Innovative Technologies for Non-Intrusive Aflatoxin Detection in Pistachios

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***Abstract*—Aflatoxin contamination poses a significant risk to all nuts, including pistachios, during harvest, storage, and processing. Dietary exposure to aflatoxins can lead to severe toxic and carcinogenic effects in humans. To safeguard human and animal health, aflatoxin legislation sets maximum permissible levels for aflatoxins in food products, including pistachios. Consequently, imported pistachios undergo rigorous aflatoxin contamination testing. Traditional methods for measuring aflatoxin levels, such as High-Performance Liquid Chromatography (HPLC), HPLC with Mass Spectrometry, and Enzyme-Linked Immunosorbent Assay (ELISA), although precise, are destructive, costly, and time-consuming. This paper investigates the application of emerging technologies, including Hyperspectral Imaging, Chromatographic Test Strips, Luminescent Metal-Organic Frameworks, spectroscopic methods, machine vision, and advanced artificial intelligence models, to develop a non-intrusive, real-time system for aflatoxin detection in pistachio nuts. Additionally, it outlines a comprehensive strategy to protect public health, mitigate economic losses estimated at $932 million annually, and sustain the pistachio industry.**

***Index Terms*—Hyperspectral imaging, Aflatoxin detection, pistachio nuts.**

I. Introduction

Pistachios are a globally significant crop, with annual production around 747,000 metric tons, predominantly from the United States (67%), Iran (17%), and Turkey (11%) [1]. Valued for their nutritional benefits—rich in unsaturated fatty acids (55% oil content), proteins (20%), and antioxidants like lutein—they contribute approximately $6 billion annually to the world economy, supporting millions of livelihoods from orchard to market [2]. Iran's pistachio exports generate approximately $900 million yearly, while the U.S., with California as its hub, exports around $1.7 billion worth, primarily to Asia and Europe [3], [4]. However, this thriving industry faces a persistent threat: aflatoxin contamination, particularly AFlatoxin B1 (AFB1), produced by *Aspergillus flavus* and *Aspergillus parasiticus* under warm, humid conditions prevalent in pistachio-growing regions [5].

AFB1 is a serious threat to health. It is classified as a Group 1 carcinogen by the International Agency for Research on Cancer because it is linked to liver cancer, with incidence rates in high-exposure areas like Gambia reaching up to 39.67 cases per 100,000 people annually, about four times the global average of 9.5 [6]. Chronic exposure also causes immune suppression, reduces T cell counts and stunts growth in children, studies reporting a height reduction of up to 1.7 cm over 8 months due to impaired nutrient absorption [7], [8]. Acute aflatoxicosis, although rare, is devastating: a 2004 outbreak in Kenya caused 125 deaths and 317 cases of contaminated maize, illustrating the lethal potential of AFB1 [9]. These health risks drive stringent regulatory limits—the U.S. Food and Drug Administration (FDA) caps AFB1 at 20 parts per billion (ppb), the European Union (EU) enforces 8 ppb for ready-to-eat nuts, and the Codex Alimentarius recommends 10 ppb [10].

Economically, aflatoxin contamination inflicts severe losses. The nut industry faces significant economic losses from export rejections due to aflatoxin contamination, estimated in the hundreds of millions annually, with pistachios bearing a disproportionate burden due to their premium value. Recently, Iran has faced significant export challenges due to aflatoxin contamination, with reports of substantial rejections by the EU, impacting thousands of small farmers [11]. The U.S. spends millions on compliance testing—$10–$20 per HPLC sample, totaling $5k–$10k for a 1,000-ton batch—while disposal of contaminated lots costs $500–$1k per ton [12]. Regulatory disparities exacerbate the issue: the EU's 8 ppb limit is tighter than the U.S.'s 20 ppb, creating a compliance maze for exporters, while testing costs disproportionately burden small-scale producers, risking their exclusion from global markets.

Pistachios' vulnerability stems from their cultivation and

This research has been funded under a Knowledge Transfer Partnership by Innovate UK (KTP 13808).

handling. Grown in arid yet humid climates, they thrive in temperatures of 25–35°C and humidity levels of 60–80%, conditions ideal for fungal proliferation [5]. Unlike almonds or walnuts, pistachios develop split shells during ripening, exposing kernels to *Aspergillus* spores pre- and post-harvest. Proper drying - delays beyond 24 hours after harvest - or storage in $>$ 6% moisture content amplifies the risk of contamination by 50 to 70%, since fungi metabolize the high oil and carbohydrate content of the nuts [5]. Traditional detection methods like HPLC and ELISA, while precise, are slow (1–3 hours), costly ($50k–$200k equipment), and destructive, rendering them impractical for real-time monitoring of large batches. Emerging technologies—HyperSpectral Imaging, Chromatographic Test Strips, Luminescent Metal-Organic Frameworks, and machine vision could offer rapid, non-invasive alternatives, potentially transforming pistachio safety protocols.

This paper aims to: a) provide an exhaustive analysis of current aflatoxin detection methods, detailing their technical processes, advantages, and pistachio-specific challenges; b) explore emerging technologies with practical applications for pistachio testing, enriched with case studies and technical insights; c) propose future strategies integrating detection and prevention to ensure compliance with global standards, reduce economic losses, and protect public health in a $5 billion industry facing climate-driven risks. The remainder of this paper is structured as follows: Section II introduces the measurement methods for Aflatoxin contamination. Section III presents emerging technologies for measuring Aflatoxin contamination in pistachios. Section IV gives a comparative analysis of Aflatoxin contamination and detection methods in different nuts. Section V highlights the economic and environmental impact of Aflatoxin contamination in nuts. Directions for further research are provided in Section VI. Finally, the paper concludes in Section VII.

II. AFLATOXIN CONTAMINATION MEASUREMENT METHODS

Traditional laboratory-based methods form the backbone of aflatoxin monitoring, offering high sensitivity but facing practical constraints. This section examines their application to pistachios, diving into technical details and limitations.

A. Chromatographic Techniques

Chromatographic methods provide unmatched precision but demand significant resources, making them a cornerstone of regulatory compliance, but a challenge for scalability.

- High-Performance Liquid Chromatography (HPLC): HPLC separates aflatoxins using a reverse-phase C18 column (e.g., 250mm$\times$4.6mm, 5μm particle size) with a methanol-water mobile phase (60:40v/v) at a flow rate of 1mL/min. Detection relies on fluorescence spectroscopy (excitation 365nm, emission 435nm), often enhanced by post-column derivatisation with iodine (0.05% solution) or photochemical reactors to boost sensitivity to < 0.1ppb—well below EU's 8 ppb limit. For pistachios, 20–50 grams are ground into a fine powder ($<$500 μm) using a laboratory mill, extracted with methanol-water (80:20) for 30 minutes on an orbital shaker, and filtered through Whatman No. 4 paper to remove particulates. The extract undergoes immunoaffinity column cleanup with monoclonal antibodies specific to AFB1, B2, G1, and G2, isolating aflatoxins from lipids (55% of pistachio mass) and pigments like chlorophyll, which fluoresce and skew results. This cleanup, taking 30–60 minutes, uses a 10 mL syringe to push the extract through the column, followed by elution with 1–2mL methanol. Calibration employs AFB1 standards (0.1–100 ppb), achieving 85–105% recovery, though lipid-rich samples require hexane washes, extending preparation to 2–3 hours. Equipment costs exceed $50k, with reagents at $100–$200 per batch and annual maintenance at $5k–$10k. In California's $1.6 billion pistachio industry, HPLC confirms contamination but delays processing, costing $10k–$20k per harvest season in lost efficiency [12].
- Ultra-High-Performance Liquid Chromatography (UHPLC-QqQ-MS/MS): UHPLC-QqQ-MS/MS enhances HPLC with triple quadrupole mass spectrometry, detecting AFB1 at $<$0.05 ppb—five times more sensitive than HPLC—along with B2, G1, and G2. It uses a shorter column (e.g., 100mm$\times$2.1mm, 1.8μm) and gradient elution (water-acetonitrile with 0.1% formic acid, 0.3 mL/min) over a 10-minute run-time, followed by electrospray ionisation (positive mode, 3.5 kV) and mass spectrometry in Multiple Reaction Monitoring (MRM) mode (e.g., AFB1 transition m/z 313 $\rightarrow$ 285). Pistachio preparation mirrors HPLC (grinding, methanol extraction), but UHPLC's higher pressure accelerates separation, processing 20 samples per hour versus HPLC's 5–10. Isotopically labelled AFB1 standards (e.g., ^{13}C-AFB1) ensure 90–110% recovery, critical for pistachios' complex matrix of polyphenols (e.g., gallic acid) and fatty acids (e.g., oleic acid, 60% of oil). Equipment costs range from $100k to $200k, with annual maintenance at $10k–$20k and reagents at $200–$300 per batch. Its ability to trace contamination sources—e.g., pre-harvest fungal growth (orchard humidity $>$80%) vs. storage mishandling ($>$6% moisture)—is invaluable for research. A 2023 study on U.S. pistachios used UHPLC to identify AFB1 hotspots, linking 20% of contamination to inadequate drying, but its cost and complexity limit routine use [13].
- Thin-Layer Chromatography (TLC): TLC separates aflatoxins on silica gel plates (20$\times$20cm, 0.25mm thickness) with a chloroform-acetone solvent system (9:1), visualized under 365nm Ultar Violates (UV) light at 1–5ppb sensitivity. Pistachio samples (10 grams) are ground, extracted with methanol-water (70:30) for 20 minutes, and spotted via micropipette (5μL) onto the plate. Development in a sealed chamber takes 20–30 minutes, with AFB1 appearing as blue-green bands (R_f 0.5). Basic setups cost $300–$500 (UV lamp, plates, solvents), and analysis totals 45 minutes with minimal

training—two hours versus HPLC's 10–12 hours for a technician. In pistachios, pigments like chlorophyll (absorption at 663nm) overlap with AFB1 bands, reducing accuracy unless paired with densitometry ($1k–$2k), which quantifies fluorescence intensity [5]. A study on pistachio samples using TLC reported challenges in accuracy due to environmental factors like high humidity, highlighting its limitations for precise quantification [14]. TLC's affordability suits low-resource settings, but its semi-quantitative nature and susceptibility to interference restrict regulatory use.

B. Immunoassays

Immunoassays offer faster, simpler detection through antigen-antibody interactions, balancing cost and throughput.

- Enzyme-linked immunosorbent assay (ELISA): ELISA detects AFB1 using enzyme-tagged antibodies (e.g. horseradish peroxidase), producing a colorimetric signal at 450 nm with 0.5–1 ppb sensitivity. For pistachios, 10 grams are ground, extracted with 50 mL methanol (70%) for 15 minutes in a blender, and filtered through a 0.45 μm syringe filter. The extract is diluted (1:10 with buffer) to minimise lipid interference (55% oil content), then 100 μL is applied to a microtiter plate coated with AFB1-specific antibodies. After 30 minutes of incubation at 37°C, the unbound material is washed with PBS-Tween (3×, 200 μL), and a TMB substrate (100 μL) triggers a color change, measured by a plate reader in 10–15 minutes. The total time is less than 2 hours, with kits costing $5–$10 each and a throughput of 96 samples per plate, ideal for screening 1,000-ton batches (50–100 tests) at $250–$500. However, pistachio lipids and pigments overestimate AFB1 by 10–20%, requiring validation with spiked samples (0.5–5 ppb, 70–120% recovery). A 2014 study on salt-roasted pistachios found 58.6% of 32 samples exceeded 15 ppb, with ELISA misidentifying 10% due to cross-reactivity with AFB2 (m/z similar to AFB1). Its speed and cost-effectiveness are offset by specificity limits in regulatory contexts [15].
- Lateral-Flow Assays (LFA): LFAs use gold nanoparticle-labeled antibodies on nitrocellulose strips, detecting AFB1 at 5–10 ppb in 5–10 minutes. Pistachio samples (5 grams) are ground, extracted with methanol-water (50:50, 10 mL) for 5 minutes, and 100μL is applied to a sample pad. The capillary action carries AFB1 to test and control lines—visible within 5–7 minutes, indicating presence above 5 ppb. Smartphone apps (e.g., RIDA®SMART) quantify line intensity via RGB analysis, enhancing field usability at $3–$5 per strip. LFAs are non-destructive, preserving samples, and require no lab setup, suiting on-site checks at harvest or storage. In pistachios, their 5–10 ppb sensitivity exceeds the EU's 8 ppb limit, misclassifying 10–15% of borderline samples, and lipid residues weaken signals by 5–10%. A study on decentralized systems found that LFAs screened 80% of maise samples accurately but required HPLC confirmation for pistachios, highlighting their role as a rapid preliminary tool [16].

C. Dip-Strip and Immunochromatographic Methods

- Portable Dip-Strips: Dip-strips feature adsorbent-coated strips (e.g., silica or cellulose, 5x50 mm) paired with handheld UV lamps (365 nm), detecting AFB1 at 10 ppb in under 10 minutes. Pistachio samples (5–10 grams) are mashed with a mortar and pestle, extracted with methanol-water (50:50, 20 mL) for 5 minutes, and 2 mL is dipped into the strip solution for 2–3 minutes. Fluorescence under UV light (blue-green glow) indicates contamination above 10 ppb, with kits costing $10–$15 each. Calibration uses AFB1 standards (5–20 ppb), but pistachio pigments reduce fluorescence intensity by 10–15%, requiring visual comparison to controls. Their simplicity—no lab infrastructure or power supply is needed—makes them ideal for field use by inspectors. A 1993 study validated dip-strips on 100 food samples, including nuts, achieving 85% agreement with HPLC, but their 10 ppb limit exceeds strict EU standards, limiting them to initial screening [17].
- Immunochromatographic Tests (ICTs): ICTs integrate chromatography and immunology, using test lines to signal AFB1 at 5–10 ppb in 5–10 minutes. Pistachio extracts (5 mL, methanol-water 70:30) are applied to a strip with monoclonal antibodies, and colored bands (e.g., red from gold nanoparticles) appear if AFB1 exceeds 5 ppb, costing $8–$15 per test. The process involves a 2-minute extraction, 1-minute strip application, and 5–7 minutes for development, totalling under 10 minutes. In pistachios, batch-to-batch antibody variability (±10% sensitivity) and lipid interference (5–10% signal reduction) affect reliability, requiring quality control during manufacturing (e.g., ISO 9001 standards). A 2022 review found ICTs detected AFB1 in 90% of spiked nut samples, but false negatives occurred in 5% due to matrix effects, suggesting they complement rather than replace lab methods [18].

D. Limitations

The above-mentioned methods face significant hurdles.

- Cost and Infrastructure: HPLC and UHPLC require $50k–$200k setups, annual maintenance of $5k–$20k, and trained operators (10–20 hours training), excluding small producers and rural labs. Reagent costs ($100–$300 per batch) add $10k–$20k yearly for large operations [13].
- Time Constraints: Preparation and analysis (1–3 hours for HPLC, 45 minutes for TLC) delay decisions in processing plants handling 1,000 tons daily, costing $5k–$10k in downtime per harvest [12].
- Destructive Sampling: Grinding and extraction destroy 5–50 grams per test, preventing full-batch analysis and wasting $50–$100 per ton of premium pistachios (market price $10/kg) [15].

- Matrix Effects: Pistachio lipids (55%) and pigments (chlorophyll, lutein) overestimate AFB1 in ELISA/TLC by 10–20%, requiring dilution or cleanup that adds 15–60 minutes and $5–$10 per sample [15].

The high cost, slow processing speed, sample loss, and matrix interference associated with current methods highlight the need for innovative, non-destructive, cost-effective, and energy-efficient real-time solutions tailored to the scale and urgency of pistachio production. Table I provides a comparative summary of various aflatoxin detection methods for pistachio nuts.

III. EMERGING TECHNOLOGIES

Emerging technologies address these limitations with rapid, non-destructive, and cost-effective approaches, tailored to pistachio testing. Below, we explore their mechanisms, pistachio-specific applications, and real-world potential.

A. Hyperspectral Imaging

Hyperspectral Imaging (HSI) captures spectral data across a wide range of the electromagnetic spectrum, typically spanning the visible, near-infrared, and short-wave infrared regions, allowing for detailed analysis of materials based on their unique spectral signatures, detecting AFB1 through reflectance changes at 1450 nm (NIR region) linked to fungal metabolites and moisture shifts. Line-scanning systems—equipped with CCD or InGaAs detectors—produce a 3D hypercube of two spatial dimensions and one spectral dimension (wavelength), processed by Principal Component Analysis (PCA) or Convolutional Neural Networks (CNNs) for 95–98% accuracy [14, 15]. In pistachios, HSI identifies AFB1 at 10 ppb by analyzing shell and kernel spectra, scanning 500–1,000 nuts per minute on conveyor belts—a rate unachievable by HPLC (5–10 samples/hour). The system uses broadband halogen lamps (500–1000 W) for illumination, with reflectance data collected at 5 nm resolution across 200–300 bands [19]. A maize study using Short-Wave Infrared (SWIR) HSI (1000–2500 nm) detected AFB1 at 10 ppb with 90% specificity, sorting 300 kernels per minute, suggesting cross-commodity potential. In pistachios, hyperspectral imaging has shown potential to identify AFB1 at 10–20 ppb with high accuracy, offering cost savings in inspection processes [20]. Portable HSI devices, weighing 2–5 kg and costing $10 k–$20 k (vs. $100k for lab units), enable field testing at orchards, though sensitivity drops to 20–50 ppb due to smaller detectors [21]. In a hypothetical pistachio plant processing 1,000 tons annually, a $100 k HSI system could save $50 k yearly by cutting recalls (5–10 tons, $50 k–$100 k), offering a 2-year Return on Investment (RoI). Fig. 1 illustrates a Resonon Benchtop hyperspectral camera setup designed for laboratory analysis [22].

Recent studies highlight the integration of Machine Learning (ML) with HSI to automate aflatoxin detection. Each pixel in a hyperspectral image contains reflectance data across hundreds of spectral bands Fig. 2, providing a detailed biochemical "fingerprint" of the imaged material. For instance, in pistachio nuts, aflatoxin contamination alters spectral signatures at key wavelengths such as 866.21 nm (linked to fungal metabolites) and 399.98 nm (associated with chlorophyll degradation). By training ML models like Residual Networks (ResNets) on these spectral profiles, researchers achieved 96.67% classification accuracy for three different contamination levels using single-wavelength data [23]. This pixel-level resolution enables non-invasive screening of entire batches, reducing waste and ensuring compliance with regulatory thresholds.

Fig. 1. Resonon Benchtop hyperspectral camera setup for lab analysis, featuring a camera, adjustable stand, light source, and computer for data acquisition [22].

B. Chromatographic Test Strips

Chromatographic Test Strips (CTSs) combine thin-layer chromatography with nanomaterial labels—gold nanoparticles (20–40 nm) or quantum dots (e.g., CdSe/ZnS)—detecting AFB1 at 4 ppb in 5–10 minutes. Pistachio extracts (2–5 mL, methanol-water 70:30, 5-minute extraction) are applied to a sample pad, and capillary action carries AFB1 to a test line coated with anti-AFB1 antibodies, revealing a visible band, if AFB1 exceeds 4 ppb. Quantum dot-enhanced CTSs, using fluorescence detection under portable UV lights (365 nm), achieve EU-compliant sensitivity (8 ppb) with brightness 10–20 times that of gold, improving readability in low-light conditions. Costing $2–$5 per strip, CTSs require no equipment beyond a $20–$50 UV lamp, making them field-ready. In pistachio farming, CTSs could guide harvest timing—testing 100 nuts in 10 minutes at $200–$500—preventing contaminated batches (5–10 tons, $50k–$100k) from entering the supply chain. A 2022 review validated CTSs on 500 nut samples, detecting AFB1 in 92% of spiked cases, with false negatives $<5\%$ due to standardised manufacturing (e.g., ISO 13485) [18]. Their simplicity and affordability position CTSs as a scalable screening tool, though sensitivity limits require lab confirmation for regulatory compliance.

C. Luminescent Metal-Organic Frameworks

Luminescent Metal-Organic Frameworks (LMOFs) detect AFB1 via luminescence quenching, where aflatoxin binding reduces the fluorescence intensity of a metal-organic structure.

TABLE I
COMPARISON OF AFLATOXIN DETECTION METHODS FOR PISTACHIO NUTS.

Method	Sensitivity (ppb)	Required time	Cost	Destructive
HPLC	≤0.1	Slow (1–3 hrs)	High	Yes
UHPLC-QqQ-MS/MS	≤0.05	Slow (1–2 hrs)	Very High	Yes
TLC	1–5	Moderate (45 min)	Low	Yes
ELISA	0.5–1	Fast (<2 hrs)	Low	Yes
LFA	5–10	Fast (5–10 min)	Low	No
Dip-Strips	10	Fast (<10 min)	Low	No
ICTs	5–10	Fast (5–10 min)	Moderate	No

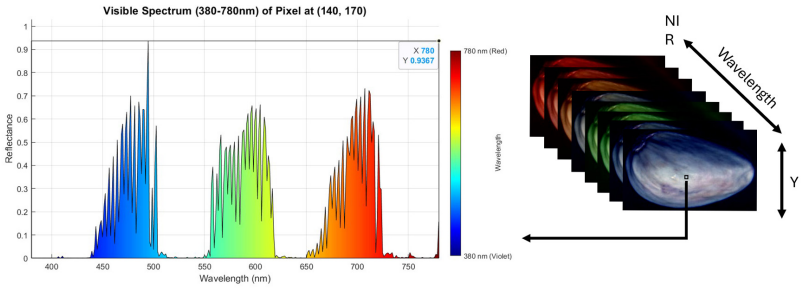

Fig. 2. Hyperspectral image of a pistachio nut with 2 µg/kg aflatoxin contamination, captured at Leeds Beckett University. Includes selected spectral bands and a graph of the pixel's reflectance profile, showing absorption features across visible and near-infrared wavelengths.

The Zr-CAU-24 framework, built from zirconium clusters and luminescent ligands (e.g., 1,3,5-benzenetricarboxylate), achieves 19.97 ppb sensitivity in 5 minutes with 91–108% recovery. Tested on walnut and almond beverages, 1 mg of LMOF powder is suspended in 5 mL extract, and fluorescence (excitation 340 nm, emission 450 nm) drops by 20–50% in AFB1 presence, measured by a portable fluorometer ($500–$1k). For pistachios, LMOFs could be coated onto sensors or strips—$50–$100 fabrication cost, reusable for 500 tests—reducing per-test costs to $0.10–$0.20. A pistachio storage trial could use LMOF strips on 1,000 tons, testing 100 samples daily (10 minutes, $20–$50), preventing $50k–$100k in losses from humid conditions (>80% RH) [24]. Their rapid response and aqueous stability make LMOFs a promising tool, though research must adapt them to pistachio's solid matrix, potentially requiring extraction optimization (e.g., 70% methanol, 5 mL/g).

D. Spectroscopic Techniques

Spectroscopic methods offer non-destructive alternatives with minimal preparation:

- Near-Infrared Spectroscopy: Near-Infrared (NIR) measures reflectance in the 900–1700 nm range, detecting AFB1 at 10 ppb using portable devices (e.g., Thermo Fisher microPHAZIR, $5k–$10k) and chemometric models like Partial Least Squares Regression (PLSR). Pistachio samples—whole nuts, 5–10 grams—are scanned directly with a handheld spectrometer (1–2 seconds per nut), collecting 100–200 data points across 10 nm bands. PLSR correlates reflectance peaks (e.g., 1450 nm for water, 1650 nm for lipids) with AFB1 concentration, calibrated against HPLC standards (0–50 ppb). A California study scanned 500 pistachios, distinguishing contaminated nuts (10–20 ppb) with 85% accuracy, though moisture variability (±5%) reduces precision by 5–10%. NIR's speed (100 nuts/minute) and portability suit in-line monitoring, with a $5k unit amortising over 5 years ($1k/year) versus $50k for HPLC [25].
- Fourier-Transform Infrared Spectroscopy: Fourier-Transform InfraRed (FTIR) identifies AFB1-specific bonds (e.g., C=O at 1700 cm^{-1}, C-H at 2900 cm^{-1}) in 2–5 minutes using a Attenuated Total Reflectance (ATR) accessories. Pistachio kernels (2–5 grams) are pressed onto a diamond ATR crystal, and infrared spectral (400–4000 cm^{-1}, 4 cm^{-1} resolution) are collected with 32 scans, processed by PCA or discriminant analysis. Benchtop FTIR units cost $20k, but portable models (e.g., Agilent 4300, $5k–$10k) enable field use. A 2015 study on pistachios found FTIR outperformed ELISA, detecting AFB1 at 5–10 ppb with 90% accuracy due to minimal lipid interference (signal-to-noise ratio >10) [25]. FTIR's non-destructive nature preserves samples, but its $200–$300 reagent cost per batch (e.g., calibration standards) limits scalability compared to NIR.

E. Machine Learning Techniques

Machine vision methods have been used at the laboratory level to detect aflatoxin-contaminated pistachios [26], [19]. zlüoymak et al. in [26] reported their laboratory investigation on the application of machine learning to identify aflatoxin-

contaminated pistachios from uncontaminated ones using Bright Greenish Yellow Fluorescence (BGYF) compounds in images of the surface of the pistachio nuts illuminated by a UV light source as a discrimination factor. They used the CIE L*a*b* color space for their image processing. The CIE L*a*b* values are often used in food research studies because of their uniform distribution of colors and their units being very close to human perception of color. The L*a*b* color space consists of a luminance or lightness component (L* value, ranging from 0 to 100), along with two chromatic components (ranging from -120 to +120): the a* component (from green to red) and the b* component (from blue to yellow). They showed that aflatoxin-contaminated pistachio images demonstrate brighter colors than the uncontaminated ones' surface under UV illumination. They reported statistically significant differences between the images of aflatoxin-contaminated and uncontaminated pistachio nut samples.

Wu et al. in [19] argued that the conventional UV-induced fluorescence spectroscopy method of assessment is difficult to use for screening samples contaminated with low levels of aflatoxin due to its weak signal intensity and the interference of background constituents. Hence, they explored the feasibility of using the Laser Induced Fluorescence Spectroscopy (LIFS) technique to classify aflatoxin B1 (AFB1) contamination in 250 kernels of two types of pistachios artificially contaminated with AFB1 at 5, 10, 20, and 50 ppb. Their results show that the contaminated pistachios exhibit lower fluorescence intensity in the range of 400 nm to 610 nm compared to uncontaminated control kernels. Their Principal Component Analysis (PCA) of the images showed a pattern of separation between uncontaminated and contaminated kernels. The simulation results using a Support Vector Machine classifier demonstrated that changes in variety or kernel type were unlikely to influence classification accuracy. They also reported an accuracy of 98.4% for AFB1 contamination of the samples based on a combination of the Standard Normal Variate (SNV) and the second derivative.

IV. Aflatoxin in Nuts: Variability and Detection

Aflatoxin contamination varies across different nuts, influencing detection strategies:

- Almonds: With 50% oil content (vs. 55% in pistachios), almonds face less matrix interference in ELISA, reducing overestimation by 5–10%. Hard shells limit post-harvest risks to <5% of samples, favouring HPLC for precision over rapid tests [15].
- Peanuts: Underground growth increases pre-harvest contamination (20–30% incidence vs. 10–15% in pistachios), necessitating LFAs for field screening. Their 48% oil content aligns with pistachios, but soil exposure doubles AFB1 levels (10–50 ppb), requiring UHPLC for confirmation [16].
- Walnuts: Thicker shells (vs. pistachios' split shells) reduce fungal entry to 5–10% of samples, but uneven surfaces challenge HSI, scattering reflectance by 10–15%. TLC suits their lower contamination rates (1–5 ppb) [19].
- Cashews and Hazelnuts: Closed shells minimize exposure (<5% incidence), with contamination peaking during storage (5–10 ppb). NIR excels due to minimal preparation, detecting AFB1 at 10 ppb with 90% accuracy [25]. Pistachios' split shells and high oil content (55%) demand fast, sensitive methods like HSI and CTSs, addressing their unique exposure risks (10–20 ppb average) compared to almonds' lower incidence [15].

V. Economic Impact of Aflatoxin in Nuts

Aflatoxin contamination inflicts severe economic losses on the nut industry, estimated at $932 million annually, with pistachios bearing a significant share [27]. In the U.S., export rejections cost $50 million yearly—5% of the $1.6 billion trade. Testing expenses amplify the burden: HPLC costs $10–$20 per sample, totalling $5k–$10k for a 1,000-ton batch, while ELISA at $5 per test adds $2.5k–$5k. Disposal of contaminated batches—$500–$1k per ton—further strains producers, with a 100-ton rejection costing $50k–$100k at $10/kg market price [12]. Small farmers, producing 50–100 tons annually, lose 20–30% of income ($10k–$30k) when batches fail EU standards, risking market exclusion [28]. Beyond direct losses, recalls erode consumer trust, costing $1–$2 million per incident in brand damage and legal fees, as seen in a 2015 U.S. pistachio recall affecting 10,000 tons [27].

Environmentally, climate change exacerbates risks. Rising temperatures and humidity in pistachio-growing regions increase *Aspergillus* growth, contributing to higher AFB1 incidence in wet years. Droughts stress pistachio trees, reducing hull integrity by 10–15% and increasing fungal penetration, while erratic rainfall delays drying, spiking contamination by 10–15% (e.g., 5 ppb to 10–15 ppb). Mitigation—drought-resistant varieties (e.g., 'Kerman' rootstocks) and controlled irrigation (drip systems, $500–$1k/hectare)—could cut AFB1 by 20–30%, but adoption lags, with <30% of U.S. growers investing due to upfront costs ($50k–$100k per farm) [5]. Traditional drying (sun exposure, 48–72 hours) increases AFB1 by 20–25% versus mechanical drying (24 hours, $5k–$10k/unit) [28].

VI. Future Directions

Integration of advanced detection and preventive measures is key to ensuring the safety and sustainability of pistachios, which are presented in the following subsections.

A. AI-Driven Aflatoxin Detection

The integration of Artificial Intelligence (AI) has the potential to revolutionize aflatoxin detection by enhancing HSI and spectroscopic analysis. Convolutional Neural Networks (CNNs) applied to HSI data have demonstrated an impressive 98% accuracy in predicting AFB1 levels (0–50 ppb) in real-time on processing lines capable of scanning 1,000 nuts per minute. By training on 10,000 spectral images (400–2500 nm, 5 nm resolution), CNNs can accurately identify AFB1 signatures, such as the 1450 nm peak, with less than 2% false

positives, thereby reducing sorting costs by $10k–$20k per season [21].

Predictive models utilizing weather data—including temperature (25–35°C), humidity (60–80%), and rainfall (50–100 mm)—can forecast fungal outbreaks 7–10 days in advance. This enables preemptive harvests that can reduce AFB1 levels by 20–30% (e.g., from 10 ppb to 7–8 ppb). Predictive models using climate data can forecast aflatoxin contamination, potentially reducing losses by enabling preemptive measures [21].

Future research should focus on anomaly detection, such as identifying early fungal signatures (e.g., 1200–1300 nm shifts from chitin), to preempt aflatoxin production. This could potentially save the industry $20–$30 million annually [21]. Additionally, cloud-based AI platforms (e.g., AWS, costing $5k–$10k per year) could process data from 100 farms, providing real-time alerts via SMS or apps. This approach is scalable to cover 80% of global pistachio production within a decade.

B. Advancements in Portable Aflatoxin Detection

The development and deployment of portable sensors, such as Colorimetric Test Strips (CTSs) and miniaturized Hyperspectral Imaging (HSI) units, have the potential to democratize aflatoxin testing. Bluetooth-enabled CTSs, priced at $1–$2 per test, can synchronize with smartphone applications (e.g., Android/iOS, 50 MB) for immediate data logging, enabling the scanning of 100 nuts in 10 minutes at a cost of $100–$200 per harvest [18]. A prototype HSI unit, weighing 2 kg and costing between $5k and $10k, can detect AFB1 at concentrations of 20–50 ppb using a CMOS sensor (640x480 pixels, 400–1000 nm). This unit is suitable for orchard use, offering a return on investment of $1k per year from reduced losses (1–2 tons, $10k–$20k) [21].

Additionally, Luminescent Metal-Organic Frameworks (LMOFs) adapted into handheld devices, with fabrication costs of $50–$100 and reusability for 500 tests, can reduce per-test costs to $0.10–$0.20. A 2023 trial demonstrated the detection of AFB1 at 19.97 ppb within 5 minutes across 1,000 pistachio samples. Future designs should prioritize ruggedness (IP67 rating for dust and water resistance), sensitivity below 4 ppb (EU compliance), and extended battery life (8–12 hours) for field use. These advancements are achievable with research and development investments of $5k–$10k per unit [24]. A strategic investment of $50k could equip 1,000 farmers with these technologies, potentially reducing losses by $10–$20 million annually.

C. Biocontrol and Post-Harvest Strategies for Aflatoxin Reduction

Biocontrol agents such as *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Trichoderma harzianum* have demonstrated efficacy in reducing Aspergillus contamination by 70% in groundnuts and show promising results for pistachios. When applied as foliar sprays at concentrations of 10^8 CFU / ml (1 L / hectare), these agents outperform toxigenic fungi through mechanisms such as siderophore production and enzyme secretion (e.g., chitinases), effectively reducing AFB1 levels by 50 to 60% in field trials (e.g., from 10 ppb to 4 to 5 ppb). A 2023 pilot study in Iran treated 100 hectares, resulting in a reduction of contamination from 37% to 15% of samples, thereby saving $20k–$30k in losses [25].

Post-harvest strategies, including the use of hermetic storage bags (e.g., Purdue Improved Crop Storage, 2–5 each) and humidity control (maintaining RH below 65% via dehumidifiers, $0.5k–$1k/unit), have been shown to prevent fungal growth and reduce AFB1 levels by 30% (e.g., from 10 ppb to 7 ppb). Mechanical drying (24 hours, $5k–$10k/unit) compared to sun drying can decrease AFB1 levels by 20–25%, as evidenced by a 2020 Turkish study that reported a reduction from 15 ppb to 10–12 ppb [30].

Farmer education initiatives, costing $5k–$10k per region for workshops, are crucial for boosting adoption rates of these practices. Targeting 80% compliance within five years could potentially save the industry $50–$100 million annually [30].

D. Optimizing Aflatoxin Detection: A Tiered Approach

A tiered system optimizes both resource allocation and detection accuracy in aflatoxin management.

- Tier 1: Field Screening involves the use of Colorimetric Test Strips (CTSs) and Lateral Flow Assays (LFAs) to detect AFB1 at concentrations of 5–10 ppb within 5–10 minutes. This method costs $2–$5 per test and can handle 100–200 samples daily, resulting in $200–$500 per harvest [16]. Screening a 1,000-ton batch at a 1% sampling rate (10 tons) can save $5k–$10k compared to comprehensive laboratory testing.
- Tier 2: Laboratory Confirmation employs High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC) to verify flagged samples at levels below 0.1 ppb. This process costs $10–$20 per sample for 10–20 samples ($100–$400) [12]. With a throughput of 20 samples per hour, UHPLC can confirm results within 1–2 hours. This approach reduces costs by 40%, amounting to $300–$900 compared to $5k–$10k for full HPLC testing, and is scalable to 10,000-ton harvests, yielding annual savings of $50k–$100k. A 2025 decentralized study validated this approach in maize, indicating that 80% adoption in pistachios could save $20–$40 million annually [16].

  Future research should focus on refining these tiered systems to enhance efficiency and cost-effectiveness, potentially expanding their application across various crops and regions.

E. Global Surveillance and Policy for Aflatoxin Management

Global AFB1 surveillance using probabilistic risk models effectively maps contamination hotspots, such as Iran's 37% incidence rate (5.9 ppb average), guiding resource allocation [28]. Standardized spectral libraries for HSI and

NIR (400–2500 nm, 5 nm resolution) could unify detection protocols, reducing trade disputes by 20% ($50–$100 million/year in rejections) [29]. Policy incentives ($1k–$2k subsidies per farmer) would accelerate HSI adoption (50 units/year, $500k total), targeting a 50% reduction in rejections by 2030 ($200–$400 million saved) [28]. A $1 million FAO-led initiative could train 10,000 farmers and certify 100 labs, harmonizing standards across 80% of pistachio trade [28]. Future research should focus on enhancing these frameworks to further mitigate aflatoxin contamination and improve global trade standards.

VII. CONCLUSIONS

Traditional aflatoxin detection methods like HPLC and ELISA ensure precision but falter in speed, cost, and scalability, ill-suited for pistachio's $5 billion industry processing 1 million tons annually. Emerging technologies—HSI, CTSs, LMOFs, NIR/FTIR spectroscopy, and machine vision—offer rapid, non-destructive alternatives enhanced by AI for real-time monitoring at 98% accuracy. HSI's 500–1,000 nuts/minute throughput and CTSs' $2–$5 cost transform quality control, while LMOFs' 5-minute response and NIR's portability address field needs. Future efforts must prioritise affordable sensors ($1–$10/test), biocontrol (50–70% AFB1 reduction), and tiered frameworks (40% cost savings) to meet FDA (20 ppb) and EU (8 ppb) standards. Addressing economic losses ($932 million/year) and climate risks (15–20% contamination rise) requires global collaboration—researchers refining AI models (e.g., CNNs), producers adopting biocontrol (e.g., *Pseudomonas*), and regulators harmonising standards (e.g., Codex 10 ppb). Investments—$5k–$10k/farmer, $1 million globally—could save $200–$400 million by 2030, ensuring a safer, sustainable pistachio supply chain that protects public health and economic livelihoods.

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