



Impact Of Temperature And Relative Humidity On The Diversity And Growth Of Seed Mycobiota In Stored Sesame Seeds

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Abstract

Storage conditions significantly influence the microbial quality and safety of oilseed crops. This study evaluated the effects of temperature and relative humidity (RH) on the diversity and growth of seed-associated fungal communities in stored sesame (*Sesamum indicum* L.) seeds. Freshly harvested sesame seeds were stored for six months under controlled combinations of three temperatures (15°C, 25°C, and 35°C) and three RH levels (50%, 65%, and 80%). Fungal populations were assessed periodically using culture-based isolation, morphological characterization, and molecular identification through ITS rDNA sequencing. Fungal growth was quantified using colony-forming unit (CFU) counts, while community diversity was evaluated using the Shannon–Weaver index. The results demonstrated a significant increase in fungal diversity and population density with increasing temperature and RH. Storage under high temperature and humidity favoured the dominance of storage fungi, particularly *Aspergillus* and *Penicillium* species, including potentially mycotoxigenic taxa. Conversely, lower temperature and RH conditions effectively restricted fungal growth and preserved seed quality. These findings emphasize the importance of controlled storage environments to mitigate fungal contamination and post-harvest losses in sesame seeds.

Keywords: Sesame seeds; Storage fungi; Relative humidity; Temperature; *Aspergillus* spp.; post-harvest storage

1. Introduction and Rationale

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops cultivated worldwide and is valued for its high oil content, nutritional quality, and medicinal properties. Sesame oil is rich in unsaturated fatty acids, lignans, and antioxidants, which contribute to its stability and health benefits (Singh et al., 1991). However, despite its inherent stability, sesame seeds are highly vulnerable to microbial deterioration during post-harvest storage, particularly under unfavourable environmental conditions (Niaz et al., 2011; Dhingra & Sinclair., (1995).

Seed-associated fungi, collectively referred to as seed mycobiota, may originate from field infections, harvesting operations, or storage environments. These fungi play a critical role in seed deterioration by reducing germination potential, altering biochemical composition, and producing secondary metabolites such as mycotoxins (Christensen & Kaufmann, 1969; Pitt & Hocking, 2009). Storage fungi such as *Aspergillus*, *Penicillium*, and *Rhizopus* species are particularly problematic in oilseeds due to

their ability to grow at low moisture levels and high temperatures (Magan et al., 2010; Frisvad et al., 2019).

Temperature and relative humidity are widely recognized as the most influential abiotic factors governing fungal growth, sporulation, and community composition in stored seeds. Elevated humidity increases seed moisture content, creating favourable conditions for fungal colonization, while high temperature accelerates fungal metabolic activity and reproduction (Sinha & Sinha, 1992). Previous studies on cereals and legumes have demonstrated strong correlations between storage conditions and fungal succession patterns (Kumar et al., 2017; Paterson & Lima, 2017). However, systematic investigations focusing specifically on sesame seeds under controlled temperature and RH regimes remain limited.

Given the economic importance of sesame and the potential health risks associated with fungal contamination, it is essential to understand how storage environments influence the diversity and growth of seed mycobiota. This study was therefore designed to evaluate the combined effects of temperature and RH on fungal communities associated with stored sesame seeds and to identify storage conditions that minimize fungal proliferation.

2. Objectives

1. To determine the effect of different storage temperatures and relative humidity levels on fungal diversity in sesame seeds.
2. To quantify fungal population growth during storage under controlled environmental conditions.
3. To identify dominant storage fungi associated with high-risk storage environments.
4. To recommend optimal storage conditions for maintaining seed quality and safety.

3. Materials and Methods

3.1. Seed Collection and Preparation

Freshly harvested sesame seeds were obtained from certified farms and transported to the laboratory in sterile containers. Seeds were manually cleaned to remove broken seeds and debris and equilibrated to a uniform moisture content of approximately 8%, as recommended for safe storage of oilseeds (FAO, 2011).

3.2. Experimental Design and Storage Conditions

A factorial experimental design was employed using three temperature levels (15°C, 25°C, and 35°C) and three relative humidity levels (50%, 65%, and 80%), resulting in nine treatment combinations (Table 1). Seeds were stored in sterile glass containers within environmental chambers for six months. Sampling was conducted at 0, 1, 3, and 6 months.

Table 1. Storage Conditions Applied in the Experiment

Treatment	Temperature (°C)	Relative Humidity (%)
T1	15	50
T2	15	65
T3	15	80
T4	25	50
T5	25	65
T6	25	80
T7	35	50
T8	35	65
T9	35	80

3.3. Isolation and Identification of Seed Mycobiota

Seed samples were surface-sterilized using 1% sodium hypochlorite and plated on Potato Dextrose Agar (PDA) supplemented with chloramphenicol. Plates were incubated at 28°C for 5–7 days. Fungal colonies were purified and identified based on morphological characteristics following standard keys (Barnett & Hunter, 1998). Molecular identification was carried out by amplifying the ITS rDNA region, and sequences were compared with NCBI GenBank databases.

Figure 1. Effect of Temperature and Relative Humidity on Fungal Population Density (log CFU/g) in Stored Sesame Seeds:

Effect of Temperature and Relative Humidity on Fungal Population Density

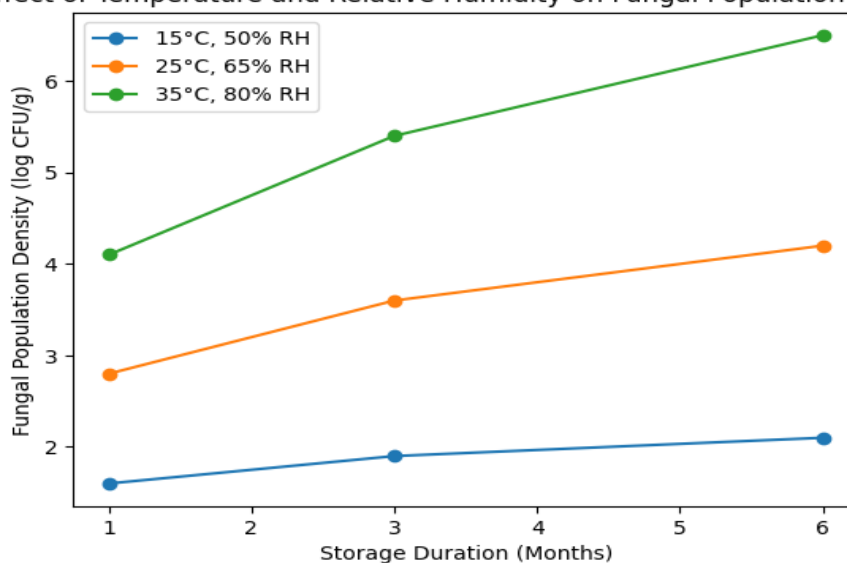


Figure 1 shows that fungal population density in sesame seeds increases with storage duration and is strongly influenced by temperature and relative humidity. The highest fungal load occurred at 35°C and 80% RH, while minimal growth was observed at 15°C and 50% RH. Moderate storage conditions (25°C and 65% RH) resulted in intermediate fungal populations, highlighting the combined effect of temperature and humidity on fungal proliferation during storage.

3.4. Quantification of Fungal Growth and Diversity

Fungal populations were expressed as log CFU/g of seeds using serial dilution techniques. Species diversity was assessed using the Shannon–Weaver diversity index, which accounts for both species richness and evenness.

3.5. Statistical Analysis

Two-way analysis of variance (ANOVA) was used to assess the effects of temperature, RH, and their interaction on fungal growth and diversity. Mean comparisons were performed using Tukey’s test at $p \leq 0.05$.

4. Results and Analysis

4.1. Changes in Fungal Diversity During Storage

At the beginning of storage, seeds exhibited low fungal diversity, primarily consisting of field fungi. As storage progressed, a shift toward storage-adapted fungi was observed, particularly under higher temperature and RH conditions. The Shannon–Weaver diversity index increased significantly at 25°C and 35°C combined with RH levels above 65%, indicating enhanced species richness and evenness (Magan et al., 2010; Magan & Aldred., 2007). An increase in fungal diversity and dominance of storage fungi under high temperature and relative humidity conditions was evident (Figures 2 and 3).

Table 2. Dominant Fungal Species Isolated from Sesame Seeds Under Selected Storage Conditions

Fungal Species	15°C–50% RH	25°C–65% RH	35°C–80% RH
<i>Aspergillus flavus</i>	Low	Moderate	High
<i>Aspergillus niger</i>	Low	Moderate	High
<i>Penicillium</i> spp.	Moderate	High	High
<i>Fusarium</i> spp.	Absent	Low	Moderate
<i>Rhizopus</i> spp.	Absent	Low	Moderate

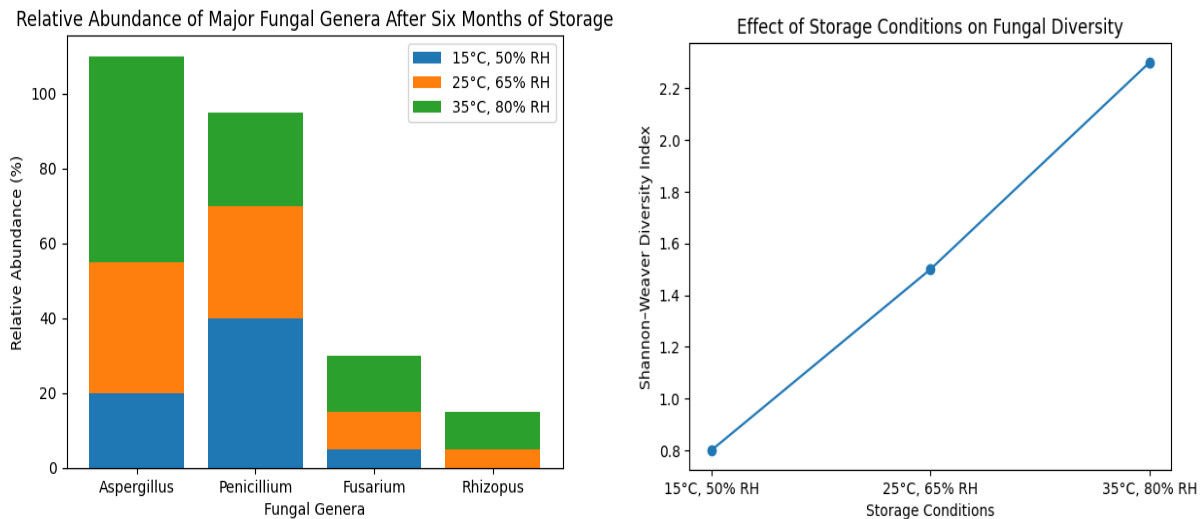
4.2. Effect of Storage Conditions on Fungal Population Growth

Fungal populations increased steadily with storage duration under all treatments; however, the rate of increase varied significantly among treatments. Seeds stored at 35°C and 80% RH exhibited the highest fungal load, reaching 6.5 log CFU/g after six months (Table 3). In contrast, seeds stored at 15°C and 50% RH maintained low fungal populations throughout the storage period. As shown in Figure 1, fungal population density increased significantly with storage duration under elevated temperature and relative humidity conditions.

Table 3. Effect of Temperature and Relative Humidity on Fungal Load (log CFU/g)

Storage Condition	1 Month	3 Months	6 Months
15°C, 50% RH	1.6	1.9	2.1
25°C, 65% RH	2.8	3.6	4.2
35°C, 80% RH	4.1	5.4	6.5

Figure: 2 &3 Relative abundance of major fungal genera isolated after six months of storage; Variation in Shannon–Weaver diversity index under different storage conditions.



Figures 2 and 3 demonstrate the effect of storage temperature and relative humidity on the composition and diversity of seed mycobiota in stored sesame seeds. Figure 2 shows that high temperature and relative humidity (35°C and 80% RH) strongly favour the dominance of xerophilic and thermotolerant fungi, particularly *Aspergillus* species, while *Penicillium* species occur under moderate to high storage conditions. *Fusarium* and *Rhizopus* species were mainly associated with elevated temperature and humidity, indicating advanced seed deterioration. Figure 3 further reveals a progressive increase in fungal diversity with increasing temperature and relative humidity, with the lowest diversity observed under cool and dry conditions (15°C and 50% RH) and the highest under warm and humid conditions. Collectively, these results indicate that unfavourable storage environments promote greater fungal diversity and dominance of potentially pathogenic fungi, increasing the risk of seed quality deterioration.

5. Discussion

The present study clearly demonstrates that temperature and relative humidity exert a strong influence on the diversity and growth of seed mycobiota in stored sesame seeds. Elevated temperature and RH favoured the rapid proliferation of storage fungi, particularly *Aspergillus* species, which are known for their xerophilic and thermotolerant nature (Pitt & Hocking, 2009). Similar trends have been reported in stored oilseeds and cereals, where warm and humid conditions accelerate fungal succession and increase contamination risks (Kumar et al., 2017; Paterson & Lima, 2017).

The dominance of *Aspergillus flavus* under high temperature and RH conditions is of particular concern due to its ability to produce aflatoxins, which pose serious health risks to humans and livestock (Williams et al., 2004; Abdel et al., 2012; Bhat et al., 2010). The comparatively low fungal populations observed at lower temperature and RH levels highlight the effectiveness of environmental control in limiting fungal growth and preserving seed quality.

6. Conclusions and Recommendations

Conclusions

The present study clearly demonstrates that storage temperature and relative humidity are critical determinants of fungal diversity and population dynamics in stored sesame seeds. Elevated temperature and moisture conditions significantly enhance fungal metabolic activity, leading to increased species diversity and higher fungal loads during storage. In particular, storage at high temperature (35°C) combined with high relative humidity (80%) was found to favor the rapid proliferation of storage fungi, including potentially mycotoxigenic species that pose serious risks to seed quality and food safety. In contrast, storage under lower temperature conditions (15–20°C) with relative humidity maintained below 60% effectively suppressed fungal growth and limited changes in mycobiota composition. These

findings underscore the importance of maintaining cool and dry storage environments to preserve sesame seed quality and minimize post-harvest losses.

Recommendations

- Sesame seeds should be stored under cool and dry conditions to minimize fungal contamination.
- Routine monitoring of storage environments is essential to ensure seed safety.
- Further research should focus on mycotoxin quantification and advanced storage technologies such as hermetic storage.

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