



# Effect of pH on the Mycelial Growth of *Aspergillus niger* and *Aspergillus flavus*

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

*Aspergillus* fungus is well-known for causing a number of secondary plant and food rots that can result in the buildup of mycotoxins, despite their significant economic contribution to the fermentation industries. In the past, even the most fundamental influences on the development and inhibition of significant mycotoxigenic fungi were only partially understood. To overcome this issue, an investigation on the impact of pH on the mycelial development of *Aspergillus* species was conducted. *A. niger* and *A. flavus* were inoculated in PDA medium with a range of pH values from 4.0 to 9.0 under in vitro conditions and then cultured for 7 days at room temperature. Results

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revealed that, the mycelial growth of *A. niger* and *A. flavus* was increased with increase in pH level up to 6 and then became static up to pH level 9 and there was no mycelial growth in pH 4. Hence, it was concluded that the use of alkaline compounds as a way of preventing their proliferation and reproductive processes may be used to inhibit the development and spore production of *A. niger* and *A. flavus*, which can affect the crops.

**Keywords:** *Aspergillus niger*; *Aspergillus flavus*; pH; mycelial growth; acid and alkaline.

## 1. INTRODUCTION

*Aspergillus* is a significant part of daily life, and the genus include the human pathogen *A. fumigatus*, the plant pathogen *A. flavus*, and the industrial workhorses *A. niger*, *A. terreus*, and *A. oryzae*. When compared to different fungal genera, the *Aspergillus* genus of filamentous fungi contains a disproportionately high number of diverse species. Mogensen et al. [1]; Perrone et al. [2]. At various times, particularly before harvesting and after-harvest processing, they might contaminate agricultural goods. *Aspergillus* species can cause changes in sensory, nutritional, and quality traits, including pigmentation, discoloration, spoiling, the emergence of off-odours, and off-flavours. But the spoilage of feed and food with mycotoxin is one of the most remarkable effects of their existence. Due to their parasitic nature, the majority of them are found on plant items as storage moulds [3]. The ecology of spoilage fungi, particularly mycotoxigenic organisms, has been found to depend significantly on pH, temperature, and the activity of water [4]. 'Hydrogen ion concentration' (pH) has been demonstrated to have an impact on fungal development, either directly by acting on the outside of cells or indirectly by affecting the availability of nutrients Abubakar et al. [5]. Studies on pH have shown that fungi may grow in a range of pHs, with the highest generation of dry mycelial weight and sporulation occurring in liquid medium at pH 5.5 and pH 6.5, respectively Saha et al. [6]; Deshmukh et al. [7]. With this context, in vitro research was done to compare the mycelial growth of *A. niger* and *A. flavus* at various pH levels.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Fungi

The study was conducted in Department of Seed Science and Technology, TNAU, Coimbatore during 2023. *Aspergillus* strains were used in this

study were collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and then the fungal cultures were subcultured in PDA medium.

### 2.2 Preparation of PDA Medium with Different pH

Peeled potato (200g) was cut into small pieces and it was boiled in 500ml of distilled water till the pieces got softened. The extract was sieved through a double-layered muslin cloth and collected in a beaker into which 20g dextrose was added. In another 500 ml of water, 20 g of agar was melted. Potato extract and melted agar were mixed together, and the final volume of the media was made up to 1000 ml by adding distilled water. 100 ml of PDA media was taken in seven separate conical flasks to adjust pH. Using 1 M NaOH or absolute glacial acetic acid, the pH of the medium was adjusted from 3 to 9 Saha et al. [6]. The pH level was tested using an electrical pH metre. The medium was sterilised for 15 mins at 15 psi in an autoclave.

### 2.3 Inoculation of *Aspergillus* sp.

Under aseptic conditions, about 20 ml of medium was poured into each sterile Petri plate. To inhibit the bacterial pathogens, a small amount of streptomycin sulphate was mixed into each plate. Mycelial discs (8mm diameter) of *A. niger* and *A. flavus* were placed in centre of the Petri plates. The Petri plates were incubated at 25±2°C for seven days, and fungus growth was monitored daily.

### 2.4 Statistical Analysis

An experiment was conducted, and its data was analyzed using a one-way analysis of variance (ANOVA) with a Completely Randomized Design. The analysis was performed using an Excel tool.

### 3. RESULTS AND DISCUSSION

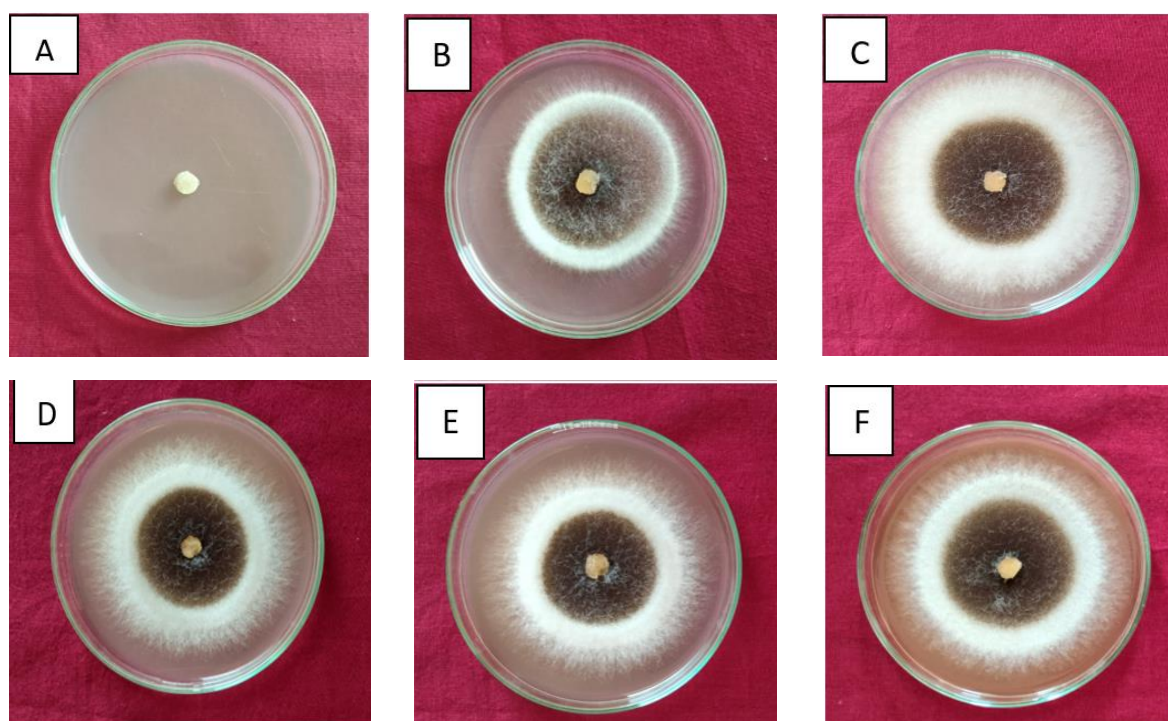
#### 3.1 Effect of pH on Mycelial Growth of *A. Niger*

In pH 3, agar did not solidify. Agar in a medium with a low pH gets hydrolyzed when autoclaved Kurtzman et al. [8]. Mycelial growth of *A. niger* was observed on seventh day after inoculation was shown in Fig.1. Results revealed that the highest mycelial growth was observed at pH 6 for *A. niger* (8.7cm) followed by pH 7 (8cm) (Fig. 3). No fungal growth was observed in pH 4. According to Zhao et al. [9], pH 5.0 to 8.0 is ideal for *Diplocarpon mali* conidial formation. Growth began to slow down above pH 6 in certain cases. This is in line with Swe et al. [10] who showed that the colony numbers per ml decrease, when the medium's alkalinity increases. The optimum pH for growth was recorded at the range of pH 5.5 – 6.5. The result indicated that slightly acidic pH to neutral pH was optimum for the growth of the organism. The optimum pH for growth was

recorded at the range of pH 5.5 – 6.5. The results were consistent with those reported by Saha et al. [6]) who discovered that a pH between 5.5 and 6.5 was ideal for growth and that the development of *Lasiodiplodia theobromae* was best at a pH range of neutral to slightly acidic. Buchanan and Ayres [11], reported that *A. parasiticus* developed best in conditions with an initial pH value of 5.0. Simultaneously, the maximum mycelial development was observed by *Botryodiplodia theobromae* at pH 6.0, whereas Pycnidial formation was effective at pH 7 Gouri Sankar et al. [12].

#### 3.2 Effect of pH on mycelial growth of *A. Flavus*

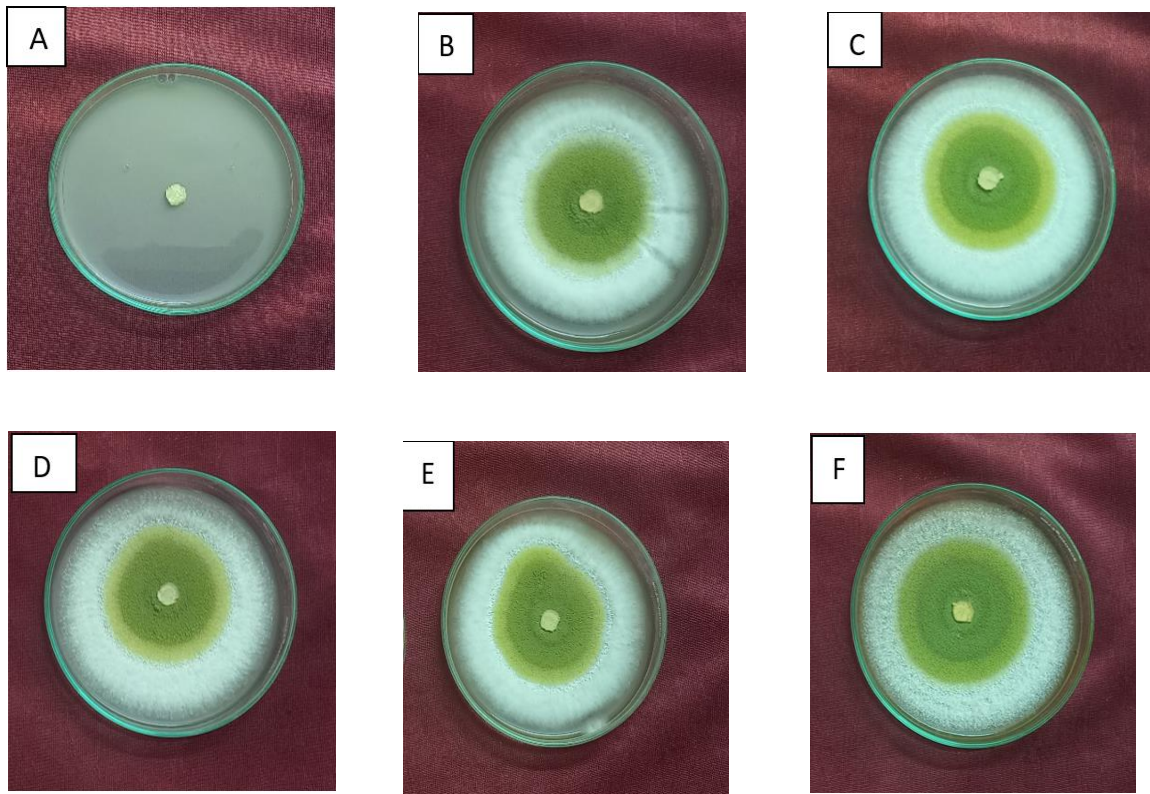
Mycelial growth of *A. flavus* was observed on seventh day after inoculation was shown in Fig. 2. The results indicated that pH 6 (8.6 cm) had the maximum mycelial growth for *A. flavus*.



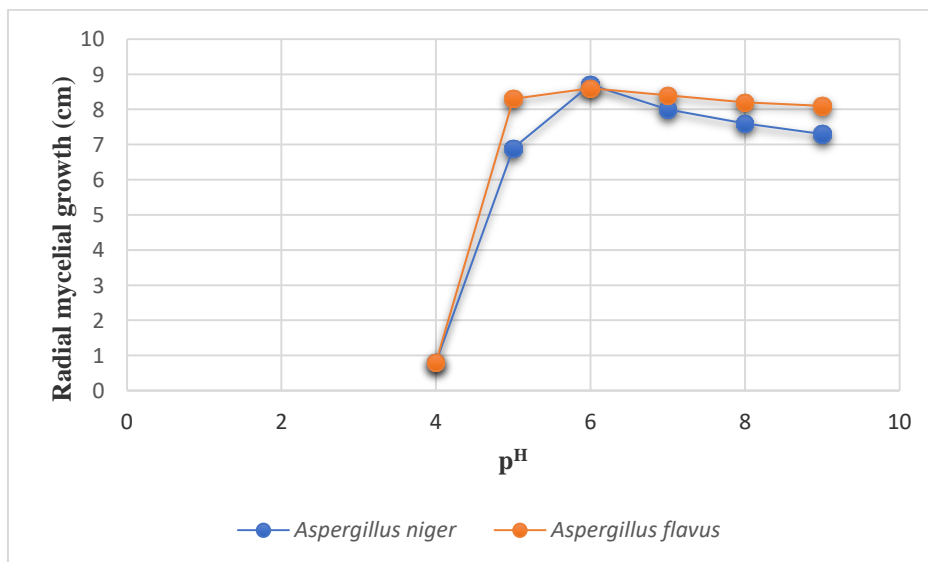
**Fig. 1. Mycelial growth of *Aspergillus niger* on seventh day in different pH. (A) pH 4, (B) pH 5, (C) pH 6, (D) pH 7, (E) pH 8, (F) pH 9**

At pH 4, no mycelial development was seen. It may be due to the fact that acetic acid produces a significant and quick decrease in the internal pH of *A. niger* and *A. flavus* conidia Stratford et al. [13]. According to Theron and Lues [14], it is

often linked to the release of protons, acidification of the cytoplasm, and elimination of the pH gradient across the membrane. Table 1 shows that there was no discernible variation in *A. flavus* mycelial development between pH



**Fig. 2. Mycelial growth of *Aspergillus flavus* on seventh day in different pH. (A) pH 4, (B) pH 5, (C) pH 6, (D) pH 7, (E) pH 8, (F) pH 9**



**Fig. 3. Mycelial growth of *Aspergillus* sp.**

values of 5 and 9. This was in contrast with the results of David et al. [15], who found that regardless of water activity ( $A_w$ ) level, the development of *A. carbonarius* obtained from wine and table grapes was impacted more

favourably at pH 4.0 and 7.0 than at pH 2.6. At pH 5.0 and 0.99 water activity ( $A_w$ ), at 33°C, Holmquist et al. [16] observed that *A. flavus* and *A. parasiticus* developed to their maximum growth rates. Similar to this, Vijay et al. [17]

noted that pH 6 and 7 were ideal for *Lasiodiplodia theobromae*'s maximal mycelial development. However, the range of acidic and alkaline conditions needed for fungi to develop is somewhat broad, ranging from pH 3.0 to pH 8.0, with the ideal pH being about pH 5.0 if nutritional needs are fulfilled Pardo et al. [18]. The results were in line with those of Olutiola [19], who noted the greater impact of pH on the development and sporulation of *A. flavus*. Both appeared to perform poorly on acidic pH 2.5–3.5 and alkaline pH 10.5 conditions, with an ideal pH of 7.5 for growth and pH 6.5 for sporulation. In general, filamentous fungi are known to be tolerant of acidic pH, and the majority of them have an ideal pH range between 5.0 and 6.0 for cellular development and a number of metabolic processes Rosfarizan et al. [20].

**Table 1. *Aspergillus sp.* mycelial growth at various pH levels**

pH	Mycelial growth (cm)	
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
4.0	0.8	0.8
5.0	6.9	8.3
6.0	8.7	8.6
7.0	8.0	8.4
8.0	7.6	8.2
9.0	7.3	8.1
Mean	6.5	7.1
SEm	0.198	0.116
CD @ 5%	0.611	0.358

#### 4. CONCLUSION

The study identified the necessary pH for *Aspergillus niger* and *A. flavus* growth. The results showed that *Aspergillus sp.* will grow in acidic to weak alkaline media. Higher pH values will hinder the growth of *Aspergillus spp.* As a result, the toxigenic fungus's ability to contaminate food and food products will gradually decline, reducing the risk to human health. Hence, different alkaline and lower acidic substances might be explored to stop the fungi's mycelia development and sporulation in order to stop their harm the crops. Further, it is necessary to do research on how various factors affect *Aspergillus* growth.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Mogensen J, Nielsen HB, Hofmann G, Nielsen J. Transcription analysis using high-density micro-arrays of *Aspergillus nidulans* wild-type and creA mutant during growth on glucose or ethanol. *Fungal Genetics and Biology*. 2006;43(8):593-603.
2. Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Samson RA. Biodiversity of *Aspergillus* species in some important agricultural products. *Studies in Mycology*. 2007;59(1):53-66.
3. Kozakiewicz Z. *Aspergillus* species on stored products. *Mycological Papers*. 1989;161:1–188.
4. Abdel-Hadi A, Magan N. Influence of physiological factors on growth, sporulation and ochratoxin A/B production of the new *Aspergillus ochraceus* grouping. *World Mycotoxin Journal*. 2009;2(4):429-434.
5. Abubakar A, Suberu HA, Bello IM, Abdulkadir R, Daudu OA, Lateef AA. Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus*. *Journal of Plant Sciences*. 2013;1(4)64-67. DOI: 10.11648/j.jps.20130104.13
6. Saha A, Mandal P, Dasgupta S, Saha D. Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. *Journal of Environmental Biology*. 2008; 29(3):407.
7. Deshmukh AJ, Mehta BP, Sabalpara AN, Patil VA. *In vitro* effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of colletotrichum gloeosporioides Penz. and sacc causing anthracnose of Indian bean. *Journal of Biopesticides*. 2012;5:46.
8. Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The yeasts*. 2011;87-110. Elsevier.
9. Zhao H, Huang L, Xiao CL, Liu J, Wei J, Gao X. Influence of culture media and environmental factors on mycelial growth and conidial production of *Diplocarpon mali*. *The Society for Applied Microbiology, Letters in Applied Microbiology*. 2010;50: 639–644.
10. Swe KH, Alimon AR, Ramin M. Effect of delaying sporulation by addition of ammonium sulphate on the fermentation of palm kernel cake based substrate by

- Aspergillus niger*. American Journal of Agriculture and Biological Sciences. 2009; 4(4):262– 265.
11. Buchanan Jr, RL, Ayres JC. Effect of initial pH on aflatoxin production. Applied Microbiology. 1975;30(6):1050-1051.
  12. Gowri Sankar T, Gopi V, Hema Bharathi Y, Gopal K, Lakshmi M. Growth of botryodiplodia theobromae an incitant of longitudinal splitting of bark and wood disease in acid lime (*Citrus aurantifolia* Swingle) as influenced by pH levels, temperature and growth media. Int J Curr Microbiol App Sci. 2016;12:756-764.
  13. Stratford M, Plumridge A, Nebe-von-Caron G, Archer DB. Inhibition of spoilage mould conidia by acetic acid and sorbic acid involves different modes of action, requiring modification of the classical weak-acid theory. International Journal of Food Microbiology. 2009;136(1):37-43.
  14. Theron MM, Lues JR. Organic acids and food preservation. CRC press; 2011.
  15. David M, Neus B, Sonia M, David A, Vicente S, Naresh M. Water relations of germination, growth and ochratoxin 'A' production by *aspergillus carbonarius* isolates from wine and table grapes from the northern mediterranean basin. Ecophysiology of ochratoxigenic moulds. Journal of Applied Microbiology. 2005;98: 839-844.
  16. Holmquist GU, Walker HW, Stahr HM. Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *A. parasiticus*. Journal of Food Science. 1983;48(3):778-782.
  17. Vijay S, Renukadevi P, Mangammal P, Krishnamoorthy SV. Effect of media, temperature and pH on the growth and Pycnidial production of *Lasiodiplodia theobromae* (Pat.), causative of black root rot in mulberry; 2021.
  18. Pardo E, Marin S, Ramos AJ, Sanchis V. Ecophysiology of ochratoxigenic *Aspergillus ochraceus* and *Penicillium verrucosum* isolates. Predictive Models for Fungal Spoilage Prevention—A Review. Food additives and contaminants. 2006; 23(4):398-410.
  19. Olutiola PO. Some environmental and nutritional factors affecting growth and sporulation of *Aspergillus flavus*. Transactions of the British Mycological Society. 1976;66(1):131-136.
  20. Rosfarizan M, Ariff AB, Hassan MA, Karim MIA. Influence of pH on kojic acid fermentation by *Aspergillus flavus*. Pakistan Journal of Biological Sciences. 2000;3(6):977-982.

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