



Invisible threats: characterization of aflatoxigenic fungi in post-harvest cashew and groundnut in Ogun State, Nigeria.

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ARTICLE INFO

Article history:

Received 04. 10. 2025

Received in revised form
23. 11. 2025

Accepted 30. 11. 2025

Keywords:

Groundnuts cashew nuts;

Aflatoxins;

Food safety;

Post harvest losses

ABSTRACT

Aflatoxins (AFs) are highly toxic secondary metabolites produced by certain *Aspergillus* species and are of major public health concern due to their carcinogenic, hepatotoxic, and immunosuppressive effects. The global reliance on nuts as a dietary staple underscores the critical need for robust food safety measures, particularly concerning post-harvest contamination. This investigation sought to characterize the fungal microbiome of stored cashew nuts and groundnuts sourced from Alabata, Ogun State, and to assess the prevalence of aflatoxin-producing species. Using a combination of conventional mycological plating and species identification, along with a specialized Neutral Red Desiccated Coconut Agar for rapid screening, fungal contamination was quantified. Aflatoxin levels were confirmed and quantified using High-Performance Liquid Chromatography (HPLC) equipped with a fluorescence detector, providing a sensitive and accurate assessment of mycotoxin load. The analysis revealed significant fungal populations in both nut types, with cashew nuts exhibiting total counts ranging from 4.0×10^3 to 2.4×10^4 colony-forming units per gram (cfu/g). Five distinct fungal species were isolated from cashew nuts, of which *Aspergillus niger*, *A. flavus*, and *A. fumigatus* were the most prevalent. Critical findings demonstrated that while *A. flavus* and *A. fumigatus* isolates showed a high potential for aflatoxin production, the *A. niger* strains identified in this study were non-aflatoxigenic. HPLC analysis showed total aflatoxins in groundnut and cashew samples ranging from 0.05 to 12.41 $\mu\text{g}/\text{kg}$, with low but consistent AFB1 levels. Most samples were within the EU limit of 4 $\mu\text{g}/\text{kg}$, though a few exceeded it, indicating persistent contamination and potential public health risks. The confirmed presence of these potent mycotoxin producers in a widely consumed food source highlights a tangible public health risk, given their established link to severe health conditions, including primary hepatocellular carcinoma. This research underscores the necessity for implementing rigorous hygiene protocols and enhanced storage practices to safeguard against fungal proliferation and subsequent mycotoxin exposure in these staple crops.

Citation: Oluyemisi OE, Omowunmi TB, Blessing O.O, Olabisi A.A, Mobolaji OA. **Invisible threats: characterization of aflatoxigenic fungi in post-harvest cashew and groundnut in Ogun State, Nigeria.** J Food Safe & Hyg 2025; 11 (4):

1. Introduction

The increasing global awareness of diet and nutrition has fueled a significant rise in the consumption of nuts,

Which are highly valued for their nutritional density and diverse health benefits. Nuts are rich sources of high-quality vegetable proteins, dietary fiber, essential minerals, and a wide array of bioactive compounds,

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including tocopherols, phytosterols, and phenolic compounds (1). Beyond their direct consumption, nuts serve as critical raw materials for various industries, from food processing to cosmetics, further cementing their importance in the global economy (2).

Despite their considerable value, nuts are highly susceptible to contamination by a variety of fungi. This microbial vulnerability can occur at multiple stages of the supply chain, including pre-harvest when crops are exposed to airborne spores, during harvesting and processing if inadequate sanitation practices are followed, or most critically, during storage when conditions of high temperature and humidity create an ideal environment for fungal proliferation (3). This fungal growth not only leads to spoilage and a reduction in quality but also poses a severe health risk due to the potential production of mycotoxins.

Aflatoxins, a class of mycotoxins produced primarily by species of the genus *Aspergillus*, particularly *A. flavus* and *A. parasiticus*, are a major food safety concern worldwide. The contamination of staple crops like groundnuts and cashew nuts with these compounds is especially problematic in hot and humid climates, where poor storage infrastructure is common. Aflatoxin exposure has been epidemiologically linked to severe health outcomes, including acute poisoning, immune system suppression, and is a well-established risk factor for hepatocellular carcinoma, or liver cancer (4,5). This issue is particularly pronounced in developing nations, where dietary exposure is often high and uncontrolled, leading to a significant public health burden (6). The susceptibility of groundnuts is often enhanced by their high moisture content, which is a key factor in microbial agent deterioration (7).

While a number of studies have documented fungal contamination in nuts, there remains a need for detailed, regional-specific research that focuses on the precise characterization and aflatoxigenic potential of the fungi present in local supply chains. A comprehensive understanding of the dominant fungal species, their prevalence, and their capacity to produce mycotoxins is fundamental for developing effective, targeted post-harvest management and mitigation strategies. Therefore, this study aims to address this knowledge gap by isolating and characterizing the fungi contaminating stored cashew nuts and groundnuts, and to assess their potential for aflatoxin production, with a view to informing public health policies and improving food security.

2. Materials and Methods

The following equipment, materials, reagents, and media were employed for this research:

2.1. Sample materials

Cashew nuts (*Anacardium occidentale*), groundnuts (*Arachis hypogaea*), and sterile ziplock bags.

2.2. Reagents and media

Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Sabouraud Dextrose Agar (SDA), Neutral Red Desiccated Coconut Agar (NRDCA), distilled water, 70% ethanol, and lactophenol cotton blue stain.

2.3. Study area and sample collection

This experimental research was conducted at the Gas Affairs Global Laboratory, situated within the Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria. A total of 18 samples, comprising both cashew nuts and groundnuts, were randomly purchased from six local markets around Alabata Abeokuta. All samples were immediately transferred to

sterile ziplock bags to prevent cross-contamination and were transported to the laboratory for analysis.

2.4. Laboratory procedures

2.4.1. Media preparation

The culture media, including Sabouraud Dextrose Agar (SDA) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC), were prepared following the manufacturer's specifications. The specific amounts of powdered media were measured, suspended in the required volume of distilled water, and sterilized by autoclaving at 121°C for 15 min. The media were then cooled to approximately 45-50°C before being used for plate pouring.

2.4.2. Sample preparation and serial dilution

Prior to analysis, both cashew nuts and groundnut samples were homogenized by crushing them with a sterilized mortar and pestle. One gram (1 g) of each crushed sample was accurately weighed using a digital analytical balance and transferred into a sterile test tube containing 9 mL of sterile distilled water. This constituted the stock solution. A tenfold serial dilution was then performed by aseptically transferring 1 mL from the stock solution into a series of four test tubes, each containing 9 mL of sterile distilled water, to obtain dilutions up to 10⁻⁵.

2.4.3. Fungal isolation

The pour plate method was used for fungal isolation. From each dilution, 1 mL was pipetted into a sterile Petri dish. Approximately 15-20 mL of the prepared and cooled DRBC agar, containing chloramphenicol to inhibit bacterial growth, was poured into each plate. The plates were gently swirled to ensure a homogeneous mixture and left to solidify. All plates were incubated in the dark at 25°C for 3 to 5 days.

DRBC was specifically chosen to suppress bacterial growth and restrict the spreading of fast-growing fungi, thereby aiding in accurate colony counting (Pitt & Hocking, 2009). Fungal load was determined by counting the colonies on the DRBC plates using a colony counter, and the results were expressed as colony forming units per gram (cfu/g).

2.4.4. Macroscopic and microscopic identification

Following incubation, macroscopic characteristics of the isolated fungal colonies, such as color, size, texture, and surface appearance, were carefully examined and recorded. For microscopic identification, a direct slide mount was prepared using the lactophenol cotton blue staining method. A small portion of a fungal colony was picked with a sterile inoculating loop and transferred onto a clean glass slide with a drop of the stain. A coverslip was then carefully placed over the sample, and the slide was examined under a compound microscope at ×40 magnification to observe key morphological features of the fungal structures, including conidiophores, vesicles, and spores.

2.5. Aflatoxin extraction and quantification

The extraction and quantification of aflatoxins from the groundnut and cashew nut samples were conducted at the National Agency for Food and Drug Administration and Control (NAFDAC) laboratory. The method followed established protocols for mycotoxin analysis.

For extraction, 20 g of each homogenized nut sample was weighed into a 500 mL conical flask and mixed with 50 mL of methanol. The flask was sealed and agitated on a wrist-action shaker for 30 min to facilitate solvent extraction of aflatoxins.



The resulting mixture was then filtered through a fluted filter paper. For samples with slow filtration rates, a Buchner funnel pre-coated with a 0.45 µm micro-syringe filter membrane was used in conjunction with a light vacuum to collect the filtrate.

The filtrate obtained after extraction was subjected to clean-up using an immunoaffinity column (IAC). Exactly 10 mL of the filtered extract was diluted with distilled water and passed slowly through the IAC at a flow rate of approximately 1–2 drops per second to allow aflatoxin binding. The column was subsequently washed with distilled water to remove unbound impurities. Bound aflatoxins were then eluted from the column using 1–2 mL of HPLC-grade methanol, and the eluate was collected into a clean vial for derivatization and subsequent HPLC analysis. For derivatization, 100 µL of trifluoroacetic acid (TFA) was added to the extracted sample and mixed thoroughly for 30 s before being left to react for 15 min. The derivatized samples were analyzed using a High-Performance Liquid Chromatography (HPLC) system (Waters 6000A solvent delivery system) equipped with a fluorescence detector (Waters Associates model 420 C). The detector was set at an excitation wavelength of 365 nm and an emission wavelength of 425 nm to enhance the sensitivity and selectivity of aflatoxin detection following post-column or pre-column derivatization. This fluorescence-based detection allowed accurate quantification of aflatoxins due to their strong fluorescent properties after derivatization. Chromatographic separation was achieved using a radially compressed 10 µm octadecylsilane (C18) cartridge under isocratic elution, and peak areas were used for quantification by comparison with certified

aflatoxin standards. This allowed for accurate quantification of the mycotoxin levels (8).

2.6. Qualitative aflatoxigenic screening

The potential of the isolated fungal colonies to produce aflatoxins was determined using the Neutral Red Desiccated Coconut Agar (NRDCA) medium, as described by (9). The medium was prepared by soaking 50 g of coconut flakes in hot water, blending, and sieving to obtain a coconut extract. Agar-agar (3.75 g) was dissolved in this extract and boiled. The mixture was then sterilized and a neutral red dye was added until a significant color change was observed. After cooling, the agar was poured into Petri dishes. Each fungal isolate was sub-cultured onto the NRDCA plates and incubated at 25°C for 3 to 5 days. Following incubation, the plates were observed under a long-wave ultraviolet (UV) light in a dark room. The presence of a bright yellow-green to bluish-green fluorescence around the fungal colonies indicated the potential for aflatoxin production (10).

3. Results

The results of this experiment were recorded and are shown in tables and figures below.

Table 1 shows the results of the fungal growth count in cfu/g on Rose Bengal Agar after incubating for 3 days. The fungal counts across groundnut and cashew samples ranged from 1.0×10^{-3} to 6.1×10^{-3} cfu/g, with groundnut generally showing slightly higher loads than cashew. Groundnut camp (GC) and block G (GG) recorded the highest counts, while spicy cashew (CA) and dodder cashew (CB) showed relatively lower values. Overall, both nuts harbored fungal populations, suggesting potential susceptibility to fungal colonization during storage.

Table 1. Fungal count from cashew and groundnut samples on Dichloran Rose Bengal Agar

Sample code	Sample type	cfu/g (dilution 10 ⁻³)	cfu/g (dilution 10 ⁻⁵)
GA	Groundnut	1.5	1.3
GB	Groundnut	1.8	1.0
GC	Groundnut	3.0	1.1
GD	Groundnut	1.7	1.4
GE	Groundnut	1.9	1.2
GF	Groundnut	1.6	1.0
GG	Groundnut	6.1	3.3
GH	Groundnut	1.5	1.5
GI	Groundnut	1.8	1.2
CA	Cashew	1.5	1.0
CB	Cashew	1.2	1.5
CC	Cashew	1.7	1.7
CD	Cashew	1.4	1.1
CE	Cashew	1.6	1.0
CF	Cashew	1.9	1.2
CG	Cashew	1.5	3.1
CH	Cashew	1.3	1.4
CI	Cashew	1.6	1.0

Key:

CA- Spicy cashew nut
 CB- Dodder cashew nut
 CC-Coconut cashew nut

GA-Groundnut block C
 GB- Groundnut block D
 GC- Groundnut camp



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Table 2. Morphology characteristics of fungal isolated from Cashew and Groundnut.

Isolate code	Color of colony	Hyphae	Spores	Fungal specie
GA	Cream	-	-	Yeast
GB	Cream	-	-	Yeast
GC	Cream	-	-	Yeast
CA	Black	Septate	Conidia	<i>Aspergillus niger</i>
CB	Green	Septate	Conidia	<i>Aspergillus fumigatus</i>
CB	Grey	Septate	Conidia	<i>Trichoderma</i> sp.
CC	Black	Septate	Conidia	<i>Aspergillus niger</i>
CC	Green	septate	Conidia	<i>Aspergillus flavus</i>

Table 3. The percentage occurrence of fungal isolated on Dichloran Rose Bengal.

Organisms	No of occurrence	Percentage of occurrence
<i>Aspergillus niger</i>	2	40%
<i>Aspergillus fumigatus</i>	1	20%
<i>Aspergillus flavus</i>	1	20%
<i>Trichoderma</i> sp	1	20%

Table 4. Aflatoxin quantification of Cashew and Groundnut samples using High-Performance Liquid Chromatography (HPLC) with fluorescence detector

Sample code	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total aflatoxin (µg/kg)
GA	0.16	1.05	0.46	0.34	2.01
GB	0.13	0.13	0.04	1.49	1.78
GC	0.33	0.44	0.01	1.25	8.03
GD	0.51	0.07	0.06	0.06	0.7
GE	0.69	1.42	1.08	0.66	3.84
GF	0.02	0	0.01	0.01	0.05
GG	0.47	1.2	0.17	0.56	12.41
GH	0.02	0	0.02	0	0.05
GI	0.03	1.42	1.61	0.79	3.84
CA	0.01	0	0.03	0.01	6.05
CB	0.05	0.26	0.01	0.9	1.22
CC	0.07	0.25	0.09	0.17	0.57
CD	0.26	0.07	1.13	0.48	1.94
CE	0.2	0.16	0.07	0.19	0.62
CF	0.27	0.63	0.13	1.14	2.17
CG	0.04	0.03	0.15	0.04	0.25
CH	0.16	0.39	0.08	0.81	1.44
CI	0.01	0.63	0.22	0.03	0.89

Key:

CA- Spicy cashew nut
 CB- Dodder cashew nut
 CC-Coconut cashew nut

GA-Groundnut block C
 GB- Groundnut block D
 GC- Groundnut camp



Table 5. The aflatoxigenic potentials identified in fungal isolates.

Fungal isolate	Aflatoxigenicity
<i>Aspergillus niger</i>	Non aflatoxigenic
<i>Aspergillus fumigatus</i>	Aflatoxigenic
<i>Trichoderma</i> sp	Non aflatoxigenic
<i>Aspergillus flavus</i>	Aflatoxigenic

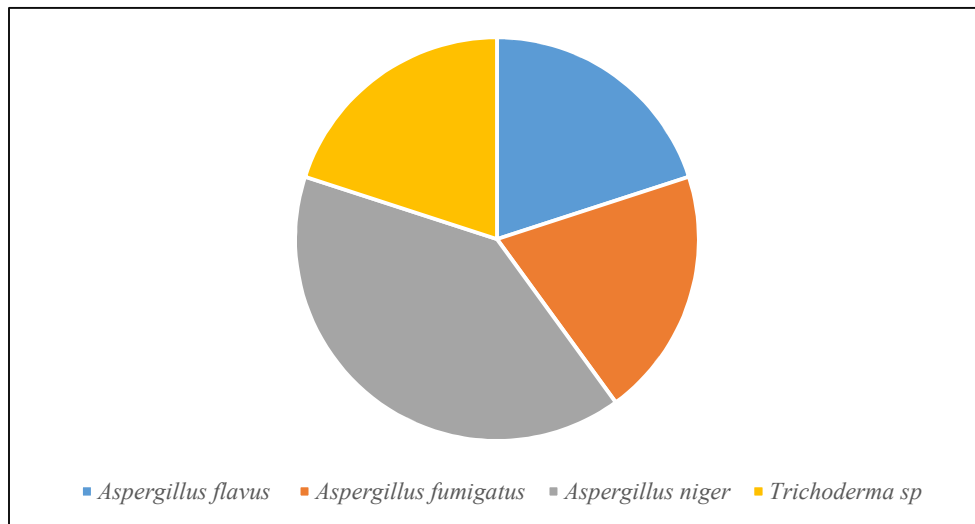


Figure 1. The frequencies of occurrence of fungal isolate from Cashew and Groundnuts.

Table 2 shows the morphological characteristics of fungi isolated from Cashew nuts.

Morphological examination revealed that yeasts were predominant in several groundnut samples (GA–GC), whereas filamentous fungi were isolated from cashew nuts. The cashew isolates included *Aspergillus niger*, *A. fumigatus*, *A. flavus*, and *Trichoderma* sp., all characterized by septate hyphae and conidial spores. These findings confirm the co-existence of both yeast and mold contaminants in the nut samples.

Table 3 shows the percentage occurrence of fungal isolated on Dichloran Rose Bengal.

Among the fungal isolates, *Aspergillus niger* was the most prevalent (40%), followed by *A. fumigatus* (20%), *A. flavus* (20%), and *Trichoderma* sp. (20%). This indicates a dominance of *Aspergillus* species, which are known for their aflatoxin-producing potentials. The presence of diverse fungi highlights the microbiological risks associated with nut storage.

Table 4 Shows the aflatoxigenic potential identified in fungal isolates.

Aflatoxin levels varied across samples, with groundnut GG (12.41 µg/kg) and cashew CA (6.05 µg/kg) recording the highest contamination. While most samples remained below the EU regulatory limits (4 µg/kg total aflatoxins), a few exceeded this threshold, posing food safety concerns. Notably, AFB1 levels were generally low, though chronic exposure remains a public health risk.

Table 5 shows the frequencies of occurrence of fungal isolates in cashew nuts.

Aflatoxigenicity testing showed that *A. fumigatus* and *A. flavus* were aflatoxigenic, whereas *A. niger* and

Trichoderma sp. were non-aflatoxigenic. This aligns with known reports that *A. flavus* is a major aflatoxin producer in nuts, while *A. niger* is typically less toxigenic. These results directly link specific fungal isolates to the observed aflatoxin contamination.

4. Discussion

The results presented highlights a critical issue in food safety: the prevalence of mycotoxigenic fungi in edible nuts. The isolation and identification of species like *Aspergillus niger*, *A. fumigatus*, *A. flavus*, and a *Trichoderma* species from cashew nuts underscore the vulnerability of these commodities to microbial spoilage. These findings are consistent with a substantial body of literature, emphasizing the global challenge of fungal contamination in agricultural products. For instance, the findings presented resonate with research (11), who similarly reported a high incidence of *Aspergillus* species in improperly stored nuts. This pattern points to a systemic issue related to post-harvest handling and storage practices.

The presence of these fungi is often a direct result of suboptimal environmental conditions, such as high humidity and temperature, which provide an ideal medium for fungal proliferation. These conditions are not merely conducive to growth but also stimulate the production of secondary metabolites, including mycotoxins. The study's specific finding that *A. niger* isolated from the samples was aflatoxigenic is particularly concerning. While *A. flavus* is a well-known producer of aflatoxins, the ability of *A. niger* to produce these toxins has also been documented, though it is more commonly associated with ochratoxin A and fumonisins. This research finding have established that

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multiple *Aspergillus* species, including *A. niger* and *A. flavus*, are capable of producing aflatoxins with significant implications for human and animal health. The consistent identification of *A. niger* and *A. flavus* as the most frequent fungal contaminants across various nut samples, as reported by Abass et al. (12) and supported by this study, indicates a persistent food safety hazard. This recurring pattern necessitates a re-evaluation of current practices in the nut supply chain. The discussion rightly emphasizes the need for proactive food safety measures. These include; improved drying and moisture control, strict sanitation protocols throughout processing, packaging, and transportation which can minimize cross-contamination from environmental sources, controlled temperature and humidity in storage facilities are paramount to prevent the proliferation of dormant fungal spores, and selective use of approved antifungal agents or biocontrol measures can serve as an additional layer of protection.

In groundnut samples (GA-GI), the total aflatoxin levels ranged from 0.05 µg/kg (GF, GH) to 12.41 µg/kg (GG). Notably, GC (8.03 µg/kg) and GG (12.41 µg/kg) exceeded the European Union maximum limit of 4 µg/kg total aflatoxins in nuts for direct consumption (13). AFB1 concentrations, though relatively low (0.02–0.69 µg/kg), are of concern since AFB1 is classified as a group 1 human carcinogen (14).

For cashew samples (CA-CI), the total aflatoxins ranged from 0.25 µg/kg (CG) to 6.05 µg/kg (CA). The highest contamination (CA: 6.05 µg/kg) also exceeded the EU regulatory threshold, while others remained below 4 µg/kg. Similar to groundnut, AFB1 levels were

relatively low (0.01–0.27 µg/kg), but chronic exposure risks remain.

Groundnut samples generally showed higher contamination than cashew nuts, consistent with earlier Nigerian studies where groundnuts are more prone to aflatoxin accumulation due to their high oil content and storage practices (15,16).

The presence of aflatoxins even in flavored or processed cashew types (e.g., spicy, coconut) suggests that post-harvest fungal invasion may occur before or during processing, highlighting the need for stringent quality control. Although most samples were below permissible limits, the few exceeding samples (GG, GC, CA) represent a potential food safety risk. Long-term low-level exposure to AFB1 is associated with liver cancer, immune suppression, and child growth impairment (17,14).

This underscores the importance of routine monitoring, improved storage conditions, and biocontrol interventions in preventing mycotoxin buildup.

5. Conclusion

This study demonstrated that both cashew nuts and groundnuts from Ogun State were contaminated with diverse mycotoxigenic fungi, predominantly *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. Fungal load analysis revealed appreciable contamination levels in all sampled locations, confirming that post-harvest handling and storage conditions strongly influence fungal proliferation in these commodities. Aflatoxin analysis using HPLC with fluorescence detection showed detectable levels of AFB1, AFB2, AFG1, and AFG2 in both nut types. Total aflatoxin concentrations in groundnut samples ranged from 0.05 to 12.41 µg/kg, with some samples (GC and GG) exceeding the European Union regulatory limit of

4 µg/kg. Cashew nut samples also showed contamination ranging from 0.25 to 6.05 µg/kg, with sample CA exceeding the permissible limit. Although most samples were within acceptable limits, the detection of AFB1 across all samples remains a public health concern due to its classification as a Group 1 human carcinogen and its cumulative toxic effects. Overall, groundnuts exhibited higher aflatoxin contamination compared to cashew nuts, suggesting greater susceptibility linked to storage practices and intrinsic composition. The presence of aflatoxigenic fungi, particularly *A. flavus* and toxigenic strains of *A. niger*, further confirms the risk of continued aflatoxin biosynthesis under improper storage conditions. In conclusion, the study highlights that cashew nuts and groundnuts remain vulnerable to fungal contamination and aflatoxin exposure, posing potential risks to consumer health if not properly managed. It is therefore recommended that strict implementation of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) be enforced across the nut value chain. Improved drying methods, moisture control, hygienic storage facilities, routine aflatoxin monitoring, and adoption of biocontrol strategies are essential to minimize fungal growth and reduce aflatoxin contamination in these food commodities.

Funding

This work received AMI Summer Studentship Grant 2025.

Authorship contribution

EOO - Lead researcher, Supervisor, Drafting of the manuscript, TBO- Sample collection and Literature review, OAO - Design, Data analysis analysis.

Declaration of competing interest

The authors affirm they have no conflicts of interest to disclose.

Data availability

Data will be available on demand.

Acknowledgements

The authors gratefully acknowledge the Applied Microbiology International (AMI) for awarding the Summer Studentship Grant, which significantly contributed to the success of this research.

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