Effect of pH on the growth of *Aspergillus niger* and *Aspergillus flavus*

Comment [R1]: incomplete

ABSTARCT

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 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. Therefore, it was determined that in order to stop A. niger and A. flavus from harming our crops, particular alkaline media may be utilised to hinder their development and sporulation.

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Key words: *Aspergillus niger*, *Aspergillus flavus*, pH, Mycelial growth.

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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.*, 2006; Perrone*et al.*, 2007). 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 (Kozakiewicz,1989). The ecology of spoilage fungi, particularly mycotoxigenic organisms, has been found to depend significantly on pH, temperature, and the activity of water (Abdel-Hadi&Magan, 2009).'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.*, 2013).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.*, 2008; Deshmukh

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et al., 2012).With this context, in vitro research was done to compare the mycelial growth of *A. niger* and *A. flavus* at various pH levels.

MATERIALS AND METHODS

Collection of Fungi

Aspergillus strains used for this investigation were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. and the fungal cultures were subcultured in PDA medium.

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Preparation of PDA medium with different pH

The following ingredients were used to make PDA media:

Potato - 200g

Dextrose - 20g

Agar - 20g

Water - 1000 ml

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Peeled potato 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 according to Saha *et al.* (2008) with slight modification. The pH level was tested using an electrical pH metre. The medium was sterilised for 15 mins at 15 psi in an autoclave.

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.

STATISTICAL ANALYSIS

Experiment was carried out by using completely randomised design. Data were analysed by using excel tool for CRD.

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RESULT AND DISCUSSION

Effect of pH on mycelial growth of *A. niger*

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In pH 3, agar did not solidify. Agar in a medium with a low pH gets hydrolyzed when autoclaved (Kurtzman *et al.*, 2011). Mycelial growth of *A. niger* was observed on seventh day after inoculation was shown in Plate 1. Results revealed that the highest mycelial growth was observed at pH 6 for *A. niger* (8.7cm) followed by pH 7 (8cm) (Fig. 1). No fungal growth was observed in pH 4. According to Zhao *et al.*, (2010), pH 5.0 to 8.0 is ideal for *Diplocarponmali* conidial formation. Growth began to slow down above pH 6 in certain cases. This is in line with Swe *et al.*, (2009) 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.* (2008) 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 (1975), 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.*, 2016).

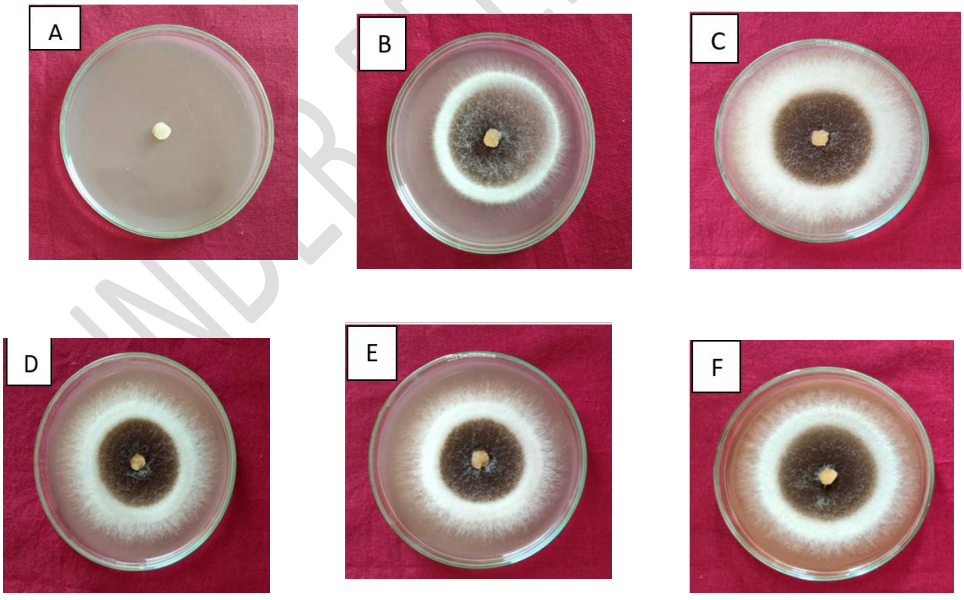

Plate 1. Mycelial growth of *Aspergillus niger* on seventh day in different pH. (A) pH 4, (B) pH 5, (C) pH 6, (D) pH 7, (E) pH 8, (F) pH 9

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Effect of pH on mycelial growth of *A. flavus*

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

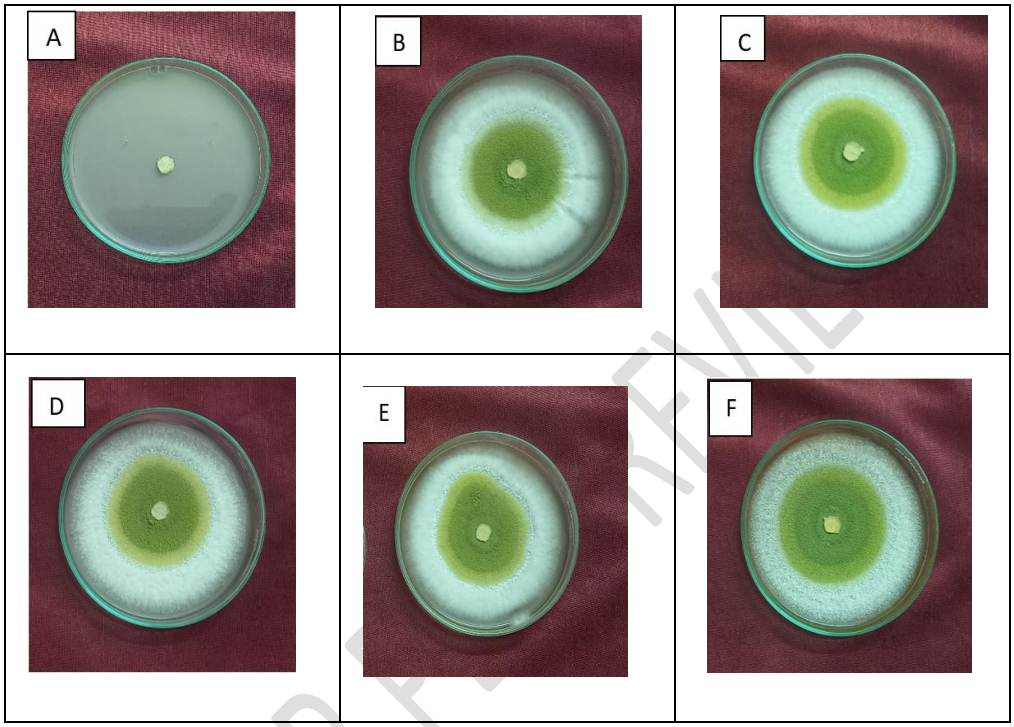

Plate 2. Mycelial growth of *Aspergillus flavus* on seventh day in different pH. (A) pH 4, (B) pH 5, (C) pH 6, (D) pH 7, (E) pH 8, (F) pH 9

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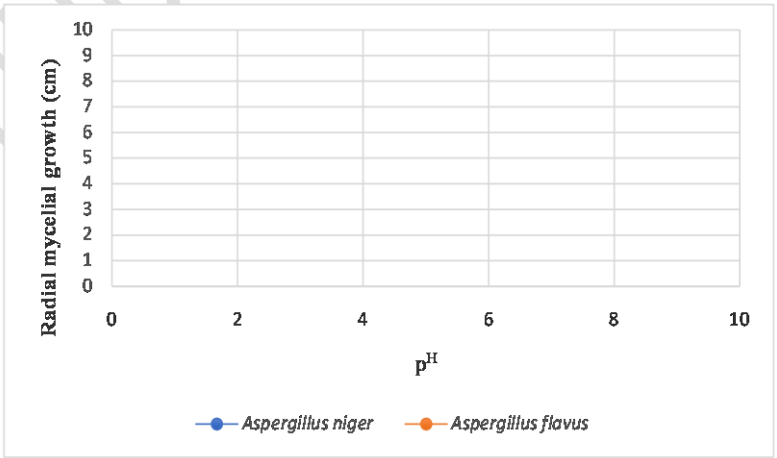

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Fig. 1. Mycelial growth of *Aspergillus sp.*

Comment [R9]:

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.*, 2009). According to Theron and Lues (2011), 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 values of 5 and 9. This was in contrast with the results of David *et al.*, (2005), who found that regardless of water activity (Aw) 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 (Aw), at 33°C, Holmquist *et al.* (1983) observed that *A. flavus* and *A. parasiticus* developed to their maximum growth rates. Similar to this, Vijay *et al.* (2021) 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.*, 2006). The results were in line with those of Olutiola (1976), 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.*, 2000).

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

pH	**Mycelial growth (cm)**	
	Aspergillus niger	***Aspergillus flavus***
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

CONCLUSION

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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

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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 media might be explored to stop the fungi's mycelia development and sporulation in order to stop their harm to our crops. Further, it is necessary to do research on how various factors affect *Aspergillus* growth.

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