepared by blending solid rice substrate with distilled water, 20% pectin and ammonium diphosphate (2:1 v/v) at pH5.5 for ten seconds in a blender and filtered through cheese cloth. Final concentration of each of the suspensions was adjusted to 10propagules/ml. The suspension was stored in bigger container packed with ice pack at 4°C (Tondje *et al.*, 2006).

Pathogenecity test

The rice seed variety (FARO 52) that was used for pathogenicity test was collected from Seed Unit of National Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria. Pathogenicity of *M. oryzae* was tested on healthy rice plants grown in the screen house located in the garden of Department of Biological Sciences, Federal University of Technology (FUT) Minna. Forty five days old FARO 52 rice plants were sprayed with (10ml/pot) mycelia suspension of the strains of blast pathogen (*M. oryzae*) by means of automizer. The plants were covered with polythene bags to provide adequate humidity. The inoculated plants were observed after 7 days for characteristic symptoms of blast (Gomathinayagam *et al.*, 2011).

Effect of different culture media on mycelia growth of *M. oryzae*

Growth rate of *M. oryzae* pathogen was assessed on four different solid media viz; Potato Dextrose Agar (PDA), Malt Extract agar (MEA) and Potato Carrot Agar (PCA) and Rice Agar (RA). Quadruplicates were maintained for each treatment. A mycelia disc (2 mm diameter) was placed at the centre of the medium in a Petri plate and incubated at 28±2°C. The diameter of the mycelial growth was measured from day 1 to 4 (Subramania *et al.*, 2013).

Results and Discussion.

Isolation, identification and pathogenicity test

The characterization and pathogenicity tests results confirmed that the fungi isolated was *M. oryzae* and authenticated as the causative organism of blast disease of rice. A total of ten (10) *M. oryzae* were isolated and given hypothetical names of MOR001-MOR010. The isolated *M. oryzae was* from the 10 farmer's field across the three local governennt areas selected. Two isolates were from Gbako (MOR 001 and MOR 02), 5 from Katcha (MOR 003, MOR 04,MOR 05, MOR 06 and MOR10) and 3 from Lavun (MOR 07, MOR 008 and MOR 09) LGAs.

Microphotograph of *M. oryzae*

The microscopic features of the isolated fungi showed that in all the ten isolates, the shape of the conidia was typically pyriform with rounded base, narrowed apex and 2-3 septate (Plate 1). The mycelia were highly branched, septate, superficial, bearing conidia at the tip and bunch at the side of the conidiophores. The conidiophores of the isolates were slender, straight, grayish white to grayish black, smooth bearing clusters of conidia which are typically of pyriform and 2-3 septate.

Effect of different media on growth of *M. oryzae*

The result of the radial growth of all the isolates on different media; Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Rice Agar (RA) and Malt Extract Agar (MEA) is presented in Figure 1. All the organisms grew very well in PDA. MOR 06 had the highest radial growth (16mm) followed by MOR07 and MOR 10 (14.5mm and 14.5mm respectively) which were not significantly different ($P<0.05$). The least growth was observed in MOR 01 (7.5mm). In PCA, MOR 08 had the highest radial growth (15mm) which was significantly ($P<0.05$) different followed by MOR 01 (12mm) and the least in MOR009 (6mm). In MEA, the highest radial growth was observed in MOR 05 (12.5mm) followed by MOR 06 and MOR 07 with 11mm and 10.5mm respectively. However, the radial growth of *M. Oryzae*was generally low in RA and were not significantly ($P<0.05$) different from one another (Figure 1).In Figure 2, the radial growth of most of the *M. oryzae* at day two of growth showed that almost all the organisms

grew very well in PDA although MOR 08 and MOR 10 had the highest radial growth of 57mm and 57.5mm respectively followed by MOR 06 and MOR07 with 50mm and 50mm respectively which were not significantly ($P<0.05$) different. The least growth was observed in MOR01 and MOR02 (35mm and 35mm) which was significantly ($P<0.05$) different. In PCA, MOR08 has the highest radial growth (57.5mm) which was significantly ($P<0.05$) different followed by MOR 10 (42.5mm) and the least was observed in MOR 07 (17.5mm). In MEA, the highest radial growth was observed in MOR 02 (50mm) followed by MOR 05 (45mm) which is not significantly ($P<0.05$) different. The least radial growth was observed in MOR 09 (17.5mm). However, the radial growth of *M. oryzae* was generally low in RA and were not significantly ($P<0.05$) different from one another (Figure 2).The radial growth of most of the *M. oryzae* at day three of growth showed that all the organisms grow very well in PDA, but MOR 03 and MOR 10 had the highest radial growth of 72.5mm and 72.5mm respectively followed by MOR 05 and MOR 06 at 67.5mm and 67.5mm respectively which were not significantly ($P<0.05$) different. The least radial growth was observed in MOR 01, MOR 02 and MOR 09 (55mm, 55mm and 55mm respectively). In PCA, MOR10 had the highest radial growth (65mm) followed by MOR 06 and MOR 09 (62.5mm and 62.5mm respectively) which was not significantly ($P<0.05$) different and the least radial growth was observed in MOR07 (30mm) which was significantly ($P<0.05$) different from MOR 03 and MOR10. In MEA, MOR05 had the highest radial growth (57.5mm) followed by MOR 03 and MOR 06 (55mm and 55mm respectively) which was not significantly ($P<0.05$) different. The least radial growth was observed in MOR004 (30mm). However, the radial growth of *M. oryzae* was generally low in RA and were not significantly ($P<0.05$) different from one another (Figure 3). The radial growth of most of the *M. oryzae* at day four of growth showed that all the organisms grew very well in PDA, but MOR010 had the highest radial growth (80mm) followed by MOR 06 (77.5mm) which were not significantly ($P<0.05$) different.The least growth observed in MOR 07 (70mm). In PCA, MOR 09 has the highest radial growth (70mm) followed by MOR03 and MOR08 (67.5mm and 67.5mm respectively) which were not significantly ($P<0.05$) different. In MEA, MOR 03, MOR05 and MOR06 were observed to have high radial growth (65mm, 65mm and 65mm respectively). The least radial growth was observed in MOR 09 and MOR 10 (50mm and 50mm respectively). However, the radial growth of *M. oryzae* was generally low in RA and were not significantly ($P<0.05$) different from one another.

The result revealed that Potato Dextrose Agar (PDA) medium and Potato Carrot Agar (PCA) medium were the most suitable media for mycelial growth of the *M. oryzae* followed by Malt Extract Agar (MEA) and least was Rice Agar (RA). The results were in agreement with earlier findings of Awoderu *et al.* (*1*991).Mijan Hossain (2000), Hossain*et al.* (2004), Meena *et al.*, (2005).Hajano *et al.* (2013) and who Lodhi *et al.* (2013), and Pandey (2014) who reported that Potato Dextrose Agar and Potato Carrot Agar were suitable media for mycelia growth of Fungi. Zhae and Simon (2006) also reported that compositions of media significantly affected the mycelia growth rate and conidial production of *Phomaexigua.* Maheshwari *etal.* (1999) and Saha *et al.* (2008) also stated that PDA is the best medium for mycelial growth of *M. oryzae*. Simple formulation of PDA and its ability to support mycelial growth of a wide range of fungi makes it the most commonly used culture media for isolation of fungi and *M. oryzae* is not an exception.

Conclusion

Most fungi thrive on PDA, and the highly supportive rate of the medium may be as a result of rich nutrients composition most especially the glucose content of the media which improves the growth of fast growing species, thus encouraging the mycelial growth. Therefore, for effective evaluation of *M. oryzae*, (causative organism) of rice blast, Potato Dextrose Agar (PDA) is the best medium for isolation.

References

Adebola, M. O. and Amadi, J. E. (2012). The

Efficacy of paecilomyces specie and Penicilliumdigitatum on Black pod Disease pathogen on the field. *European Journal of Applied Science,* 4(3), 101-104

Akator, k., Awande, S., Adjata, D., Séré, Y. andSilue, D. (2013). Rice common disease in West Africa and integrated management of rice diseases: *Rice Pathology Consulting Firm* (ccpr) 260

Alam, M. S., Begum, M. F., Sarkar, M. A., Islam, M. R. andAlam, M. S. (2001). Effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botryodiplodiatheobromae*Pat.*Pakistan Journal of Biological Sciences*, 4(10), 1224-1227.

Aremu, M. B., Sanni, A. A. andMaji, A. T. (2013). Microbiological Quality Assessment of Rice Sold by Food Vendors around some Health Centres in Ilorin Metropolis. *International Journal of Applied Research and Technology,* 2(11), 26 – 35.

Awoderu, V. A., Esuruso, O. F., and Adesoun, O. O. (1991). Growth and Conidia production in race NG-5/1A-65 of Pyriculariaoryzae. Cav. In-vitro. *Journal of Basic Microbiology,* 31(3) ,163-168

Canno P. F. and Kirk P. M. (2007).*Fungal Family of the world*. Wallingford UK, CABI,Singapore. p456

Englander, L., Browning, M. andTooley.P. W. (2006). Growth and sporulation of *Phytophthoraramorum in vitro* in response to temperature and light. *Mycologia*, 98(3): 365-373.

Getachew, G., Alemu, T.andTesfaye, K. (2014).Morphological, Physiological and Biochemical studies on Pyriculariagrisea isolates causing blast disease on finger millet in Ethopia.*Journal of Applied Bioscience,* 74, 59-71

Ghazanfar, M.U., WaqasWakil, S.T. andSaleem-il-yasin (2009). Influence of various fungicides on the management of rice blast disease. *Mycopathology,* 7(1), 29-34

Gilbert, M.J., Soanes, D.M. and Talbot, N.J., (2004). Functional Genomic Analysis of the Rice Blast Fungus *Magnaporthegrisea*. *Applied Mycology and Biotechnology*, 4, 331-352

Gomathinayagam, S., Balasubramanian, N., Shanmugaiah, V., Rekha, M., Manoharan, P. T. andLalithakumari, D. (2011). Molecular characterization of carbendazim resistance of plant pathogen (*Bipolarisoryzae*), Fungicides -Beneficial and Harmful Aspects, *InTech* Available from: http://www.intechopen.com/books/fungicides-beneficial-and harmfulaspects/molecular-characterization-of-carbendazim-resistance-of-plant-pathogen-bipolaris-oryzae.1,307-451, ISBN: 978-953

Goyal, P., M. Chahar, A.P.,Mathur, Kumar, A.andChattopadhyay, C. (2011). Morphological and cultural variation in different oilseed brassica isolates of *Alternariabrassicae*from different geographical regions of India. *Indian Journal of Agricultural Science*, 81(11): 1052-1068.

Hajano, J., Lodhi, A. M., Khanzada, M. A., Rajput, M. A. and Shah, G. S. (2013). Influence of Abiotic factors on the vegetative growth and sporulation of *M. oryzae* couch. *Pakistan Journal of Phytopathology,* 25(01), 65-70

Hossain, M. M., Kulkarni, S. andHegde, Y. R. (2004).Physiological and Nutritional studies on *Pyriculariagrisea*, the causal agent of Blast of Rice.*Karnataka Journal of Agricultural Science*, 17(4), 851-853

Jamal-U-deenHajano, A. M., Muntaz, A. P., Alikhanzada, A. andSerwarshah, G. (2012).Invitro evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *Magnaportheoryzae* couch. *Pathology Journal of Botanist* 44(5), 1775-1778

Khanzada, M. A., Rajput, A. Q. andShahzad, S. (2006). Effect of medium,

temperature, light and inorganic fertilizers on in vitro growth and sporulation of *Lasiodiplodiatheobromae*isolated from mango. *Pakistan Journal Botany*, 38(3), 885-889

Kuhn, D. M. and Ghonnoum, M. A. (2003). Indoor mold, toxigenic fungi, and *Stachybotryschartarum*: Infectious disease perspective. *Clinical Microbiology Review*, 16(1), 144-172.

Kumara, K. L.W. and Rawal, R. D. (2008). Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indianisolates of *Colletotrichumgloeosporioides*causing anthracnose disease of papaya (*Carrica papaya* L). *Tropical Agricultural Research and Extension,* 11, 7-12.

Lodhi, A. M., Khanzada, M. A., Rajput, M. A. and Shah, G. S. (2013). Influence of Abiotic factors on the vegetative growth and sporulation of Magnoportheoryzae. Couch. *Pakistan Journal of Phytopathology*. 25, 65-70

Maheshwari, S. K.., Singh, D. V. and Sahu, A. K. (1999). Effect of several nutrient media, pH and carbon sources on growth and sporulation of *Alternaria alternate.Journal of Mycopathology Research* 37, 21-23.

Meena, P.D., Chattopadhyay C., Kumar, V. R., Meena, R. L.andRana, U. S (2005). Spore behaviour in atmosphere and trends in variability of *Alternariabrassicae* population in India. *Journal Of Mycology and Plant Pathology* 35, 511

Mew, T.W. and Gonzales, P. (2002).*A Handbook of Rice Seed borne Fungi*.International Rice Research Institute, LosBanós, Philippines. 83

MijanHossain, M. D. (2000). Studies on blast disease of rice caused by *Pyriculariagrisea* (Cooke) Sacc. in upland areas. *Msc Thesis, University of Agricultural Sciences Dharwad*52-53

Pandey, S. (2014).Invitro studies of various culture media, PH, Carbon and Nitrogen sources on growth of *M.oryzae* causing rice blast. *India Journal of Tropical Biodiversity,* 22(2), 194-198

Quroshi, S. U. and Meah, M. B. (1991).Studies on physiological aspects of *Botryodiplodiatheobromae*Pat., causing stem-end rot of mango. *Bangladesh Journal of Botany*, 20(1), 49-54.

Saha, A., Mandal, P., Dasgupta, S. and Saha, D. (2008). Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodiatheobromae* (Pat.). *Journal of Environmental Biology*, 29(3), 407-410

Scardaci, S. C., Webster, R. K., Greer, C. A., Hill, J. E., William, J. F., Mutters, R.G., Brandon, D. M., McKenzie, K. S. and Oster, J. J. (1997). *Rice blast: A new disease in California*. Agronomy Fact Sheet Series. Department of Agronomy and Range Science, University of California, Davis. http://agronomy.ucdavis.edu/uccerice/AFS/agfs0297.htm. Retrieved; November 2015

Seebold, K.W., Datnof, J. L. E., Correa-Victoria, F. J., Kucharek, T.A. and Snyder, G.H. (2004). Effects of Silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Diseases,* 88, 253-258.

Sharma, G. And Pandey, R. R. (2010). Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. *Journal of Yeast& Fungal Research* 1(8), 157-164

St-Germain, G. And Summerbell, R. (1996).Identifying Filamentous Fungi – *A Clinical Laboratory Handbook*, 1st Edition. Star Publishing Co., Belmont,California.

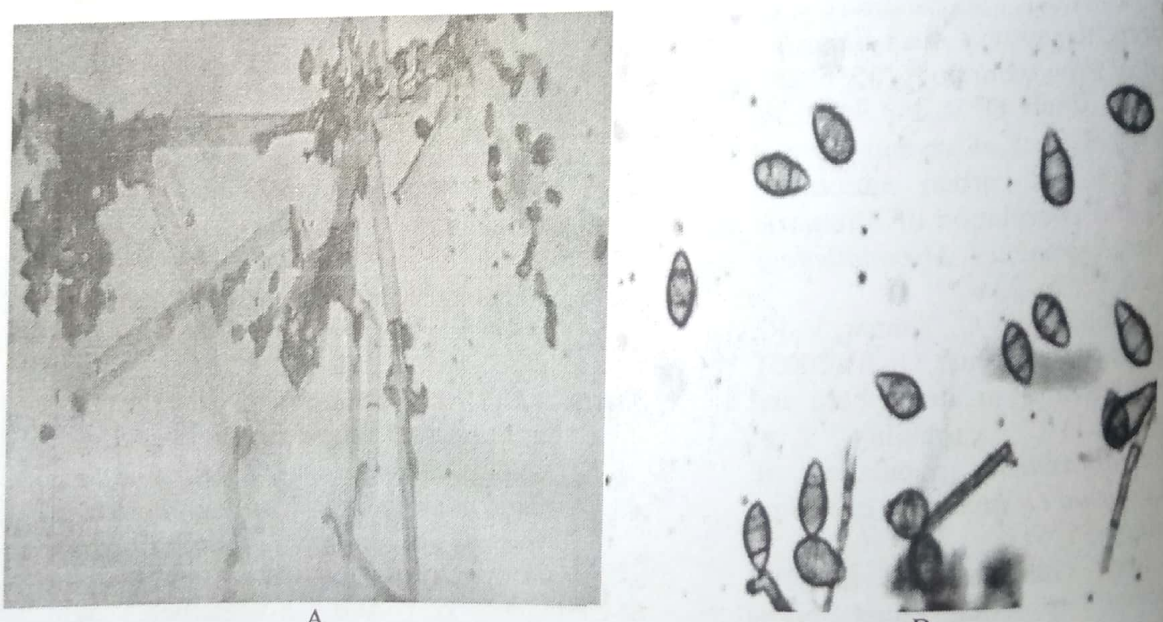
Subramania, G., Murugesan, R. and Lydia, O. (2013). Spot diagnosis of fungicide (Carbendazim) resistance in rice using PCR with reference to

Pyriculariaoryzae; *International journal of Science, Environment and Technology*, 2(5), 1039-1059

Tondge, P. R., Berry, P., Hebber, G., Samuel, J. H., Bowers, S., Weise, E. Nyemb, D., Begonde, J. F andFontem, D. (2006). Bioassay of Geniculesporium species for Phytophthoramegaarya, biological control on cocoa pod hus pieces. *African Journal of Biotechnology*, 8, 648-652

Xiao, C. L. andSitton, J. W. (2004).Effects of culture media and environmental factors on mycelial growth and pycnidial production of *Potebniamycespyri*. *Mycological Research*, 108 (8), 926-932

Zhae, S. and Simon, F. S. (2006). Effect of culture media, temperature, pH and bio-herbicidal efficacy of *Phomaexigua*, a potential biological control for salal (*Gaultheria shallon*).*Biocontrol Science and Technology* 6: 1043-1055



A B

Plate I; A: Conidia attached to conidiophores, B: Conidia showing the septate/ cross wall

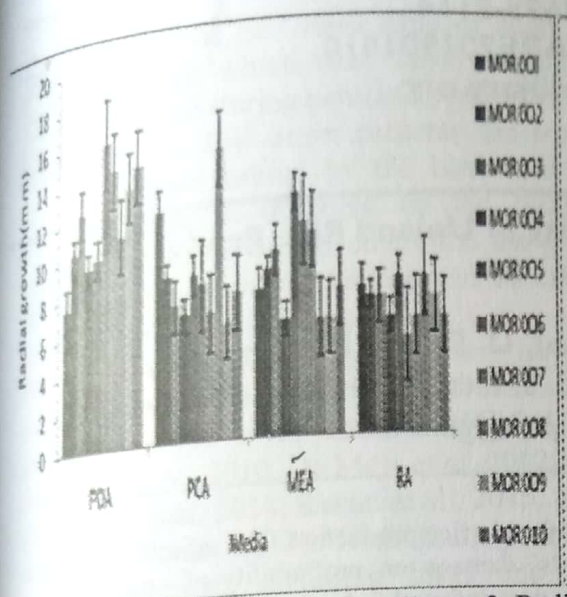


Figure 1; Radial growthat Day 1

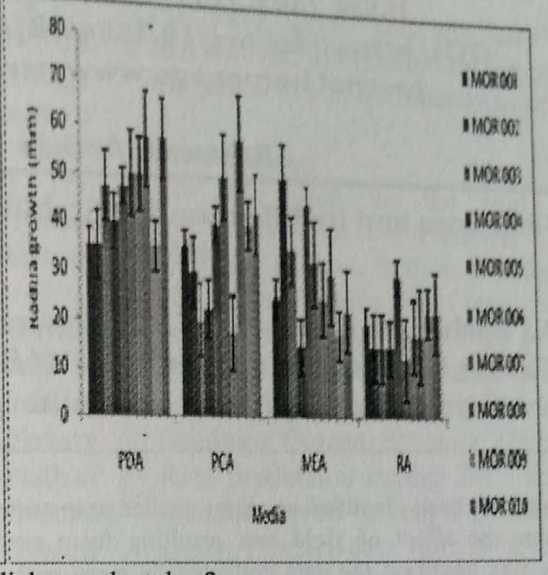


Figure 2; Radial growth at day 2

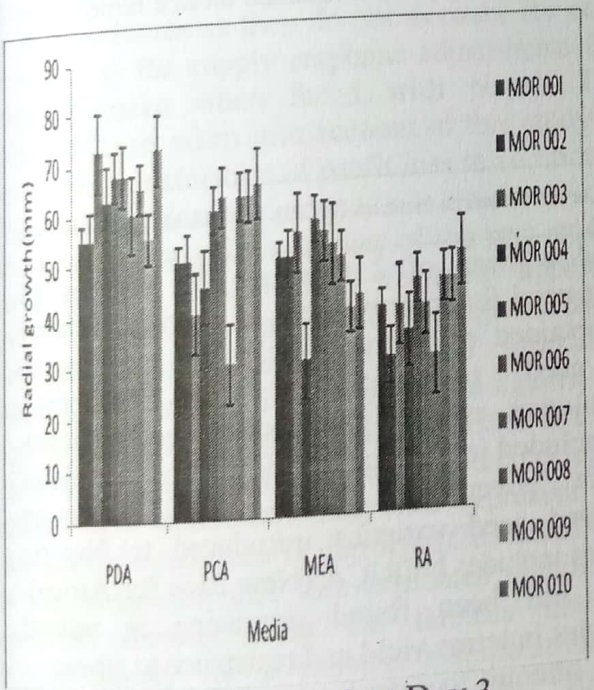


Figure 3; Radial growth at Day 3

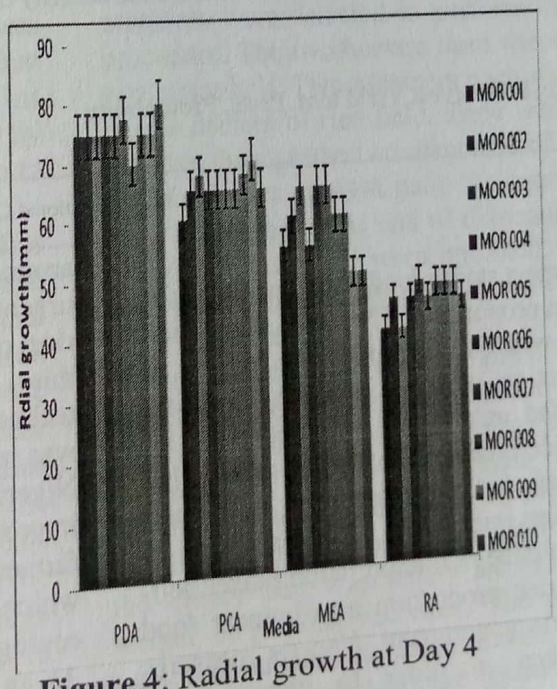


Figure.4; Radial growth at Day 4

Footnote: Potato Dextrose Agar = PDA, Potato Carrot Agar = PCA, Rice Agar = RA, Malt Extract Agar = MEA), *Magnaportheoryzae*=MOR