*Official publication of Pakistan Phytopathological Society*
**Pakistan Journal of Phytopathology**
ISSN: 1019-763X (Print), 2305-0284 (Online)
https://pjp.pakps.com

**RESEARCH ARTICLE**

# Impact of Abiotic Factors on *In Vitro* Development of *Aspergillus flavus* Isolated from Sesame Seeds

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*Article History:*
*Submitted: July 05, 2024; Revised: July 19, 2024; Accepted for Publication: September 07, 2024.*

A B S T R A C T

Plant pathogen's growth and development are influenced by abiotic conditions, which impact sesame crop production. *Aspergillus flavus* was selected for this research based on its prevalence and disease incidence in sesame. Under *in vitro* conditions, the development of *A.flavus* was investigated at various temperatures, pH levels, light intensity, nitrogen, and carbon sources. The data was collected after 3, 7, and 10 days of incubation on potato dextrose agar (PDA). The best sporulation was observed at 25°C, with a colony diameter of 3.5 cm. Mycelia development of 1.1 cm and an uneven colony border were observed during a continuous dark phase. *A. flavus* could develop moderate sporulation at 5.0 pH with colony diameter of 2.2 cm. Among carbon sources, sucrose promoted maximal mycelial growth of 1.55 cm with the best sporulation. Sodium nitrate, as a nitrogen source, also showed a maximum diameter of 4.1cm. The findings of this study showed certain levels of abiotic factors that promote the development of *A. flavus*. By avoiding these abiotic factors, farmers and researchers can increase the quantity and quality of sesame crop production.

**Keywords**: *Aspergillus flavus*, abiotic factors, aflatoxins, sesame, *in vitro*.

## INTRODUCTION

Sesame (*Sesamum indicum* L.), known as the queen of oilseed, is one of the oldest oilseed crops belonging to the *Pedaliaceae* family, sown in subtropical and tropical places in Asia, Africa, and South America. The major sesame production is relatively higher in China (1312 kg/ha), Myanmar (543 kg/ha), and India (341 kg/ha) than in the remaining part of the third world. These countries also reported about 80% of the world's exports in 2014. In 2017, sesame shares were more than USD 1300 million in global trade (Usman *et al.,* 2022). Sesame seeds are small in size, ovate, pearl-shaped, and have hilum, testa, cotyledons, endosperm, and embryo. The seeds contain an exalbuminous nature, and their color ranges from white to light brown and dark brown to black (Arun and Mahabeer, 2023).

The chemical composition of sesame seeds reveals that the seed is a considerable source of oil (44–58%), protein (18–25%), carbohydrate (13.5%), ash (5%), vitamin (A, B1, B2, and E) and minerals (phosphorus and calcium) (Kahyaoglu and Kaya, 2006; Arun and Mahbeer, 2023). Due to its chemical composition, this plant is currently employed as a food source and for medicinal uses. Sesame seeds and oil are used for various purposes, including cooking, salad dressing, garnish, snacks, flavoring agents, margarine production, and as a raw material in paints, varnishes, soaps, fragrances, and

pesticides (Quasem *et al.,* 2009). Sesame seeds contain antibacterial, antioxidant, anti-inflammatory, antiviral, and antifungal characteristics in their natural state. However, several deteriorating microorganisms (fungi) have caused issues with seed production and storage (Arun and Mahabeer, 2023).

Many *Aspergillus* species can produce aflatoxins, but *A.flavus* is the most common cause of contamination worldwide, which poses a severe threat to consumer's health (Klich, 2007; Mobeen *et al.,* 2011; Mariana *et al.,* 2023). Acute health effects include liver cirrhosis and mortality, but chronic exposure can lead to cancer and has been associated with stunting in children and immune system suppression (Probst *et al.,* 2007). Aflatoxin contamination in crops is a complex process that starts in the field and is triggered by environmental and biological factors such as host vulnerability, heat and high-temperature stress, insect attack, and fungal species (Williams, 2006). Contamination may begin or continue after harvest if crops are stored in favorable conditions, which enhances fungus proliferation and aflatoxin production. Aflatoxins have been the topic of intensive research to better understand the biology, epidemiology, and prevalence of aflatoxin-producing fungi, as well as to create effective disease management techniques to prevent negative health, trade, and environmental consequences (Probst *et al.,* 2007).

A variety of abiotic factors limit productivity, resulting in a variety of diseases as well as poor crop management, i.e., lack of water in the soil suppresses germination of the seeds. *Aspergillus* fungus can thrive and multiply anywhere on the earth, in a variety of climates ranging from desert to tropical moist to temperate. For active growth of *A. flavus*, temperatures ranging from 25°C to 33°C are required. It can, however, thrive at temperatures between 30°C and 40°C. Furthermore, many farms do not employ basic food processing techniques or have enough food storage facilities. As a result, when abiotic levels such as temperature, pH, and light intensity are above the optimum level, economic losses from *A. flavus* contamination might be as high as 100% (Klich *et al.,* 2007; Kumar *et al.,* 2017).

To employ management techniques, this study focused on the optimum levels of abiotic factors and favorable substrate, i.e., nitrogen source and carbon source that allow *A. flavus* to survive and enhance their development in an *in vitro* environment, which causes contamination and results in the loss of sesame crops.

## MATERIALS AND METHODS

**Collection of fungi:** The identified strain of *A.flavus* was collected from the Mycology Laboratory, Department of Botany, PMAS-Arid Agriculture University Rawalpindi. The fungus was preserved at 4°C until used.

**Preparation and sterilization of culture media:** Unpeeled potatoes (200 g) were dipped in 1000 ml of tap water and boiled at 100°C. After boiling, 200 g of potatoes were filtered through cheesecloth in a beaker. Afterward, 20 g of Agar and 15 g of dextrose were added to the potato infusion and mixed thoroughly before pouring into a Simax media bottle. Erlenmeyer flasks (500 ml) were filled with 250 ml of the PDA media and autoclaved for 15 minutes at 121°C and 15 psi pressure (Usman *et al.,* 2023; Yaqoob *et al.,* 2024).

**Fungal inoculation and growth of fungi on PDA:** Petri dishes containing PDA media were inoculated with fungus from a ten-day-old culture. The inoculated dishes with *A. flavus* were incubated at three different temperatures: 20°C, 25°C, and 30°C (Hathout *et al.,* 2014). Petri dishes were also inoculated with fungus having PDA of different pH levels (5, 7, and 8), and these pH levels were maintained by a digital pH meter, 0.1N sodium hydroxide, and 0.1N hydrochloric acid. Some Petri dishes inoculated with fungus were exposed to continuous 24 hr light, 24 hr dark, and alternate cycles of 12 hr light and 12 hr dark (Hubballi *et al.,* 2010). In another experiment, three carbon sources (glucose 13.5 g/l, sucrose 12.5 g/l, starch 12.5 g/l) were added individually as a component of carbon source in Petri dishes containing PDA and maintained at 27°C. In some Petri dishes, two nitrogen compounds (Sodium nitrate 8.5g/l, ammonium nitrate 8.5g/l) were mixed as an ingredient of nitrogen source in a PDA medium and kept at 27°C (Rajpoot *et al.,* 2015). The diameter of the colonies on the same axis was measured directly to determine the linear growth of the test fungus. Colony color, colony diameter, and margin of the colony were also examined (Hubballi *et al.,* 2010). All the results were recorded after 3 days, 7 days, and 10 days of inoculation. All the experiments were performed in triplicates.

**Statistical analysis:** The mean values were assessed by using two-way ANOVA (Analysis of Variance). The statistical significance between the means was based on LSD (least significant difference) at $P > 0.05$.

## RESULTS

**Impact of different temperature levels on the growth of *A. flavus*:** Fungal growth and its development are influenced by the temperature (Figure 3). The current findings showed the development of *A. flavus* from minimum to maximum at 20°C, 25°C, and 30°C. Among all of these temperatures, fungal growth was statistically significant. After 10 days of incubation, the fungal colony expanded, and good sporulation was seen at 25°C with a light-yellow substrate color and smooth margin (Figure 1). The best mycelial diameter of 3.5cm was observed at 25°C, which was determined to be the ideal temperature for the development of *A. flavus*. Whereas the highest temperature, such as 30°C, had a negative impact on sporulation (Figure 2).

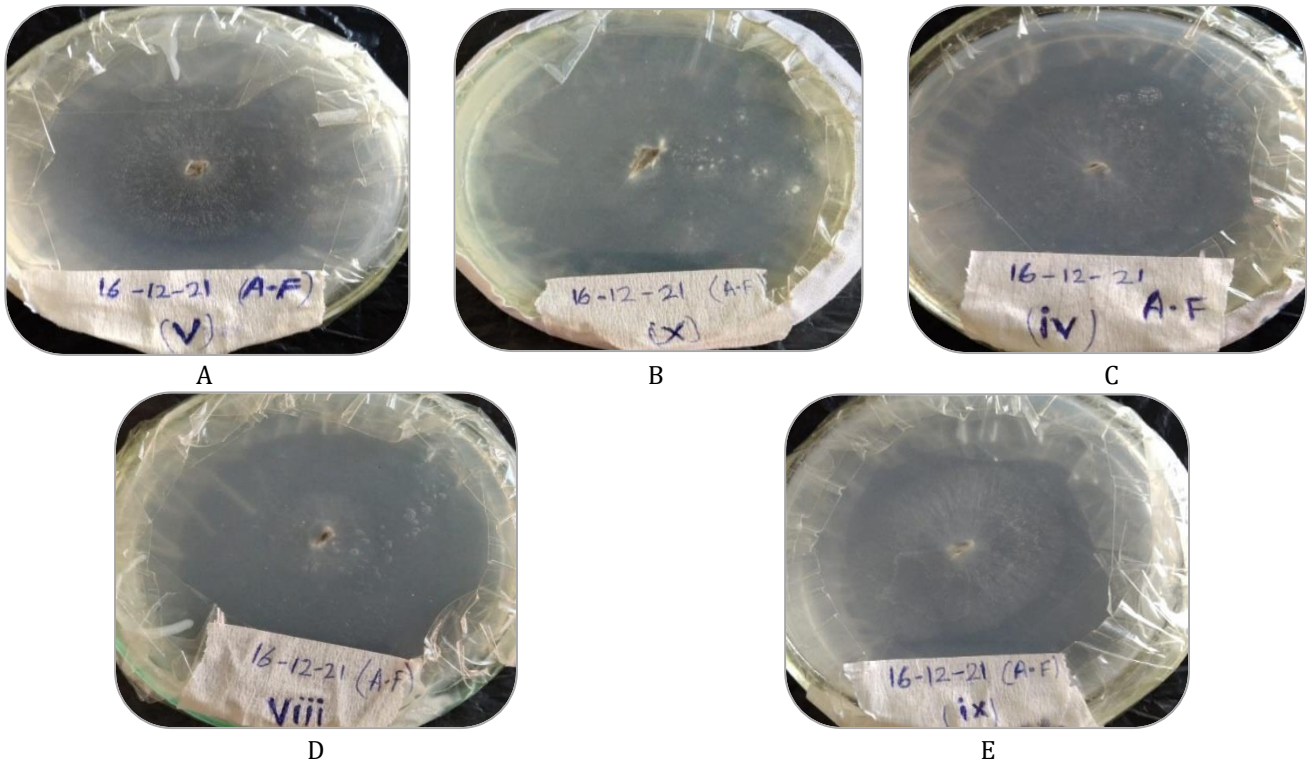
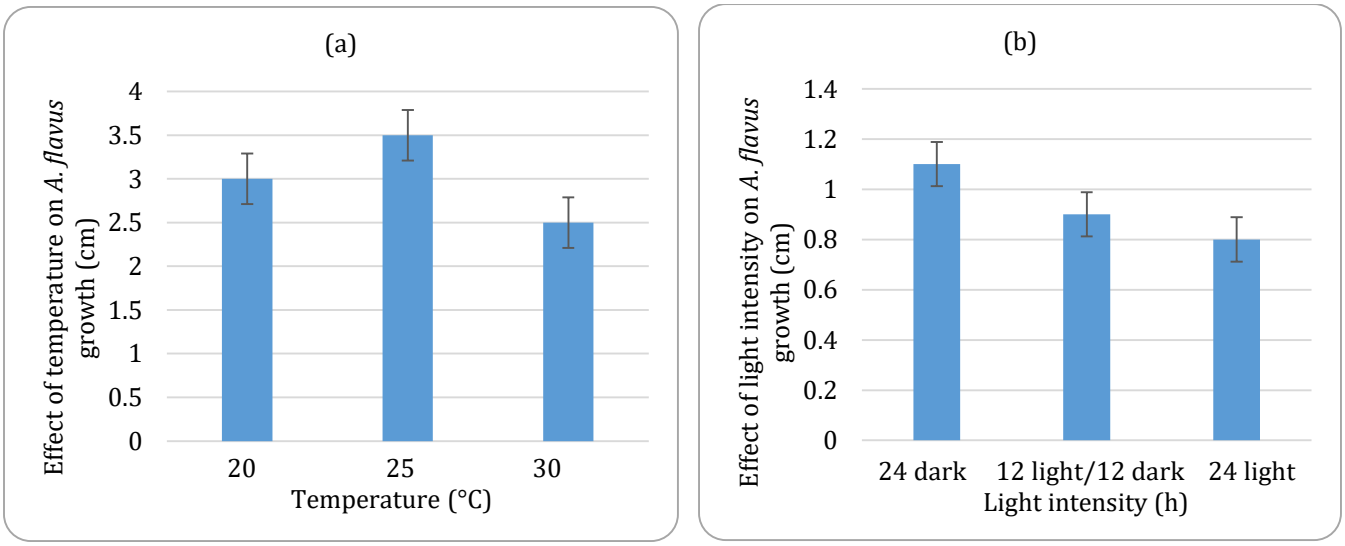



Figure 1. Colony growth of *A. flavus* on different abiotic factors after 10 days of incubation: (A) temperature, (B) light, (C) pH, (D) carbon source, (E) nitrogen source.



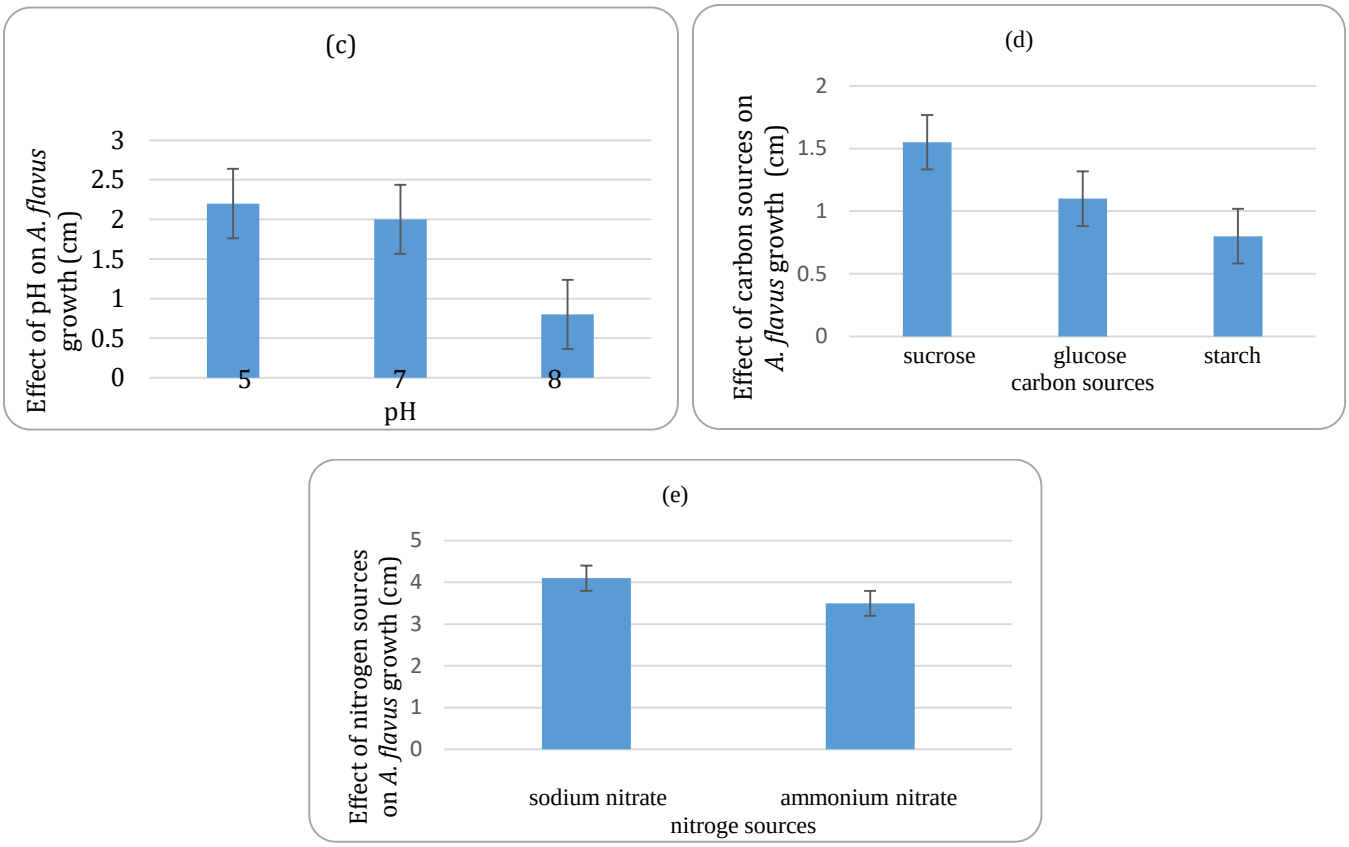



Figure 2. Effect of different abiotic factors on *A.flavus* growth after 10 days of incubation: (a) temperature, (b) light, (c) pH, (d) carbon sources, (e) nitrogen sources.

**Impact of different light intensities on the growth of *A. flavus*:** *A. flavus* mycelial development is significantly influenced by light (Figure 3). After 10 days of incubation, the fungus was exposed to a continuous 24-hour dark phase, which resulted in the maximum mycelia growth (1.1cm) of *A. flavus*. The colony color of *A. flavus* differed from light grey on the front and light brown on the back sides, having inadequate sporulation and even edges (Figure 1). The other treatments, such as the 24 h light phase, showed the minimum mycelia development (0.8) as compared to the 12 h light + 12 h dark phase (0.9) treatment (Figure 2).

**Impact of different carbon sources on the growth of *A. flavus*:** Carbohydrates are essential nutrients for the growth and development of fungi (Figure 3). After 10 days of incubation, the maximum mycelia development of *A. flavus* was recorded by sucrose (1.55 cm) followed by glucose of 1.1 cm and starch of 0.8 cm as the carbon sources (Figure 2).

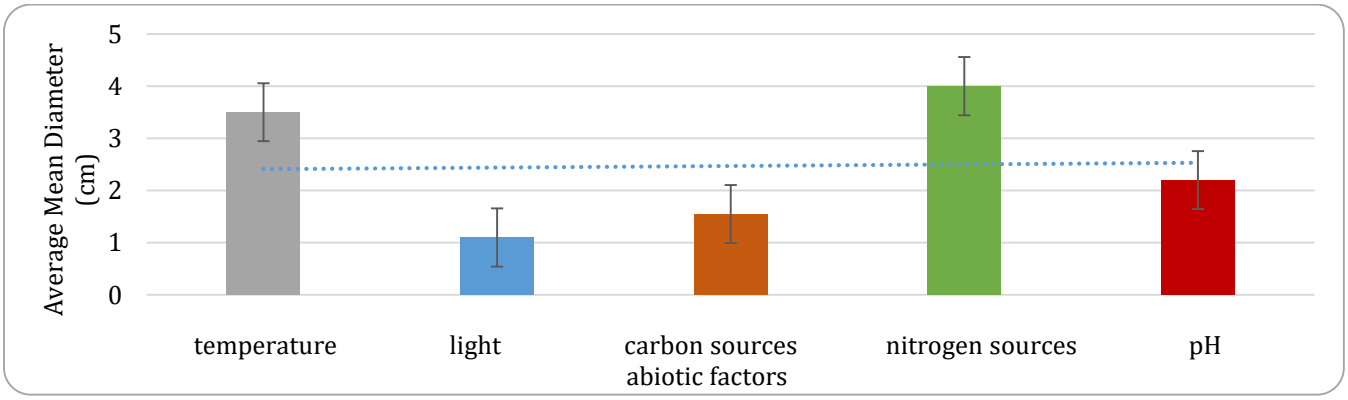



Figure 3. Colony growth of *A. flavus* on different abiotic factors after 10 days of incubation.

Table 1. Colony characteristics of *A. flavus* under different abiotic factors after 10 days of incubation.

| | | | Colony Characteristics | | | |
|---|---|---|---|---|---|---|
| Sr. No | Abiotic factors | Levels | Color on front | Color on back | Sporulation | Margin |
| 1 | Temperature | 20 | Light green | Blackish brown | Moderate sporulation | Smooth |
| | | 25 | Light yellow | Light green | Good Sporulation | Smooth |
| | | 30 | Green | Light grey | Poor sporulation | Irregular |
| 2 | Light intensity | 24 dark | Light grey | Light brown | Inadequate sporulation | Smooth |
| | | 24 light | Light green | Light yellow | Poor sporulation | Smooth |
| | | 12 light, 12 dark | Light yellow | Light yellow | Poor sporulation | Smooth |
| 3 | pH | 5 | Light green | Blackish green | Strong sporulation | Smooth |
| | | 7 | Blackish grey | Light brown | Moderate sporulation | Irregular |
| | | 8 | Green | Light yellow | Poor sporulation | Smooth |
| 4 | Carbon sources | Sucrose | Green | Light yellow | Inadequate sporulation | regular |
| | | Glucose | Light green | Light yellow | Good Sporulation | Irregular |
| | | Starch | Light grey | Brown | Poor sporulation | Irregular |
| 5 | Nitrogen sources | Sodium nitrate | Greenish grey | Blackish brown | Moderate sporulation | Smooth |
| | | Ammonium nitrate | Light yellow | Yellow | Moderate sporulation | Smooth |

**Impact of nitrogen sources on the growth of *A. flavus*:** In this experiment, the use of two distinct nitrogen sources for the growth of *A. flavus* was investigated (Figure 3). After 10 days of incubation, sodium nitrate was shown to be the most significant nitrogen source, which enhanced the colony diameter of 4.1 cm, while ammonium nitrate revealed the lesser mycelia development of 3.5 cm (Figure 2). At all nitrogen sources, moderate sporulation and smooth margins were observed (Figure 1).

**Impact of different pH levels on the growth of *A. flavus*:** The current analysis reveals a great deal of variation in colony diameter. *A .flavus* prefers the acidic and neutral pH ranges, while alkaline medium results in poor mycelia development (Table 1). After 10 days of incubation period, the maximum mycelial growth of 2.2 cm and sporulation of *A. flavus* was observed at a pH level of 5.0, followed by pH 7.0 and pH 8.0 (Figure 2).

**DISCUSSION**

Sesame is a vital source of protein and oil. *A. flavus* has a negative effect on sesame crop production and its storage. The current study focuses on the determination of abiotic factors such as temperature, pH levels, light intensity, nitrogen, and carbon sources, which affect the growth of *A. flavus* in *in vitro* circumstances. Inoculation of fungal strains and their growth on PDA under abiotic conditions were observed. The diameter of the colonies on the same axis was measured directly to estimate the linear growth of the fungus after 3 days, 7 days, and 10 days of inoculation. The highest growth of *A. flavus* was investigated at 25°C temperature, which enhanced the colony growth rate (Gebremeskel *et al.,* 2022). Hubballi *et al.* (2010) revealed that *Alternaria alternata* showed the maximum growth at 25°C on the PDA medium. After 9 days of incubation, Kumar *et al.* (2015) found that the *Alternaria solani* produced maximum growth at 25°C temperatures, which supports the present findings.

The maximum mycelial development was recorded at a continuous dark phase (24h), which was contradictory to the results of (Hubballi *et al.,* 2010). He reported the maximum fungal growth at alternate cycles (12 h light + 12 h dark) as compared to continuous dark and light phase treatments.

Further, this study found that sucrose is the best carbon source for fungal growth and sporulation. A study by Rajpoot *et al.* (2015) also evaluated the best growth and sporulation of *T. pseudokoningii* by supplementing sucrose and glucose as a carbon source. This study also investigated the impact of nitrogen sources on biomass production and spore germination and evaluated that sodium nitrate is the best source of mycelial growth. Similar outcomes were recorded by Jayaswal *et al.* (2003) at various nitrogen sources.

According to Bais *et al.* (2019), 4 and 8 pH significantly decreased the colony growth rate and sporulation of *A. solani*. In this study, good sporulation was observed at 5.0 pH, which supports the current findings.

**CONCLUSION**

In conclusion, this study sheds light on the effect of abiotic factors on the growth of *A. flavus* infecting sesame seeds. The colony growth rate of *A. flavus* under different abiotic conditions was observed, such as nitrogen sources > temperature > pH > carbon sources >, and light after 10 days of incubation at different levels. The mycelial development of *A .flavus* can be controlled by avoiding these levels of abiotic factors such as temperature ≤ 30, pH ≤ 8, dark phase (24 h) of light intensity, and carbon source, i.e., sucrose and sodium nitrate as nitrogen source. These findings are crucial for devising targeted fungus control strategies and sustainability of sesame yield production and storage.

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| Salyha Kalsoom | : | Performed the experiments |
| Brian G. Nayyar | : | Conceived and designed the experiments/Critical Review |
| Ayesha Sarwar | : | Write up/data analysis |
| Tahseen Fatima | : | Contributed reagents/materials/analysis tools |
| Wajiha Seerat | : | Write up/Critical Review |