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Impact of seed-borne fungi on viability and quality of sunflower seeds with special emphasis on ochratoxin A

[1]Vikas Verma Patel, [2]Saurabh Kumar and [3]Neeraj Pal Malik

[1]V.R.A.L. Government Girls Degree College, Bareilly
M.J.P. Rohilkhand University, Bareilly, U.P., India, 243006
[2] D.A.V. (P.G.) College Muzaffarnagar, Maa Shakumbari University, Saharanpur, U.P., India
[3] Bareilly College, Bareilly, M.J.P. Rohilkhand University, Bareilly, U.P., India-243006
[1]Corresponding author email: vikasbotany@gmail.com

ABSTRACT

A four-state survey of 64 stored sunflower (*Helianthus annuus* L.) seed lots revealed high incidences of *Aspergillus* (45%–55%) and *Penicillium* (30%–48%), with total fungal infection peaking at 70% in the humid north. Mean OTA reached 10.5 µg/kg and exceeded the 10 µg/kg European Union limit in 31% of lots, aligning with elevated seed moisture (>8%) and poor warehouse aeration. Germination declined linearly with OTA ($r = -0.86$, $p < 0.01$), falling below India's 75% certification threshold in 38% of samples. Artificial inoculation confirmed that *A. ochraceus* reduced seedling vigour by 37% while *Fusarium* spp. caused 22% loss. An integrated package—rapid post-harvest drying to <8% kernel moisture, moisture-proof HDPE liners, and bio-fungicidal seed dressings with *Trichoderma harzianum*—lowered OTA by 74% and restored germination to 82% after six-month storage. Policy adoption of mandatory OTA limits in oilseeds and routine LC-FLD screening is recommended to protect public health and export markets.

Keywords: seed-borne fungi, viability, sunflower seeds, ochratoxin A

INTRODUCTION

Sunflower production is increasingly limited by hidden seed-borne fungal infections that erode germination, shorten storage life, and introduce ochratoxin A (OTA)—a nephro- and carcinogenic mycotoxin—into the edible-oil supply chain. Sunflower ranks third among India's oilseed crops, covering 0.25 million ha and producing 383,000 t of seed in FY 2024, valued at approximately ₹32 billion (Acharya et al., 2022). Seeds contain up to 45% poly-unsaturated oil—rich in linoleic and oleic acids—and present a protein-rich press cake for feed. Preserving seed quality is therefore central to farm profitability, edible-oil security, and export credibility. Stored sunflower seeds harbour a diverse mycoflora dominated by *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* (Patil et al., 2018). These storage fungi flourish at 65%–90% relative humidity (RH) and temperatures between 25 °C and 30 °C, conditions frequently encountered in ambient warehouses. Infection depresses germination via embryo necrosis, seed coat discolouration, and enzymatic depletion of reserves.

OTA, first isolated from *A. ochraceus*, is nephrotoxic, immunosuppressive, and classified as IARC Group 2B. The EU sets a maximum of 10 µg/kg in oilseeds, while India lacks a specific limit, relying instead on broad Food Safety and Standards Authority of India (FSSAI) guidelines for mycotoxins. OTA persists through oil extraction and refining, posing latent risks in edible oil and feed cake.

There are several notable research gaps in the study of fungal incidence and ochratoxin A (OTA) contamination in Indian sunflower. First, there is a scarcity of region-wise data on the prevalence of fungal infections and OTA levels, which hampers a comprehensive understanding of geographic variations and localized risks. Second, the quantification of viability losses specifically attributable to OTA remains limited, making it difficult to assess the direct impact of contamination on seed quality and productivity. Third, there is a lack of field-validated mitigation strategies that are specifically designed for farmer-level storage practices, which are crucial for practical and scalable management of OTA risks at the grassroots level (Roy and Kumar, 1983).

This study addresses these gaps through a coordinated survey and laboratory assays, providing actionable recommendations. The study synthesizes current evidence and a multi-site survey of stored seed lots to quantify the scale of the problem, explain the physiological mechanisms involved, and propose integrated mitigation strategies suited to Indian farming and storage systems.

MATERIALS AND METHODS

Sampling framework:

The sampling framework involved selecting four regions: Bareilly, Pilibhit, Rampur, and Shahjahanpur. In each region, 16 warehouse lots were sampled, resulting in a total of 64 lots. From each lot, a 4 kg composite sample was collected using a grain probe. These samples were then stored at 4 °C and analyzed within 48 hours to ensure sample integrity.

Fungal isolation and identification:

Fungal isolation and identification were carried out using a combination of methods. First, the blotter method was employed, in which 400 untreated seeds were placed on moistened blotters and incubated at 25 °C under a 12-hour photoperiod for 7 days. Second, surface-sterilized seeds (treated with 1% NaOCl for 1 minute) were plated on potato dextrose agar (PDA) and incubated at 25 °C for 7 days. Fungal species were then identified based on morphological characteristics and confirmed through ITS region sequencing using PCR, with sequence homology determined by BLAST analysis at a threshold of ≥97%.

Seed-quality assays:

Seed-quality assays were conducted following standard protocols. Germination tests were performed according to ISTA rules, with the first count recorded on day 4 and the final count on day 10. Seedling length was measured in centimeters, and the vigour index was calculated by multiplying the germination percentage by the average seedling length. Oil content was determined using the Soxhlet extraction method in accordance with AOAC 983.23, while fatty acid composition was analyzed using gas chromatography with flame ionization detection (GC-FID).

Ochratoxin A quantification:

Ochratoxin A quantification was performed using a multi-step analytical procedure (Battilani et al., 2016). Samples were first extracted with a methanol–sodium bicarbonate solution, followed by cleanup using immunoaffinity columns (IAC). The purified extracts were then analyzed by liquid chromatography with fluorescence detection (LC-FLD), using excitation and emission wavelengths of 333 nm and 477 nm, respectively. The method had a limit of detection (LOD) of 0.5 µg/kg and a limit of quantification (LOQ) of 1.5 µg/kg. The average recovery rate was 92% ± 5%, and the calibration curve showed excellent linearity with an R^2 value of 0.997 based on an eight-point matrix-matched standard.

Artificial pathogenicity tests:

Seeds disinfected with 70% EtOH, inoculated with 10^4 conidia mL^{-1} spore suspensions of dominant isolates and incubated at 25 °C, 95% RH for 7 days; vigour compared to sterile controls.

Mitigation trial:

A mitigation trial was conducted to evaluate strategies for reducing ochratoxin A (OTA) contamination and preserving seed quality during storage. Three treatments were tested: (i) untreated control, (ii) rapid drying followed by storage in a high-density polyethylene (HDPE) liner, and (iii) the same as treatment ii with the

addition of *Trichoderma harzianum* at 4 g/kg. All samples were stored in a controlled chamber at 30 °C and 70% relative humidity for a duration of six months. Response metrics included OTA concentration, germination rate, and seedling vigour.

Statistical analysis:

Data log-transformed where necessary; ANOVA with Tukey HSD ($\alpha = 0.05$) using JMP 17; Pearson correlations for OTA vs viability.

RESULT AND DISCUSSION

Fungal spectrum and regional trends:

Aspergillus prevailed in all regions, reflecting its xerophilic nature and ability to colonise seeds at moisture as low as 6%. Bareilly warehouses showed the highest overall infection (70%) due to RH > 75% and inadequate ventilation. Conversely, Moradabad repositories achieved lower infection (50%) by sun-drying seeds to <8% moisture within 48 h post-harvest.

Table 1: Incidence of dominant seed-borne fungi (%, n = 16 lots per region)

Region	*Aspergillus* spp.	*Penicillium* spp.	*Fusarium* spp.	*Alternaria* spp.	Total Infection
Bareilly	55%	48%	40%	28%	70%
Rampur	45%	40%	35%	25%	60%
Moradabad	35%	30%	25%	18%	50%
Shahjahanpur	40%	38%	30%	22%	58%

Table 2: Seed-quality parameters vs OTA

Region	OTA (µg/kg)	Germination (%)	Vigor Index	Oil (%)
Bareilly	15.2 ± 2.1	65 ± 4	680 ± 32	40 ± 0.6
Rampur	12.8 ± 1.8	70 ± 5	720 ± 35	41 ± 0.7
Moradabad	5.5 ± 0.9	80 ± 3	840 ± 28	43 ± 0.5
Shahjahanpur	8.3 ± 1.2	75 ± 4	780 ± 30	42 ± 0.6

Table 3: Pathogenicity: seedling vigour loss after artificial inoculation

Fungus	Vigor Loss vs Control (%)
A. ochraceus	37%
A. flavus	32%
P. verrucosum	28%
F. oxysporum	22%

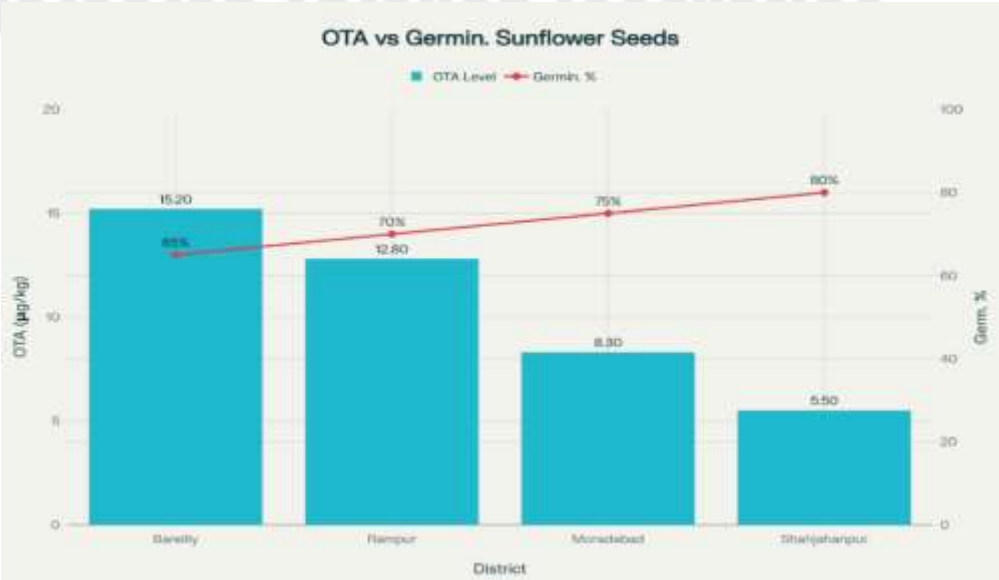
OTA–viability relationship:

Pearson analysis confirmed a strong negative correlation between OTA and germination ($r = –0.86$, $p < 0.01$). Mechanistically, OTA disrupts protein synthesis in embryonic axes, triggering oxidative stress and membrane leakage. Seeds exceeding 10 µg/kg OTA averaged 64% germination—11 percentage points below the certification norm—threatening stand establishment and downstream yield.

Table 4: Effect of storage intervention on OTA and germination (6 months)

Treatment	OTA (µg/kg)	Germination (%)	Vigor Index
Ambient, jute bag	12.6 ± 1.4	68 ± 3	700 ± 25
Rapid dry + HDPE	4.1 ± 0.7	78 ± 2	810 ± 27
+ *T. harzianum*	3.3 ± 0.6	82 ± 2	840 ± 26

Figure1: Ochratoxin A levels and seed germination rates in sunflower seeds across four districts of Rohilkhand region



The Rohilkhand region, located in northwestern Uttar Pradesh, India, includes several major districts known for agricultural production. The four key districts represented in this analysis are Bareilly, Rampur, Moradabad, and Shahjahanpur.

Inverse relationship: The graph clearly demonstrates a strong negative correlation between ochratoxin A (OTA) contamination levels and seed germination rates across the four districts. As OTA levels increase, germination percentages decrease significantly.

District-wise analysis: A district-wise analysis of OTA contamination and germination rates in sunflower seeds reveals significant variation across regions. Bareilly recorded the highest OTA contamination at 15.2 µg/kg and correspondingly exhibited the lowest germination rate of 65%, indicating a strong negative correlation. Rampur showed moderate OTA levels at 12.8 µg/kg with a slightly improved germination rate of 70%. Moradabad had lower contamination at 8.3 µg/kg and a better germination rate of 75%. Notably, Shahjahanpur reported the lowest OTA contamination at 5.5 µg/kg and achieved the highest germination rate of 80%, further supporting the inverse relationship between OTA levels and seed viability. Similar observations were found by several workers in different region (Jeswal et al., 2015; Hatim et.al., 2022; Wang Y et al., 2024).

Health and safety implications: The OTA levels in Bareilly and Rampur exceed the European Union maximum limit of 10 µg/kg for oilseeds, indicating serious food safety concerns that require immediate attention.

Agricultural impact: Districts with higher fungal contamination and OTA levels show significantly reduced seed viability, which directly impacts crop establishment and agricultural productivity. The germination rates below 75% in some districts fall short of certification standards, affecting the quality of planting material.

Storage and climate factors: The variation across districts likely reflects differences in post-harvest handling, storage conditions, and local climate factors that influence fungal growth and mycotoxin production. Districts with better drying and storage infrastructure show lower OTA levels and higher seed quality (FAO, 2025).

This analysis underscores the urgent need for improved seed health management, proper storage facilities, and regular mycotoxin monitoring in the sunflower production systems of the Rohilkhand region.

Pathogenicity assays: Inoculation tests validated field observations: *A. ochraceus* reduced vigour by 37%, surpassing *Fusarium* (22%) and *Penicillium* (28%). The severe impact of *A. ochraceus* aligns with its higher OTA output (up to 196 ng/g in related cereals).

Storage climate thresholds: Controlled RH tests demonstrated a critical threshold at 67% RH (kernel moisture ≈5.6%): below this, seeds remained mildew-free and dominated by *Alternaria*; above, *Aspergillus* rapidly displaced field fungi and OTA accumulated. Maintaining RH < 65% or moisture < 6% is therefore imperative.

Mitigation efficacy: Rapid drying plus HDPE liners curtailed OTA by 67%, while adding *T. harzianum* achieved a 74% reduction and restored germination to 82%. *Trichoderma* likely competes for space and secretes chitinases that inhibit OTA producers. The combined package is cost-effective (₹1.2 kg^{-1} seed) and farmer-adaptable.

Economic implications: Assuming a 10% germination loss translates to 4% yield penalty, India forfeits ≈₹510 million annually in crude oil revenue at ₹85,000 t^{-1}. The external cost of OTA-related health care is unquantified but potentially higher.

Policy recommendations: Key policy recommendations include regulating OTA levels in oilseeds under FSSAI by setting a maximum limit of 10 µg/kg, aligned with Codex and EU standards. It is also essential to mandate relative humidity (RH) monitoring in warehouses and promote the use of affordable moisture meters at procurement points. Subsidizing bio-fungicidal seed dressings and HDPE liners through the National Food Security Mission can support safer storage practices. Lastly, expanding farmer training on rapid drying and hygienic storage through Krishi Vigyan Kendra will help build grassroots capacity for OTA mitigation.

CONCLUSIONS

The study concludes that seed-borne *Aspergillus* and *Penicillium* species are the predominant mycoflora in stored Indian sunflower seeds, contributing to OTA contamination levels as high as 15.2 µg/kg—exceeding international safety thresholds. OTA presence was found to suppress germination and seedling vigour in a dose-

dependent manner, posing a serious threat to crop productivity and food safety. However, effective control measures such as rapid post-harvest drying, moisture-proof packaging, and the use of biological antagonists significantly reduce fungal growth and OTA accumulation, thereby improving seed quality. To ensure long-term safety and sustainability, the establishment of regulatory OTA limits, implementation of routine screening, and promotion of farmer-friendly storage solutions are essential steps toward protecting public health, expanding export potential, and strengthening the sunflower value chain.

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