

Factors Affecting Growth and Pycnidial Production of Aquatic Pycnidial Fungi

OMKATHOUM HASSAN KHATTAB

Department of Plant and Microbiology, Helwan University, Cairo, Egypt

E-mail: Ekhtab555@yahoo.co.uk

ABSTRACT

Clypeopycnis aeruginascens and *Ascochyta* sp. were isolated from dead twigs of *Phragmites australis*, *Cyperus papyrus* and *Eclipta prostrata*. In this study the effects of temperature, pH, culture media and light on mycelial growth and pycnidial production of *C. aeruginascens* and *Ascochyta* sp. were evaluated. The fungus grew from 4 - 30°C, with optimum growth at 25°C and no growth and pycnidial production at 40°C and 50°C. The fungus grew at pH 5 - 7, while no mycelial growth and pycnidial production was observed above pH 7 even after 10 days of incubation. Pectin agar and gelatin agar media were not suitable for either mycelial growth or pycnidial production. PDA, MEA and Dox's media were most suitable for mycelial growth and pycnidial production of aquatic pycnidial fungi (*C. aeruginascens* & *Ascochyta* sp.). Pycnidial production was enhanced by fluorescent light. MEA, CDA and PDA media were most suitable for production of pycnidia and conidia under light or dark.

Key Words: Aquatic fungi; Pycnidial fungi; *Clypeopycnis aeruginascens*; *Ascochyta* sp.

INTRODUCTION

Aquatic pycnidial fungi occur commonly on a submerged shoots of aquatic macrophytes such as *Phragmites*, *Carex* and *Schoenoplectus*. These hosts provide substrates for many pycnidial fungi (Webster & Descals, 1981). With the exception of studies by Kim *et al.* (2005), McQuilken *et al.* (1997) and Nebane and Ekpo (1992), little is known about the enzymatic capabilities and decomposition activities of these fungi.

Ascochyta causes leaf, stem and pod spot on *Lathyrus ochrus*, *Cyperus sp.* *Phaseolus vulgaris*, *Pisum sativum* and *Trifolium subterraneum*. The type species of *Ascochyta* has been fully described and illustrated by Punithalingam and Holiday (1972) and a detailed study of its conidiogenesis has been reported by Boerema and Bollen (1975). *Clypeopycnis aeruginascens* petrak, presents on dead twigs of *Phragmites australis*, *Cyperus papyrus*, *Rumex dentatus*, *Rorripa palustris* and *Persicaria salicifolia*.

Morgan-Jones *et al.* (1972) published a partial revision of the genus *Bartilinia tassi* labeling the conidiogenous cells as annellides. However, NagRoj (1993) re-examined the type and other species in the genus using light microscope and found that the ontogeny of conidiogenesis is holoblastic. *Bartalima robillardoides* Tassi is one of the major leaf spots for tamarinal (*Tamarindus indica* L.) an economically important tree in Asia, Africa and South America (Gunasena & Hughes, 2000). The objective of this study was to provide information on effects of culture media and various environmental factors including temperature, pH and light on mycelial growth and pycnidial production of *Clypeopycnis aeruginascens* and *Ascochyta* sp.

MATERIAL AND METHODS

Culture Media

1.0% Malt extract agar medium. Malt extract agar (MEA) medium (1%) for isolation of aquatic pycnidia was used with the following composition (g L⁻¹); malt extract 1, agar 15 and distilled water 1000 mL. For control of bacterial growth, 0.1% crystalviolet was added to the warm agar medium (Descals *et al.*, 1977). Antibiotic (1 mL) was pipetted into the bottom of the petri-dish before the warm medium was poured. In another study, 2% MEA was used for growing aquatic pycnidial fungi and measurement of optimum temperature for growth, with the following composition: composition (g L⁻¹): malt extract, 20; agar, 15; distilled water 1000 mL. It is stored in 15 mL amounts in McCartney bottles after sterilization at 121°C for 15 min.

Isolation of pycnidia from twigs. *Clypeopycnis aeruginascens* and *Ascochyta* sp. were isolated from the twigs of *Phragmites australis*, *Cyperus articulatus* and *Cyperus alopecroides* that grow on the edges of the river Nile in Qanater City. Identification of pycnidial fungal colonies, were carried out according to Sutton (1980). Previously collected pycnidia from the edges were observed on twigs incubated for one month at 25°C. A single pycnidium found on twigs was crushed in a drop of sterile water on a flamed microscope slide by using a flamed dissecting needle. Conidia were dispersed in the drop and picked up in a freshly down-out sterile fine pasteur pipette and transferred into 0.1% MEA with 0.1 mL crystalviolet and incubated at 25°C for 24 h in all cases. The residue of pycnidia and conidia on the isolation slide was preserved by adding a drop of Lacto-fuchsin stain and a cover slip.

Effect of temperature on mycelial growth and pycnidial production. To investigate the effect of temperature on the growth of pycnidia and conidia, 1 mL of a spore suspension was added to warm 2% MEA agar medium and poured into Petri-dishes aseptically and the plates were incubated at 25°C for 7 days. Disks of agar mycelium were cut with a flamed cork-borer and transferred to petri-dishes containing 2% MEA. These plates in triplicate were incubated at 4, 15, 25, 30, 40 and 50°C for 10 days. Colony diameter was measured daily along two axis and the mean values were recorded. Pycnidia development and colony morphology were visually evaluated weekly.

Effect of pH on mycelial growth and pycnidial production. After preparation of the malt extract agar media, their suitable volumes were adjusted at different pH 3, 4, 5, 6, 7, 8 and 9 using 1 N HCl or 1 N NaOH. Media were sterilized, poured in petridishes and incubated at 25°C for 7 days. The growth of conidia was assessed by measuring the diameter of colony and production of pycnidia and colony morphology were recorded weekly.

Effect of culture media on mycelial growth and pycnidial production. Mycelial growth and production of pycnidia were examined by measuring the colony radial growth rates of individual strains of *C. aeruginascens* and *Ascochyta* sp. grown on the following culture media: Potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan), 2% MEA media, Czapek-Dox agar (CDA): 2 g NaNO₃, 1 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 0.01 g FeSO₄·7H₂O, 30 g sucrose and 15 g agar in 1 L distilled water, Protease media (GA) (Ammar *et al.*, 1991): gelatin 10; agar 20; 0.2 M; phosphate buffer; 1 L distilled water; cellulose media (Cl) (Bland & Douglas, 1977): Urea, 0.3; KH₂PO₄, 2; (NH₄)₂SO₄, 1.4; MgSO₄·7H₂O, 0.3; Cellulose, 5; Peptone, 1; FeSO₄·7H₂O, 1 m; MnSO₄·7H₂O, 1.6 m; ZnSO₄·7H₂O, 1.7 m; COCl₂, 2 m and Distilled water, 1 L. pectolytic medium (CVP) (Liao & Wells, 1987): glycerol, 0.5; yeast extract, 0.2; MgSO₄·7H₂O, 0.292; CaC₁₂·H₂O, 0.0147; poly galacturonic acid, 0.4; agar, 1.5; distilled water, 100 mL. Plates containing one of these six media were inoculated in triplicate in darkness at 25°C. The colony diameters and other characteristics in each plate were measured as described above.

Effect of culture media and light. The effect of light on mycelial growth and pycnidial production was evaluated on the same six media used in the media effect studies. Plates with two isolates in triplicate were incubated at 25°C, under fluorescent light for 7 days. Colony characteristics were measured as described above. Pycnidia development on different media was evaluated weekly.

Statistical analysis. Experimental design was completely randomized design in factorial arrangement. LSD was used for comparing treatment means (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

Effect of temperature. The rate of mycelial growth of two

isolates followed similar trends in response to changes in temperature (Fig. 1). The rate of mycelial growth increased as temperature increased up to 25°C and decreased rapidly afterwards. Slight changes in colony morphology were observed at 4 and 30°C, being slower at 4°C with fluffy aerial mycelium. At 30°C colony sometimes was not completely circular, but with thin mycelium. The average growth at this temperature was 3 - 4 cm. Optimum growth occurred at 25°C for two isolates, with an average growth of 7.1 cm d⁻¹. No colony growth was noted at 40°C and 50°C after 7 days of incubation.

The rates of mycelial growth of the 2 species were significantly different ($P < 0.001$), which was temperature dependent. The pycnidia were produced on MEA medium and were scattered on the plate after 7 days at 25°C. These pycnidia, while they were produced after 10 days at 15°C and 30°C. These results were in agreement with that of Xiao and Rogers (2004). They tested *Sphaeropsis pyripitrescens* mycelial growth response at temperatures 0 – 40°C and noted 20°C as optimal mycelial growth temperature. Kim *et al.* (2005) grew six isolates of *S. pyripitrescens* from -3° to 25°C and noted optimum growth at 20°C whilst no growth at 30°C. Zhao *et al.* (2006) noted differential responses of *Ramularia rhei* and *Ascochyta rhei* to temperature. *R. rhei*

Fig. 1. Effect of temperature on radial of *Clypeopycnis aeruginascens* and *Ascochyta* sp. On malt agar media values are the means of data from two runs of the experiment (Three replicate at each temperature)

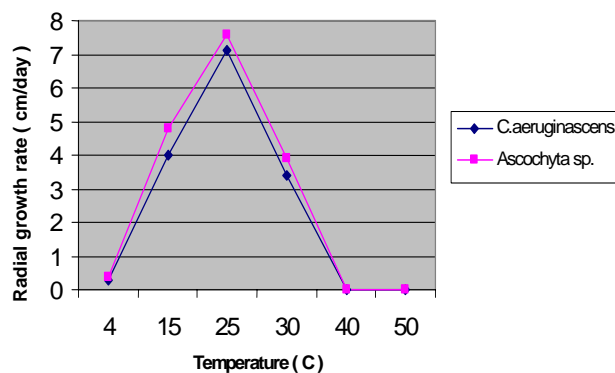


Fig. 2. Effect of pH on radial growth of *Clypeopycnis aeruginascens* and *Ascochyta* sp. on malt agar media values are the means of data from two runs of the experiment (Three replicate at each pH)

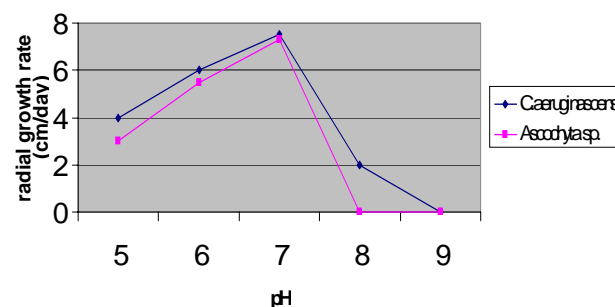


Table I. Effect of different media on mycelial growth and pycnidial production. Colony diam. (cm) after growth for 7 days on the different media

Media Isolates	PDA		CDA		MEA		CL		GA		CVP	
	My.	Py	My	Py	My	Py	My	Py	My	Py	My	Py
<i>Clypeopycnis aeruginascens</i>	6.5	+	6.5	+	8.2	+	3	-	2.2	-	3.5	-
<i>Ascochyta</i> sp.	8.4	+	7.5	+	7.8	+	3.2	-	3.1	-	2.2	-

PDA= potato dextrose agar; CDA= Czapek-Dox agar; MEA= malt extract agar; CL = cellulose agar; GA = proteolytic agar; CVP = pectolytic agar; My= mycelial growth; Py= pycnidial production; - =non present; + = present

Values are the means of data from the two runs of the experiment (three replicate plates of each medium)

was better adapted to > 25°C, with an optimum growth around 20°C, whereas *A. rhei* was more adapted at > 15°C, with an optimum > 25°C. Overall, conidia of *R. rhei* germinated and subsequent colonies grew at greater rates than those of *A. rhei* on leaf discs at < 25°C. Roger *et al.* (2006) found that the number of pycnidia of *Mycosphaerella pinodes* on pea leaves increased with from 5 to 20°C, but decreased between 20 and 30°C.

Effect of pH. The two isolates *Clypeopycnis aeruginascens* and *Ascochyta* sp. followed similar trends in response to changes in pH (Fig. 2). Optimum growth occurred at pH with an overall average of 7.5 cm colony diameter after 7 days of incubation at 25°C. A notable decline in growth occurred when pH increased from 7 to 9 for *C. aeruginascens*, but *Ascochyta* sp. showed no growth at pH 8 and 9. The pycnidia produced on MEA were scattered after 7 days at pH 5, 6 and 7. The optimum pH for mycelial growth of most fungi is 5 - 6.5 (Ingold, 1973). Rodriguez *et al.* (1985) reported that optimum growth of *Sphaeropsis tumefaciens* is pH 4. Kim *et al.* (2005) recorded that optimum pH for mycelial growth of *S. pyripitrescens* was 3 - 4. However, the effect of pH on mycelial growth and conidial germination was not significant from pH 5 to 10 (Zhae & Simon, 2006).

Effect of culture media. The radial mycelial growth rates of *C. aeruginascens* and *Ascochyta* sp. were significantly ($p < 0.0001$) affected by culture media. *C. aeruginascens* and *Ascochyta* sp. were grown on all media (Table I). MEA, PDA and CDA were most favorable for rapid growth mycelium and pycnidial production of both the isolates. At 25°C, colonies on these three media reached the edge of the plates after 7 days of incubation. The fungus formed circular, white and compact colonies with few or no aerial hyphae. *Ascochyta* sp. had highest radial growth on PDA. On VPC, CL and GA did not favor radial mycelial growth. The mycelial growth was poor with thin mycelium, although two isolates showed no pycnidia growth on CL, CVP and GA media. Kim *et al.* (2005) found that cornmeal agar was not suitable for either mycelial growth or pycnidial production of *Sphaeropsis pyripitrescens*, although it is suitable for mycelial growth of other *Sphaeropsis* species such as *S. sapinea* (Swart *et al.*, 1991) and *S. tumefaciens* (Rodriguez *et al.*, 1985).

Under both light and dark, the production of pycnidia and conidia was noted on all media except on GA and CVP

media for *C. aeruginascens* and *Ascochyta* sp. (Table II). Although the fungus grew on all media, pycnidia were not produced on CVP, CL and GA media. The fungus grown on MEA, PDA and CDA media started to form pycnidia after 7 days of incubation under dark and 5 days under light. On PDA, pycnidia of two isolates were aggregated in the center of the plate in the dark, whereas they were more uniformly under light. On MEA, Pycnidia were scattered on the plate under light or in dark CDA media, produced white, fluffy aerial mycelium at the edge of the plate after 10 days of incubation. Pycnidia formed only on the fluffy mycelial masses under light. In the dark after 2 weeks of incubation, mycelia covered entire plates CL, CVP and GA media. It is that light exposure and types of media enhance the production of pycnidia.

In conclusion, enhanced production of pycnidial under fluorescent light was dependent on types of media generally agrees with reports for some fungi in Sphaeropsidales (Nebane & Ekpo, 1992; McQuilken *et al.*, 1997). Kim *et al.* (2005) reported that the effect of light on pycnidial production was medium dependent and pycnidial and conidial production on OMA was reduced under continuous light as compared to complete darkness. Fluorescent light significantly enhanced sporulation of the fungus on most agar media tested (Zhae & Simon, 2006). They found that the type of culture media significantly affected mycelium growth, sporulation and conidial germination.

Table II. Radial growth (cm/day) and pycnidial production of *Clypeopycnis aeruginascens* and *Ascochyta* sp. on six media at 25°C, under light and dark condition

Media	C. aeruginascens				Ascochyta sp.			
	Light		Dark		Light		Dark	
	My.	Py	My.	Py	My.	Py	My.	Py
MEA	8	+	8.2	+	7.5	+	7.8	+
PDA	7.5	+	6.5	+	8.7	+	8.4	+
CDA	7.8	+	6.5	+	8.1	+	7.5	+
CVP	2.8	-	3.5	-	5.1	+	2.2	-
CL	4	-	3	-	4.5	-	3.2	-
GA	3.6	-	2.2	-	3.2	-	3.1	-

MEA = malt extract agar; PDA = potato dextrose agar; CDA = Czapek-Dox agar; CVP = pectolytic medium; CL = cellulose agar medium, GA = proteolytic medium, My = mycelial growth, Py = pycnidial production, - = non present, + = present.

Values are the means of data from the two runs of the experiment (three replicate plates of each medium)

REFERENCES

- Ammar, M.S., S. El-louboudy and U.M. Abdul-Raouf, 1991. Distribution, total viable bacteria and identification of the most potent proteolytic bacterial strains isolated from Aswan city. *Azhar J. Microbiol.*, 11: 224–38
- Bland, S.M. and E.E. Douglas, 1977. Semi-quantitative plate assay for determination of cellulose production by *Trichoderma uiride*. *Appl. Environ. Microbiol.*, 33: 179–83
- Boerema, G.H. and C.J. Bollen, 1975. Conidiogenesis and conidial septation as differentiating criteria between phoma and *Ascochyta*. *Persoonia*, 8: 111–44
- Descals, E., J. Webster and B.J. Dyko, 1977. Taxonomic studies on aquatic hyphomycetes. I *Lemoniera* de Wildeman. *Trans. British Mycol. Soc.*, 69: 89–109
- Gunaseena, H.P.M. and A. Hughes, 2000. *Tamarind*. International Center for Underutilised Crops, Southampton, U.K
- Ingold, C.T., 1973. *The Biology of Fungi*, P: 176. Hutchinson and Co. Ltd. London
- Kim, Y.K., C.L. Xiao and J.D. Rogers, 2005. Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. *Mycol.*, 97: 25–32
- Liao, C.H. and J.M. Wells, 1987. Association of pectolytic strains of *Xanthomonas compestris* with soft rots of fruit and vegetables at retail markets. *Phytopathol.*, 77: 418–22
- McQuilken, M.P., S.P. Budge and J.M. Whipps, 1997. Effects of culture media and environmental factors on conidial germination, pycnidial production and hyphal extension of *Coniothyrium minutans*. *Mycol. Res.*, 101: 11–7
- Morgan-Jones, G., T.R. NagRaj and B. Kendrick, 1972. Genera coelomycetarum. V. *Alpakesa* and *Bartalinia*. *Canadian J. Bot.*, 50: 877–82
- NagRaj, T.R., 1993. *Coelomycetous Anamorphs with Appendage-bearing Conidia*. Mycologue Publication, Waterloo, Ontario
- Nebane, C.L.N. and E.J.A. Ekpo, 1992. Effect of culture media, temperature and light on radial growth and pycnidium production of cowpea isolates of *Phoma bakeriana*. *Ann. Appl. Biol.*, 121: 537–44
- Punithalingam, E. and P. Holiday, 1972. *CMI Descriptions of Pathogenic Fungi and Bacteria* Nos. 333, 335, 339.
- Rodriguez, S.D., R. Rodriguez and P.L. Melendez, 1985. Effect of culture media, temperature and pH on growth of *Sphaeropsis tumefaciens*. Hedges. *J. Agric. Univ. Puerto Rico*, 69: 391–6
- Roger, C., B. Tivoli and L. Huber, 2006. Effect of temperature and moisture on disease and fruit body development of *Mycosphaerella pinodes* on pea (*Pisum sativum*). *Pl. Pathol.*, 48: 1–9
- Snedecor, G.W. and W.G. Cochran, 1980. “*Statistical Methods*” 7th edition, pp: 225–69. Iowa State University Press, Ames, Iowa
- Sutton, B.C., 1980. *The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Commonwealth Mycological Institute, Kew, U.K
- Swart, W.J., M.J. Wingfield, M.A. Palmer and R.A. Blanchette, 1991. Variation among South African isolates of *Sphaeropsis sapinea*. *Phytopathol.*, 81: 489–93
- Webster, J. and E. Descals, 1981. Morphology, distribution and ecology of conidial fungi in fresh water habitats. In: Cole, C.G. and W.B. Kendrick (eds.), *The Biology of Conidial Fungi*, pp: 295–355. Academic Press, New York and London
- Xiao, C.L. and J.D. Rogers, 2004. A postharvest fruit rot in d’Anjou pears caused by *Sphaeropsis pyriputrescens*. *Pl. Dis.*, 88: 114–8
- Zhae, S. and F.S. Simon, 2006. Effect of culture media, temperature, pH and bio-herbicide efficacy of *Phoma exigua*, a potential biological control agent for salal (*Gaultheria shallon*). In: *Biocontrol Science and Technology*, 6: 1043–55
- Zhao, Y.B., W. Grout and X. Xu, 2006. Effect of temperature on germination and hyphal growth from conidia of *Ramularia rhei* and *Ascochyta rhei*, causing spot diseases of rhubarb (*Rheum rhaponticum*). *Pl. Pathol.*, 55: 664–70

(Received 25 September 2006; Accepted 10 October 2006)