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Research Paper

# Influence Of Culture Condition And pH On Growth And Production Of Brown Pigment from Alternaria alternata

Microbiology

**KEYWORDS :** *Alternaria alternata,* media, pH, mycelial growth, pigment production

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## ABSTRACT

*Alternaria spp. grows over a wide range of pH, temperature and simple sugars. However, limited reports are available on the combined influence of pH, temperature and simple sugars on mycelial growth and pigment production. In the present investigation, the influence of culture condition and pH was investigated on mycelial growth and production of brown pigment from Alternaria alternata in 6 media at pH 4-9. Among 3 natural and 3 synthetic media investigated for mycelial growth and production of brown pigment from A. alternata, natural medium Sorghum Extract maintained at 28oC ± 2oC under stationary condition favoured maximum mycelial growth and brown production of pigment. Among all pH studied, pH 6 on day 9 supported maximum mycelial growth (12.92 ± 0.03 g L$^{-1}$) in Sorghum Extract medium and maximum brown pigment (14.3 ± 0.86 mg g-1) in Maize Grain Extract.*

## INTRODUCTION

The growth and production of secondary metabolites from micro organisms is predominantly dependant on the composition of growth medium and culture conditions (Bills, 1995). The influence of various types of growth medium and condition is imperative to investigate and produce different secondary metabolites in microbes (Larsen *et al*., 2005). Use of several agro-industrial residues like wheat bran, wheat straw, corn cob, etc. has been explored to design cost effective production processes (Li *et al*.2007a; Katapodis *et al*. 2007; De Souza *et al*. 1999).

In the present investigation, the influence of culture condition and pH was investigated in 3 natural (MGE, GHE, SE) and 3 synthetic media (CS, FB and ME) for maximum mycelial growth and production of pigment from *A.alternata.*

## MATERIALS AND METHODS

### Alternaria culture conditions

*A. alternata*, MTCC 2724 strain was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. The culture was revived on sterile Potato Dextrose Agar (Himedia, India) medium, incubated at 25 °C, and the 21st day mycelial culture served as inoculum for biomass and pigment production studies. The mycelial growth and production of pigment from *A. alternata* MTCC 2724 was studied in 3 Natural media such as MGE, GHE, SE and 3 Synthetic media (CS, ME, FB) maintained at 28°C ± 2°C for 21 days. Erlenmeyer flasks (250 mL) containing 50 mL of respective media were taken individually and the pH value was adjusted to 4, 5, 6, 7, 8 and 9 using 1M HCl and IM NaOH. The flasks containing medium were autoclaved at 121°C for 15 min. The flasks were inoculated with 6 mm mycelial discs of the 21 day old *A. alternata* MTCC 2724 and incubated at 28°C ±2°C at stationary condition. At every three days interval the flasks containing *A. alternata* MTCC 2724 was withdrawn and the mycelial mat was harvested by filtration using Whatman no.1 filter paper. The harvested mycelial mat was processed for the determination of dry weight and pigment content using the method as described by Gadd (1982). All the experiments were maintained in triplicates and the data was analyzed statistically using One-Way Anova.

### Estimation of mycelial dry weight of A. alternata MTCC 2724

Mycelial mat recovered from Whatman no.1 filter paper was washed with distilled water and air dried on a pre weighed filter paper at 40 for 24 h and the dry weight was recorded based on the following formula.

$$DW\ (g\ L^{-1}) = W_2 - W_1$$

where,

DW – Dry weight of the mycelial mat (g L-1)

W1 – Initial weight of filter paper (g)

W2 – Final weight of filter paper with mycelial mat (g)

### Quantification of pigment of A. alternata MTCC 2724

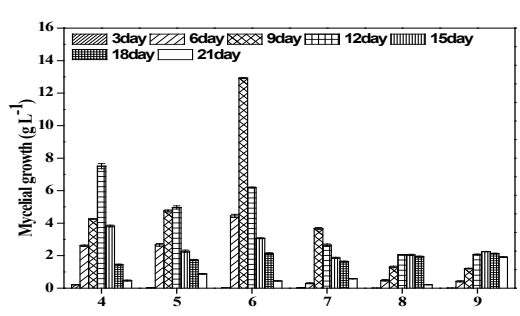
The harvested mycelial mat was air dried at 40 °C for 24 h. The obtained dry mycelium was extracted and estimated for pigment content following the method as described by Gadd (1982). Melanin pigment obtained from Sigma Aldrich served as standard for quantification of the pigment content.

## RESULTS

### Influence of pH on mycelial growth and brown pigment from A. alternata MTCC 2724 in 3 Natural media

The growth and production of pigment by *A. alternata* MTCC 2724 were studied at different pH such as 4, 5, 6, 7, 8 and 9 under 3 natural media. Among 6 different pH, pH 6 in SE medium supported maximum mycelial growth of A. alternata (12.92 ± 0.03 g L-1) on 9th day (Fig. 1) followed by pH 5 on 9th day with mycelial growth (7.59 ± 0.02 g L$^{-1}$) in MGE medium (Fig. 2). At pH 9 on 9th day, the least mycelial growth (4.28 ± 0.01 g L-1) was recorded in GHE medium (Fig. 3). Among 6 different pH, pH 6 on 9th day recorded maximum pigment (14.3± 0.86 mg g-1) in MGE (Fig. 4) followed by pH 9 on 12th day in SE medium (13.40 ± 0.11 mg g-1) and at pH 4 on 12th day least pigment production (12.9 ± 0.17 mg g-1) was recorded in GHE (Fig. 5 & Fig. 6).

**Fig: 1: Effect of pH on mycelial growth (dry wt.) of *A. alternata* MTCC 2724 in SE medium**



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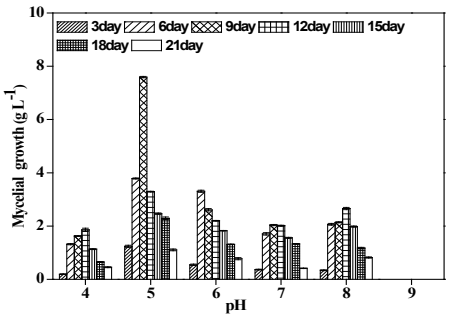
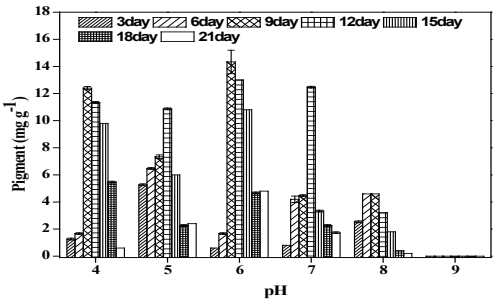



**Fig: 2: Effect of pH on mycelial growth (dry wt.) of** A. alternata **MTCC 2724 in MGE medium**

**Fig: 3: Effect of pH on mycelial growth (dry wt.) of** A. alternata **MTCC 2724 in GHE medium**



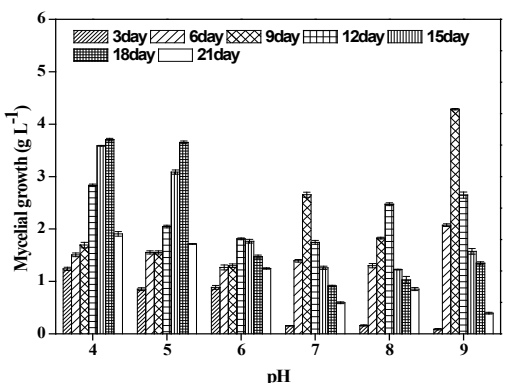




**Fig: 4: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in MGE medium**

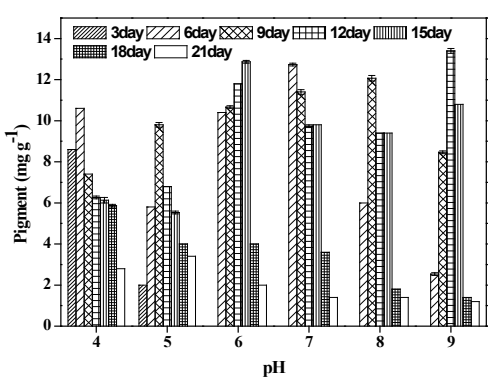



**Fig: 5: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in SE medium**

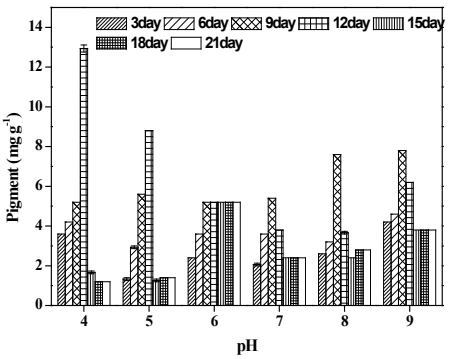



**Fig: 6: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in GHE medium**

**Influence of pH on mycelial growth and brown pigment from** *A. alternata* **MTCC 2724 in 3 Synthetic media**

The growth and production of pigment by *A. alternata* MTCC 2724 were studied at different pH such as 4, 5, 6, 7, 8 and 9 under 3 synthetic media. Among 6 different pH, pH 9 on 12th day supported maximum mycelial growth of *A. alternata* (11.51± 0.04 g $L^{-1}$) in CS (Fig. 7) followed by pH 4 on 12th day with mycelial growth (10.45 ± 0.01 g $L^{-1}$) in FB medium (Fig. 8).

At pH 9 on 18th day the least mycelial growth (7.00 ± 0.02 g $L^{-1}$) was recorded in ME medium (Fig. 9). Among 6 different pH, pH 9 supported maximum pigment production (11.26 ± 0.13 mg $g^{-1}$) on 6th day in FB medium (Fig. 10) followed by pH 4 on 9th day in ME medium (9.93 ± 0.06 mg g-1) and pH 7 in CS medium on 9th day, least pigment production (7.33 ± 0.06 mg $g^{-1}$) was recorded (Fig. 11 & Fig. 12).

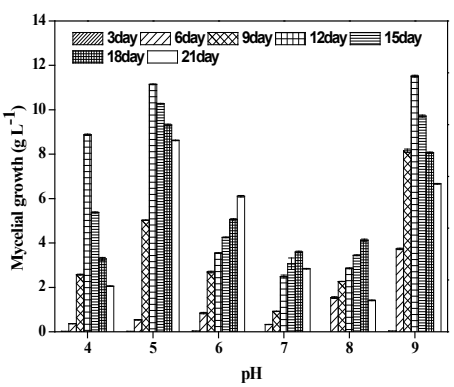



**Fig: 7: Effect of pH on mycelial growth (dry wt.) of** *A. alternata* **MTCC 2724 in CS medium**

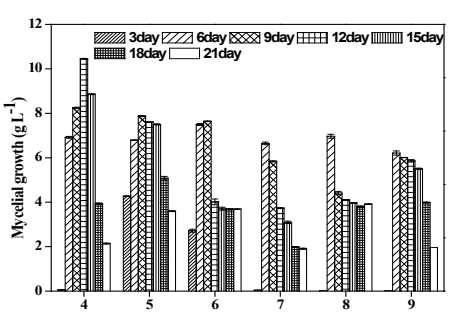



**Fig: 8: Effect of pH on mycelial growth (dry wt.) of** *A. alternata* **MTCC 2724 in FB medium**

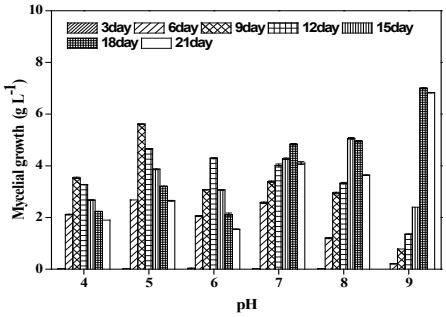



**Fig: 9: Effect of pH on mycelial growth (dry wt.) of** *A. alternata* **MTCC 2724 in ME medium**

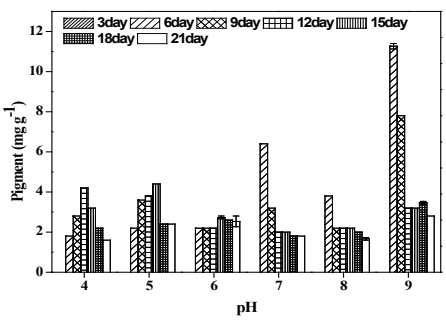



**Fig: 10: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in FB medium**

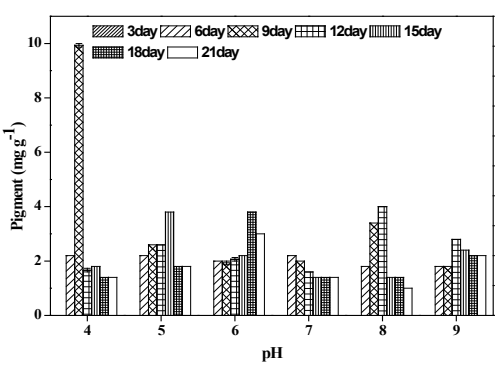



**Fig: 11: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in ME medium**

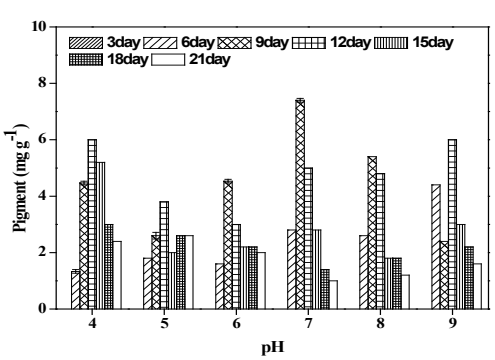



**Fig: 12: Effect of pH on production of pigment from** *A. alternata* **MTCC 2724 in CS medium**

**DISCUSSION**

The growth patterns of fungi on different natural and synthetic media have been investigated by many researchers (Sumathi, 2008 & Chandrasekarenthiran, 2010). Among 5 isolates of *Pycnoporus sanguineus* investigated, *P. sanguineus* (MCRCPS02) exhibited rapid mycelial growth (9.0 cm) on 6th day (Chandrasekarenthiran, 2010). Hamano & Kilikian, 2006 reported highest production of red pigment (20.7 U) in a medium containing 10 g $L^{-1}$ glucose, 5 g $L^{-1}$ corn steep liquor and 7.6 g $L^{-1}$ monosodium glutamate. Conidia of *A. alternata* strains require more nitrogen than carbon for better growth (Siddiqui & Bajwa, 2008) and sorghum grains have been reported to favour better growth and metabolite production in a study conducted by Burroughs *et al*. (1976). In the present investigation, among 3 natural (MGE, GHE, SE) and 3 synthetic media (CS, FB and ME) investigated for maximum mycelial growth and production of pigment from *A. alternata*, natural medium SE favoured maximum mycelial growth (12.92 ± 0.03 g $L^{-1}$) whereas maximum pigment (14.33 ± 0.86 mg g $L^{-1}$) was recorded at pH 6 on 9th day in MGE medium which corroborates with the earlier study conducted by Chandrasekarenthiran 2010 on *P.sanguieneus* where maximum mycelial growth (6.42 ± 0.2 g $L^{-1}$) on 12th day in Potato Dextrose broth whereas maximum production of the pigment (0.90U.g $L^{-1}$) was recorded in Potato Dextrose Yeast Extract broth on 15th day in a study conducted by Sumathi (2008) on *Ganoderma lucidum* where maximum mycelial growth of *G. lucidum* was recorded in natural medium such as maize grain extract among the ten media tested.

**CONCLUSION**

Cultivation processes involving filamentous fungi have been optimized for decades to obtain high product yields (Grimm *et al*., 2005). Submerged culture has potential advantages over solid substrate cultivation for higher mycelial production in a compact space and shorter incubation time (Bae *et al*., 2000). It is evident from the above study that natural medium at pH 6 on day 9 favoured maximum mycelial growth and production of brown pigment as compared to synthetic media. From the above study it is also concluded that the media composition, culture condition and pH should be investigated to design an effective production process.

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