

INFLUENCE OF CULTURE MEDIA AND LIGHT REGIMES ON THE GROWTH OF HELMINTHOSPORIUM FULVUM

ABSTRACT

The effects of different culture media and light regimes on the growth of *H. fulvum* were investigated. The growth of the fungus was monitored on five culture media. They include potato dextrose agar, yeast extract agar, nutrient agar, corn meal agar and Czapek-dox agar media. The results on the effect of culture media indicated that potato dextrose agar, Czapek-Dox agar and nutrient agar media were the best media for the growth of *H. fulvum*. While corn meal agar medium supported the least growth of the fungus. It was also discovered that there is no significant difference in the growth of the fungus under the light regimes tested. The results showed that light has no effect on the growth of *H. fulvum*.

Key words: Media, Light, Growth, *Helminthosporium fulvum*

INTRODUCTION

Helminthosporium fulvum belongs to the family Dematiaceae. They are species of worldwide distribution. The fungus is black in colour with erect conidiophores, bearing large multiseptate, smooth and cylindrical conidia. The fungus causes several diseases of plants such as tomatoes, rice, wheat, oats, barley and sugar cane. Chinoko and Naqvi (1989) reported *H. fulvum* as the most pathogenic fungus associated with spoilage of tomato fruits in southern Nigeria. The fungus was also found to cause preharvest rot of tomato fruits in Sokoto (Ibrahim, 2002).

The growth of fungi is controlled by many factors. Culture media and light are some of the important factors influencing the growth of fungi. Every living organism requires food for its growth and reproduction and fungi are not an exception. Culturing of fungi under laboratory conditions implies that the medium should contain all the essential elements and compounds they required for growth and other life processes. However, no medium is equally suitable for all fungi.

Ibrahim (2002) reported that the best growth of *Monascus ruber* was obtained in potato dextrose agar than in Czapek-Dox agar medium. Potato dextrose agar and Sabouraud dextrose agar media were found to be most suitable for the growth of *Fusarium oxysporum*, *Clypeopycnis auruginascens* and *Aschochyta* sp (Omkathoum, 2006; Selvi and Sivakumar, 2013). Similarly, Alam et al. (2001) and Suleiman et al. (2013) obtained best growth of *Butryodiplodia theobromae* and *Phythium aphanidermatum* on potato dextrose agar medium.

Muhammad et al. (2005) discovered that the growth of *Phytophthora cactorum* was best in continuous light condition as compared to continuous dark and 12 hour light alternating with 12 hour dark. The exposure of *Alternaria alternata* to alternate 12 hour light and 12 hour dark resulted in maximum growth as compared to continuous light or dark (Huballi et al., 2010). It was discovered by Abubakar (1985) that light had no effects on the growth of *Aspergillus niger*. Light had also been found to have no effect on growth of *Verticillium dahliae*, *Rhizoctonia solani* and *L. theobromae* (Kazanda et al., 2006; Saha et al., 2008).

The aim of this paper was to investigate the effect of culture media and light on the growth of *Helminthosporium fulvum*.

MATERIALS AND METHODS

Isolation of fungus

The surface sterilized rotten tomato fruits were cut open with sterile scalpel. Pieces were cut out from the freshly exposed areas of the advancing margin of the affected tissues. These were then placed individually and aseptically on prepared potato dextrose agar (PDA) plates and incubated at room temperature under day/night light regime for 5 days. The isolates were subcultured to obtain pure culture of the isolates. The isolates were identified by reference to Evin (1969) identification scheme and *H. fulvum* was among the isolated fungi. The fungus was then used for growth studies.

Effect of different culture media on growth of *H. fulvum*.

The culture media used are potato dextrose agar (PDA), yeast extract agar (YEA), nutrient agar (NA), corn meal agar (CMA) and Czapek-Dox agar (CDA) media. The required powder of each was made into a harmonized suspension with distilled water to form a litre medium. The media were sterilized in an autoclave at 121 °C for 15 minutes. The agar media were poured in sterilized petri dishes. They were inoculated with 2mm agar discs of 7 days old culture of *H. fulvum* at the center and incubated at 30 °C for 4 days. Three replicate plates were harvested daily per media. Mycelial growth was determined by measuring the extent of growth along several radii from the point of inoculation. The average mean values were determined.

Effect light regimes on growth of *H. fulvum*

Sterilized Petri dishes containing potato dextrose agar medium were inoculated with 7 day old culture of *H. fulvum*. Replicate plates were incubated under two light regimes; continuous darkness and alternating 12 hours light and 12 hours darkness. The set up were incubated at 30 °C for 4 days. Mycelial growth was determined by measuring the extent of growth along several radii from the point of inoculation. The growth was assessed daily.

Statistical analysis

The data obtained was analyzed using χ^2 distribution analysis at 0.05% level of significance.

RESULTS AND DISCUSSION

The present study revealed that all the culture media tested supported the growth of *H. fulvum* (Table 1). By the end of one day incubation period potato dextrose agar and nutrient agar media yielded the best growth followed by yeast extract agar media and least growth was recorded in Czapek-Dox agar medium. The same trend was maintained at end of 2 day incubation period. The growth of fungus was found to be highest in potato dextrose agar medium after 3 days of incubation. It was followed by growth in yeast extract and nutrient agar media respectively. Corn meal extract agar medium exhibited the least growth. At the end of 4 days incubation period the growth in potato dextrose agar, Czapek-Dox agar and nutrient agar medium were the best and comparable. The lowest growth was recorded in corn meal agar medium (Table 1). This shows that *H. fulvum* was able to produce sufficient growth on a wide range of media. Similarly, it was discovered that potato dextrose agar medium supported the best growth of *Pycularia grisea*, *Trichoderma terrean*, *Colletotrichum gloeosporoides* and *Beaveria bassiana* (Mello et al., 2004; Santhamizhelsvia et al., 2010; Kibar and Peksen, 2011; Netam et al., 2013). In another investigation it was also found to yield maximum mycelial growth of *Metarrhizium anisopliae*, *B. bassiana*, *B. brongniatii*, *Lasiodiplodia theobromae* and *Fusarium pallidoroseum* (Letchoumanne and Manisegar, 1996; Sharma et al., 2002; Saha et al., 2008).

On the effect of light on growth of the fungus observable growth was first noticed after 24 hours of incubation. However no significant differences were observed in the growth of the fungus under the two light regimes. This is an indication that the light regimes tested have no effect on the growth of *H. fulvum* cultured on potato dextrose agar medium (Table 2). Similarly Abubakar (1985) reported that light have no effects on the growth of *Aspergillus niger*. Light had also been found to have no effect on growth of *Verticillium dahliae*, *Rhizoctonia solani* and *L. theobromae* (Kazanda et al., 2006; Saha et al., 2008). Likewise, Shehu and Bello (2011) observed that light had no effect on growth of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. oryzae* and *A. candidum*. But this is contrary to the finding of Huballi et al. (2010) who discovered that *Alternaria alternata* produced better growth when cultured in alternate (12 hrs light and 12 hrs darkness) light regimes than in continuous light or total darkness. Likewise Muhammad et al. (2005) reported that *Phytophthora cactorum* when cultured in light produced better growth than when cultures are kept in total darkness. Also Anukumar (2006) and Hassanzedah (2012) reported that alternate 12 hour light and 12 hour dark supported best growth of *Alternaria alternata*, *A. solani*, *Anthrobotrys oligospora* and *Trichoderma hirsutum*.

CONCLUSION

The results of this study revealed that the growth of *H. fulvum* was influenced by different culture media and potato dextrose agar, nutrient agar and Czapek-Dox agar media were the best media for the growth of this fungus. But light regimes tested showed no effect on

the life of *H. fulvum*. Further studies will be carried out to expose the effects of other factors on the growth of this pathogenic fungus.

ACKNOWLEDGEMENTS

The authors are thankful to Mal. Abdurrahman Barau of mycology laboratory Usmanu Danfodiyo University, Sokoto for allowing us use their laboratory facilities and his technical assistance during the conduct of the research work.

REFERENCES

1. Abubakar, A. (1985). Physiological studies on *Aspergillus niger* van Tiegham causing storage rots of onion (*Allium cepa*) in Nigeria. M.Sc. Thesis, University of Sokoto, Nigeria.
2. Alam, M. S., Begum, M. F., Sakan, M. A. and Islam, M. C. (2001). Effect of temperature, pH, light and media on growth, sporulation, formation of pigment and pycnidia of *Butryodiplodia theobromae*. *Pakistan Journal of Biological Sciences* 1: 1224-1227
3. Anukumar, K. (2006). Studies on *Alternaria solani* causal agent of early blight of tomato. M. Sc. (Ag) Thesis, University of Agricultural Science Dharwad
4. Chinoko V. D. and Naqvi S. H. Z. (1989). Studies on fungi associated with postharvest rot of tomato (*Lycopersicon esculentum*) in southern Nigeria. *Nigerian Journal of Botany*: 2,9-19.
5. Evin, H. B. (1969). *Atlas and Manual of Plant Pathology*. Plenum press, New York, London: 116 - 254
6. Hassazadeh, M., Muhammadifer, M., Sahebany, S. and Etabarian, H. R. (2012). Effect of cultural conditions on biomass production of nematophagous fungi as biological agents. *Egyptian Acad. Jour. Biol. Sci.* 5(1):115-126
7. Ibrahim, M. (2002). Physiological studies on some fungal isolates associated with preharvest rot of tomato (*Lycopersicon esculentum* (Mill) var *pyreformeae* Alefpea tomato) fruits in selected local government areas of Sokoto state, Nigeria. M.Sc. Thesis, Usmanu Danfodiyo University, Sokoto.
8. Kazanda, M. A., Rajapat, A. Q. and Sharzad, S. (2006). Effect of media, temperature, light and inorganic fertilizer on invitro growth and sporulation *Lasioidiplodia theobromae* isolated from mango. *Pak. Jour. Bot.* 38 (3): 885-889
9. Letchoumanne, S. and Manisegar, S. (1996). *Fusarium pallidoroseum* on rice leaf fold. *Indian Journal of Entomology*, 58 (4): 364- 368
10. Mello, A. F. S., Machado, A. C. Z. and Bedendo, I. P. E. (2004). Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperature and light regimes. *Sci. Agric. (Piracicaba Braz)* 61 (5): 542-544
11. Muhammad, I. F., Abdulbasit, A. G., Mohammad, A., Muhammad, H. N. and Jaffar, A. K. (2005). Effect of light/darkness, nitrogen and different fungicides on the growth of *Phytophthora cactorum* causing root rot of apricot. *Journal of Applied and Environmental Science* 1(2):32-35
12. Netam, R. S., Bahadur, A. N., Tiwari, R. K. S. and Tiwari, U. (2003). Effect of different culture media, carbon sources, nitrogen sources, temperature and pH level on growth and sporulation of *Pycularia grisea* isolate from finger millet. *Research Journal of Agricultural Sciences* 4(1) : 83-89
13. Omkathoum, H. K. (2006). Factors affecting growth and pynidial production in aquatic pycnidial fungi. *Journal of Agriculture and Social Sciences* 2 (4): 234-237
14. Saha, A., Mandal, P., Dasgupta, S. and Saha., D. (2003). Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasioidiplodia theobromae* Pat Griffon Maubi. *Jour. Env. Biol.* 29 (3): 407-410
15. Santhamizhlselvan, P., Alica, J., Sujeeta, R. P. and Jeyalakshi, A. (2010). Growth, sporulation and biomass production of native entopathogenic fungal isolates on suitable media. *Journal of Biopesticides*, 3 (2): 466 -469
16. Selvi, K. V. and Silvakumar, T. (2013). Isolation, identification and characterization of *Fusarium* species from mangrove habitat of Pichavaram, Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences* 3(1): 33- 49
17. Sharma, S, Gupta, R. B. C. and Yadava, C. P. S. (2002). Selection of suitable media for mass multiplication entamophagus fungal pathogens. *Indian Journal of Entomology* 6(3); 2254-2261
18. Shehu, K. and Bello, M. T. (2011). Effect of environmental factors on the growth of *Aspergillus* species associated with stored millet grains in Sokoto. *Nigerian Journal of Basic and Applied Sciences*, 19 (2) : 218-223
19. Suleiman, M. N., Emua, S. A. and Ayodele, S. M. (2011). Growth and physiological studies on root rots fungus of cowpea. *European Journal of Experimental Biology* 1(4): 181- 187

Days	Culture media/Mycelial growth (mm)				
	PDA	YEA	NA	CMA	CDA
1	25	24	25	18	15
2	30	27	29	27	26
3	39	38	38	32	37
4	45	45	45	39	45

$$X^2 = 1.25, df = 12, P < 0.05$$

Table 1: Effect of culture media on growth of *H. fulvum*

Days	Light regime/Mycelial growth (mm)	
	Total darkness	alternate light and darkness
1	23	18
2	33	30
3	45	45

$$X^2 = 0.33, df = 2, P > 0.05$$

Table 2: Effect of light regimes on growth of *H. fulvum*

¹K. Shehu and ²M. Ibrahim

¹Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto

²Department of Biology, Shehu Shagari College of Education, Sokoto