***Original Research Article***

**Occurrence of Seed-Borne Fungi in Selected Sunflower (*Helianthus annuus*) Varieties in Abuja, Nigeria**

**ABSTRACT**

**Study Design**: Experimental in the Laboratory

**Place and Duration of Study:** This study was conducted at the Crop and Environmental Protection Laboratory, University of Abuja, Abuja-Nigeria

**Aim:** Investigated the occurrence of seed-borne fungi in seeds of five sunflower varieties in Abuja, Nigeria.

**Methodology:** Seed samples were inoculated in Sabouraud Dextrose Agar where four improved sunflower varieties (SAMSUN1-4) and one of local variety (SKYSCRAPER) were plated and incubated to isolate colonies at temperature of 28 o C with duration of 5-7 days. The morphological identification of fungal isolates was performed, and the percentage of their presence was determined. At the 3 days after incubation (3DAI) there was prevalence of Fusarium oxysporum (54.4%), *Aspergillus niger* (33.2%), *Aspergillus flavus* (6.8%) and *Penicillium chrysogenum* (5.6%).

The frequency of occurrence (PF) for each fungus species was calculated. Data collected were subjected to ANOVA and their means separated using Duncan Multiple Range Test (DMRT) at P ≤ 0.05.

**Result:** It was shown that dominant fungal species identified at 3DAI were - *Aspergillus flavus (6.8%), Aspergillus niger (33.2%), Fusarium oxysporum (54.4%)* and *Penicillium chrysogenum (5.6%).* Notably, it is also worth noticing that *A. niger* showed a growth of 2.09% at 4DAI and 1.90% at 5DAI. *F. oxysporum increased from* 274 and 306, representing 50.37% and 49.04% of the total isolates respectively.

**Conclusion:** The presence of pathogenic and mycotoxigenic fungal species in seeds of the sunflower varieties highlights the potential implications for both agriculture and human health. Sunflower contains a great quantity of A. alternata (aflatoxigenic fungi and A. flavus (mycotoxigenic fungi) seeds. These observations validate the fact that an improved seed treatment, seed storage and fungal surveillance are the aspects that can be deployed to increase agricultural productivity and food safety.

**Keywords:** Sunflower, Seed-borne, Fungi, Contamination*,*

#  Introduction

Sunflower (Helianthus annuus) L. is a globally important oilseed crop, ranks as a top source of vegetable oil cultivated across diverse agro-ecological zones, substantially contributing to global food security and national economies. Native to North America, sunflower's introduction to Europe in the 16th century, and its prominence in the 19th-century within the Russian Empire, led to the development of many modern varieties (Yi *et al*., 2025). Sunflower cultivation in Nigeria is expanding beyond monomodal rainfall regions in the north. Recent studies have demonstrated its adaptability to southwestern Nigeria's forest-savanna transition zones (Adeleke and Babalola, 2020), where bimodal rainfall patterns support biannual cultivation cycles and increased productivity of sunflower.

Sunflower oil is rich in linoleic acid (omega-6), monounsaturated oleic acid (omega-9), and vitamin E, which have significant nutritional benefits and oxidative stability (Haynes, 2016). The high content of healthy unsaturated fatty acids in the oil makes it a superior alternative to saturated fats (Adeleke and Babalola 2020). Beyond its primary use for oil, sunflower has diverse economic values: seeds are consumed as snacks, used for butter, and in confectionery, while the cake from pressed oil is used as animal feed (Adeleke and Babalola, 2020). Industrially, sunflower oil is integral component in the manufacture of soaps, paints, and lubricants, and the plant has also been used in phytoremediation to extract heavy chemicals from contaminated soils (Chauhan and Mathur, 2020). In Nigeria, seeds are consumed fresh or roasted and are traditionally used for managing hypertension, ulcers, erectile dysfunction, and diabetes. Furthermore, sunflowers are crucial for apiculture and biodiversity as a vital honeybee forage (Malunjkar *et al*., 2024).

Crop farming in Africa is challenged by crop diseases and post-harvest contamination, especially in Nigeria. While sunflower (Helianthus annuus L.) is gaining attention as an oilseed, its potential is underexplored due to limited data on its susceptibility to fungal infections and mycotoxin contamination. Sunflower seeds, used for food and feed, are particularly prone to fungal attacks and mycotoxin contamination under favourable conditions (Cao *et al*., 2022).

Globally, sunflower is impacted by fungal pathogens like Alternaria helianthi, Fusarium verticillioides, and Sclerotinia sclerotiorum, which compromise seed quality and yield (Gulya *et al*., 2010; Yang *et al*., 2024). Nigeria's humid and warm climate favours fungal growth and notably, Aspergillus species, producers of hazardous aflatoxins, are a concern due to their role in food spoilage and health risks upon consumption (Ráduly *et al*., 2020).

The severity of mycotoxin contamination is greater in developing nations like Nigeria due to inadequate regulatory enforcement and a lack of awareness among small-scale farmers and consumers regarding the dangers of mycotoxin. This leads to poor pre- and post-harvest practices that increase the risks of contamination (Ezekiel *et al*., 2014).

Furthermore, comprehensive data on mycotoxin contamination in crops like sunflower remain scarce, particularly in the North-Central region of Nigeria, where various agricultural activities are practised.

This study was therefore carried out to determine the seed-borne fungi associated with seeds of selected sunflower varieties in Abuja, Nigeria.

2. Materials and Methods

## **2.1. Experimental location**

Experiments were conducted at the Crop Protection Laboratory, University of Abuja. University of Abuja is located at Latitude 9 ͦ 3.471’ N and Longitude 7 ͦ 29.705’ E. Abuja is located within the centre of the country, within the Federal Capital territory (FCT). It lies on 477m above sea level and features a [tropical wet and dry climate](https://en.wikipedia.org/wiki/Tropical_wet_and_dry_climate) and experiences three weather conditions annually. The FCT has an area extent of 8,000 Km2 and lies between latitudes 8.25oN and 9.21oN of the equator and longitudes 6.45oE and 7.39 °E of the Greenwich Meridian (NearWeather.com, 2025).

**2.2. Source of seeds**

Experiments were conducted to determine the seed-borne pathogens present in seeds of 4 sunflower varieties obtained from Institute for Agricultural Research, Samaru, Zaria, namely SAMSUN1, SAMSUN2, SAMSUN3, and SAMSUN4, and a 5th variety, SKY SCRAPER, obtained from the local market (Abuja Local) in the FCT.

### 2.3. Isolation and Identification of Fungi

Fungi were isolated and cultured following the protocols described by (Mancini *et al*., 2016) and (Liamngee *et al*, 2016). For each seed variety, 100 seeds were randomly selected and surface-sterilized using a 5.25% sodium hypochlorite solution. The seeds were subsequently rinsed aseptically with ten successive 100 mL volumes of sterilized distilled water and air-dried under sterile conditions. From each batch, 10 seeds were randomly chosen and evenly spaced on Sabouraud Dextrose Agar (SDA) plates, then incubated at 28 °C for 1 to 5 days. Emergent fungal colonies were subcultured to obtain pure isolates. Identification of the isolates was conducted using morphological and microscopic characteristics based on the updated identification keys provided by (Frisvad *et al.,* 2019) and (Guermech *et al*., 2025). Pure cultures were maintained on SDA slants and stored at 4 °C for subsequent analyses.

**2.4. Determination of Frequency of Occurrence**

The frequency of occurrence (PF) for each fungal species was calculated by applying the
following formula given by (Harrigan, 1998):

$$PF= \frac{Number of seeds on whch fungus appeared}{Total number of seeds} ×100$$

The percentage of seeds infected by fungi was then calculated.

## 2.5. Statistical Analysis

All data collected were analyzed using the analysis of variance and SPSS (2021) software package. Data was analyzed using descriptive and inferential statistics.

**3. Results**

### 3.1. Occurrence of seedborne fungi isolates on sunflower varieties (3DAI)

Seed health tests revealed the presence of four prominent fungal species (*Aspergillus flavus*, *Aspergillus niger,* *Fusarium oxysporum* and *Penicillium chrysogenum*) with variability in the occurrence of fungal isolates among the sunflower varieties (Table 1). At 3 days after infection (DAI), SAMSUN2 showed no occurrence of *Aspergillus flavus* but relatively high occurrences of *Aspergillus niger* and *Fusarium oxysporum*. In contrast, SAMSUN1 and SAMSUN3 had moderate occurrences of all four fungal species. *Fusarium oxysporum* appeared to be the most prevalent fungal species across all varieties, with frequencies ranging from 50 to 60. *Aspergillus niger* also showed relatively high frequencies, particularly in SAMSUN4 and SKY SCRAPER. Interestingly, *Penicillium chrysogenum* exhibited lower occurrences overall, with most varieties having frequencies below 30. There were no occurrences of *Aspergillus flavus* and *Penicillium chrysogenum* in SAMSUN2.

**Table 1. Occurrence of Seedborne fungi isolates on the seeds of Sunflower Varieties at 3DAI**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variety** | **Isolates** | **Frequency**  | **%tage incidence** |
| SAMSUN1 | *Aspergillus flavus* | 10 | 2.0 |
| *Aspergillus niger* | 25 | 5.0 |
| *Fusarium oxysporum* | 54 | 10.8 |
| *Penicillium chrysogenum*  | 24 | 4.8 |
| SAMSUN2 | *Aspergillus flavus* | 0 | 0.0 |
| *Aspergillus niger* | 24 | 4.8 |
| *Fusarium oxysporum* | 60 | 12.0 |
| *Penicillium chrysogenum*  | 4 | 0.8 |
| SAMSUN3 | *Aspergillus flavus* | 10 | 2.0 |
| *Aspergillus niger* | 30 | 6.0 |
|  | *Fusarium oxysporum* | 54 | 10.8 |
|  | *Penicillium chrysogenum*  | 0 | 0.0 |
| SAMSUN4 | *Aspergillus flavus* | 10 | 2.0 |
|  | *Aspergillus niger* | 37 | 7.4 |
|  | *Fusarium oxysporum* | 54 | 10.8 |
|  | *Penicillium chrysogenum*  | 0 | 0.0 |
| SKY SCRAPER | *Aspergillus flavus* | 4 | 0.8 |
|  | *Aspergillus niger* | 50 | 10.0 |
|  | *Fusarium oxysporum* | 50 | 10.0 |
|  | *Penicillium chrysogenum*  | 0 | 0.0 |

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### 3.2. Frequency Distribution of Fungal Isolates at 3 Days After Infection (DAI)

The frequency distribution of fungal isolates in sunflower samples at 3 Days after infection (DAI) showed the prevalence of different fungal species, including *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum,* and *Penicillium chrysogenum*, along with their respective percentages of occurrence (Table 2). *Fusarium oxysporum* was the most prevalent fungal isolate, with a frequency of 272, accounting for 54.4% of the total isolates, indicating its dominance as a fungal species in sunflower samples at 3DAI. *Aspergillus niger* follows with a frequency of 166, representing 33.2% of the total isolates, indicating its significant presence as well. *Aspergillus flavus* and *Penicillium chrysogenum* had lower frequencies of 34 and 28, respectively, comprising 6.8% and 5.6% of the total isolates, respectively.

**Table 2. Frequency Distribution of Fungal Isolates 3DAI**

|  |  |  |
| --- | --- | --- |
| **Fungal Isolates** | **Frequencies of Occurrence** | **%tage incidence** |
| *Aspergillus flavus* | 34 | 6.8 |
| *Aspergillus niger* | 166 | 33.2 |
| *Fusarium oxysporum* | 272 | 54.4 |
| *Penicillium chrysogenum* | 28 | 5.6  |
| **Total** | **500** | **100** |

### 3.3. Frequency and Percentage Distribution of Fungal Isolates on Sunflower Seeds at 4DAI and 5DAI

Results show that at 4DAI and 5DAI, there was a noticeable increase in the frequency of fungal isolates across most varieties (Table 3). *F. oxysporum* increased from 56 to 63 in SAMSUN1 and SAMSUN3. SAMSUN2 and SKY SCRAPER recorded the lowest frequencies of *Aspergillus flavus* (2 to 5 and 3 to 6) between 4DAI and 5DAI respectively, while *P. chrysogenum* consistently had the lowest frequencies across all varieties except SAMSUN1 which was significantly higher than the other varieties (26 to 30) at both 4DAI and 5DAI. In contrast, SAMSUN4 and SKY SCRAPER exhibited relatively higher frequencies of *A. niger* within the period. *A. flavus* in SAMSUN1, SAMSUN3 and SAMSUN4 increased at the same rate, from 12 to 14 between 4DAI and 5DAI respectively, but there was a significant increase in *P. chrysogenum* in SAMSUN1 compared with the other varieties.

**Table 3: Occurrence of Seedborne fungi isolates on Sunflower Seeds at 4DAI and 5DAI**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variety** | **Isolates** | **Frequency 4DAI**  | **%tage incidence** | **Frequency 5DAI** | **%tage incidence** |
| SAMSUN1 | *Aspergillus flavus* | 12 | 2.23 | 14 | 2.25 |
|  | *Aspergillus niger* | 27 | 5.02 | 32 | 5.15 |
|  | *Fusarium oxysporum* | 56 | 10.41 | 63 | 10.15 |
|  | *Penicillium chrysogenum* | 26 | 4.83 | 30 | 4.83 |
| SAMSUN2 | *Aspergillus flavus* | 2 | 0.37 | 5 | 0.81 |
|  | *Aspergillus niger* | 26 | 4.83 | 30 | 4.83 |
|  | *Fusarium oxysporum* | 62 | 11.52 | 68 | 10.93 |
|  | *Penicillium chrysogenum* | 6 | 1.12 | 9 | 1.45 |
| SAMSUN3 | *Aspergillus flavus* | 12 | 2.23 | 14 | 2.25 |
|  | *Aspergillus niger* | 32 | 5.95 | 37 | 5.96 |
|  | *Fusarium oxysporum* | 56 | 10.41 | 63 | 10.15 |
|  | *Penicillium chrysogenum* | 2 | 0.37 | 5 | 0.81 |
| SAMSUN4 | *Aspergillus flavus* | 12 | 2.23 | 14 | 2.25 |
|  | *Aspergillus niger* | 39 | 7.25 | 42 | 6.76 |
|  | *Fusarium oxysporum* | 56 | 10.41 | 63 | 10.15 |
|  | *Penicillium chrysogenum* | 3 | 0.56 | 6 | 0.96 |
| SKY SCRAPER | *Aspergillus flavus* | 3 | 0.56 | 7 | 1.14 |
|  | *Aspergillus niger* | 52 | 9.67 | 57 | 9.18 |
|  | *Fusarium oxysporum* | 52 | 9.67 | 57 | 9.18 |
|  | *Penicillium chrysogenum* | 2 | 0.37 | 5 | 0.81 |

There was a slight increase in the number and proportion of *Aspergillus flavus* isolates between 4DAI and 5DAI with frequencies of 41 and 54, representing 7.62% and 8.70% of the total isolates, respectively (Table 4). *Aspergillus niger* remained a dominant fungal isolate, with a notable increase in absolute numbers and a slight decrease in the proportion of the total fungal community, with frequencies of 176 and 198 at 4DAI and 5DAI, representing 32.71% and 31.88% of the total isolates, respectively. *Fusarium oxysporum* was the most prevalent fungal isolate within the period. While its absolute numbers increased, its relative abundance also decreased as the proportion of other isolates (particularly *Penicillium chrysogenum*) increased more noticeably, with frequencies of 282 and 314, representing 52.42% and 50.56% of the total isolates, respectively. *Penicillium chrysogenum* showed proportional increase among the isolates between 4DAI and 5DAI, having frequencies of 97 and 55, representing 7.25% and 8.86% of the total isolates, respectively.

**Table 4: Frequency and Percentage Distribution of Fungal Isolates at 4DAI and 5DAI**

|  |  |  |
| --- | --- | --- |
| **Fungal Isolates** | **4DAI** | **5DAI** |
|  | **Frequency** | **Percentage (%)** | **Frequency** | **Percentage (%)** |
| *Aspergillus flavus* | 41  | 7.62% | 54  | 8.70% |
| *Aspergillus niger* | 176  | 32.71% | 198 | 31.88% |
| *Fusarium oxysporum* | 282  | 52.42% | 314 | 50.56% |
| *Penicillium chrysogenum* | 39 | 7.25% | 55 | 8.86%  |
| **Total** | **538** | **100%** | **621** | **100%** |

##  4. Discussion

This investigation revealed the presence and varying proportional distributions of Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, and Penicillium chrysogenum within sunflower seed mycobiota over five days. F. oxysporum and A. niger consistently constituted the predominant fungal populations at 4 and 5 days after emergence (DAI). Minor shifts in the relative proportions of fungal isolates between time intervals suggest differential growth rates or competitive dynamics under experimental conditions, with a notable increase in P. chrysogenum and a slight decrease in F. oxysporum. The observed proliferation of F. oxysporum at 4 and 5 DAI highlights crucial implications for disease management strategies. This warrants further understanding of these distinctions for targeted disease management interventions (Guzman *et al*., 2021) and (Khalid *et al*., 2014).

The identification of seed-borne pathogens presents significant agricultural and public health concerns. Such pathogens can serve as primary inoculum sources, leading to seedling blight, compromised stand establishment, and mature plant diseases (Agrios, 2005). The detection of A. flavus is particularly critical due to its implications for food safety and agricultural economics. Its presence necessitates rigorous monitoring for aflatoxin contamination in sunflower seeds, as contaminated batches face strict rejection, incurring substantial economic losses and posing public health risks (Bennett and Klich, 2003).

Specifically, A. flavus strains are known producers of aflatoxins which are potent carcinogenic mycotoxins. Aflatoxin B1, is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Khalid *et al*., 2014). The presence of F. oxysporum also indicates risks of seed rot and potential mycotoxin contamination, given that some Fusarium species are recognized plant pathogens and mycotoxin producers (Pitt and Hocking, 2014).

Minimising mycotoxin exposure is paramount for public health, as these compounds detrimentally affect both human and animal health, simultaneously compromising food security and nutritional access (Khalil *et al.*, 2014). Uncontrolled mycotoxin-induced infections can lead to fatal outcomes (Fisher *et al*., 2020). Consequently, these findings are critical for assessing the quality and safety of sunflower seeds for both human consumption and livestock feed production. Precise identification of fungal species is the foundational step towards developing targeted and effective management strategies for sunflower production.

## 5. Conclusion

This study significantly advances our understanding of fungal pathogen impact on sunflower seed varieties, with implications for agriculture and public health in Nigeria. The heightened prevalence of F. oxysporum and A. flavus indicates potential risks to plant health, food safety (mycotoxin contamination), and human/animal health (infections). The abundance of A. niger calls for concern in its potential for infections and mycotoxin production. While the increase in P. chrysogenum suggests a shift in fungal community structure, these identified fungi collectively pose potential health risks via mycotoxin production, infection, or allergic reactions, requiring further investigation into factors determining severity. These findings underscore the critical need for proper sunflower seed handling, storage, and processing to mitigate fungal growth and mycotoxin contamination in Nigeria. Further research is necessary to assess specific consumption risks from contaminated seeds and to implement targeted management strategies, including mycotoxin monitoring, plant disease incidence assessment, and evaluating health impacts on exposed individuals. Future work should prioritise molecular identification and sustainable management strategies for seed-borne pathogens

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that NO generative AI technologies (ChatGPT, COPILOT, Gemini, etc) and text-to-image generators were used during the writing or editing of this manuscript.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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