

**First evidence of SO₂-releasing bags controlling fungal growth, aflatoxins
and cyclopiazonic acid contamination in unshelled peanuts.**

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Running head title: SO₂ bags for fungal and mycotoxin control.

Impact statement: This study provides the first evidence that SO₂-releasing storage bags effectively control fungal growth and eliminate mycotoxin contamination in unshelled peanuts under poor storage conditions. Given the global importance of peanuts as a climate-resilient crop and cash crop, particularly in low- and middle-income countries, this application offers a practical and scalable solution to reduce post-harvest losses, enhance food safety, and improve market access. The findings support the adoption of SO₂-releasing bags to protect public health and strengthen food supply chains in regions most vulnerable to mycotoxin contamination.

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Abstract

Peanuts (also known as groundnuts; *Arachis hypogaea* L.) are a globally significant cash crop but are highly susceptible to fungal contamination, particularly by *Aspergillus* section *Flavi*, which can contaminate the product with mycotoxins, including aflatoxins. This contamination poses serious food safety concerns, especially in low- and middle-income countries, limiting access to international markets. This study assessed the efficacy of slow-release SO₂-bags compared to plastic bags (control) in reducing fungal growth and mycotoxin contamination in stored unshelled peanuts. Naturally contaminated and *Aspergillus flavus*-inoculated peanuts were stored at water activity (a_w) levels of 0.85 and 0.95 for 15 days at 25°C. Fungal populations were monitored, aflatoxins and cyclopiazonic acid were analysed using LC-MS/MS qTRAP. SO₂-releasing bags completely inhibited fungal growth in naturally contaminated peanuts and significantly reduced *A. flavus* population with effectiveness dependent on water activity. Additionally, SO₂-releasing bags suppressed mycotoxin production. This study demonstrates, for the first time, the effectiveness of SO₂-releasing bags in preventing fungal spoilage and mycotoxin contamination in post-harvest peanuts, offering a promising solution for enhancing peanut safety and quality.

Keywords: groundnuts, mycotoxins, *Aspergillus flavus*, applied mycology

Introduction

Peanuts is a cash crop and an excellent edible oil-seed legume (Akram et al., 2018). The world annual production of unshelled peanuts was 54M tonnes in 2023, with over 90% of peanuts produced in developing countries (FAOSTAT, 2023; FAO 2002). Peanuts are a valuable crop nutritionally as they are a very good source of vegetable protein and contain a significant amount of unsaturated fats and magnesium (Arya et al., 2016). They have been associated with various positive health effects (Parilli-Moser et al., 2022). The high levels of proteins in peanuts make them suitable to produce Ready-to-Eat Therapeutic Food (Diouf et al., 2016) which are used to support the eradication of malnutrition amongst children in developing countries (Schoonees et al., 2019).

Peanuts production is faced with several constraints including drought stress, fungal, bacterial, viral diseases, and insect pests (Kortei et al., 2022). Among these, colonisation of peanuts by mycotoxins-producing fungi within the *Aspergillus* section *Flavi* represents a food safety problem resulting in significant health and economic losses due to concentration of mycotoxins higher than maximum limits established by food safety authorities (Acur et al., 2020; Sultan et al., 2010). *Aspergillus flavus*, an opportunistic plant pathogen, is considered the main representative species within the *A.* section *Flavi* group. This species is able to produce aflatoxins (AFs) and cyclopiazonic acid (CPA). Among AFs, aflatoxin B₁ (AFB₁) is classified as a Group I human carcinogen according to the International Agency for Research on Cancer being associated with hepatocellular carcinoma (IARC, 1993). CPA is a neurotoxic compound often co-occurring with AFs, being produced by *A. flavus* and other species within the *Aspergillus* section *Flavi* (Ostry et al., 2018).

In many developing regions, inadequate agricultural practices and poor storage conditions exacerbate aflatoxin accumulation from harvest through to the final product (Pitt et al., 2013; Cervini et al., 2022), prompting many countries to establish maximum regulatory limits for

mycotoxins in food products, including peanuts and peanut-based products (Van Egmond et al., 2007). For instance, for raw peanuts entering the European Union (EU), the level of total AFs and AFB₁ must be less than 4 and 2 µg/kg, respectively (Commission Regulation (EU) 2023/915). On the contrary, no maximum regulatory limits exist for CPA in food products. Nevertheless, these restrictive levels are often impossible to achieve, especially in low- and middle-income countries, where lack of awareness of mycotoxins, poor regulatory framework and lack of good agricultural and storage practices are predominant conditions (Bereka et al., 2022; Bila et al., 2025; Tadesse et al., 2025). In many African countries, existing post-harvest interventions, such as the use of Purdue Improved Crop Storage bags, remain costly and inconsistently adopted (Nakoma Ngoma et al., 2025; Kamilu et al., 2025) despite there is evidence for their use to be more profitable for small-scale farmers as compared to traditional storage technologies, even in the short-term (Aker et al., 2023). As a result, significant food losses and exposure to these hazardous compounds continue to occur. Therefore, it is essential to continue developing and promoting the adoption of other effective mitigation strategies including post-harvest and storage technologies.

Amongst these, sulphur dioxide (SO₂) and its sulphites (e.g. sodium and potassium sulphites) have been commonly used in a wide range of foods including fruits, vegetables, fruit juices and concentrates, syrups, wines and jams and to a lesser extent prawns, fish, minced meats, sausages, and mushrooms. In Europe, their maximum level is regulated by Commission Regulation (EU) No 1129/2011 where they are listed with E-numbers from E220 to E228 (EU, 1129/2011). They are known to exert antimicrobial and fungistatic actions on food during storage. Sodium metabisulphite (NaMBS) is often used as a post-harvest antifungal agent for table grapes' storage, during processing of raisins and grape juice, and during grape crushing for wine production (Magan and Aldred, 2007; Prabhakar and Reddy, 2000). NaMBS pads which release SO₂ have been widely used to control fungal spoilage caused by *Botrytis cinerea*

(grey mould) on grapes during storage (Domingues et al., 2018; Habib et al., 2021; Morgan et al., 2019; Youssef et al., 2020). They are composed of laminated plastic membranes containing NaMBS which is encapsulated in a wax matrix. These pads release SO₂ through a dual-phase mechanism: an initial burst within the first 48 hours provides a fungicidal effect, followed by a continuous low-level release that maintains a fungistatic action. The release of SO₂ is triggered by water vapour generated from the commodity's respiration and high relative humidity, which reacts with the NaMBS (Tessara, Berrysis, 2021). Despite their proven effectiveness in high-value horticultural commodities, the potential application of slow-release SO₂ for controlling fungal growth and mycotoxin contamination in raw commodities such as peanuts remains largely unexplored. Therefore, this study aimed to investigate whether NaMBS pads adapted into small bags which slowly release SO₂ could effectively control fungal spoilage, *A. flavus* colonisation and associated mycotoxin production in unshelled peanuts, particularly under high water activity conditions known to favour contamination.

Materials and methods

Peanut samples

Unshelled peanuts (*Arachis hypogaea* L.) from a local market in the UK were used as naturally contaminated peanuts for this study. The water activity (a_w) was 0.35 and they were kept at 4°C in resealable zip-lock polyethene bags until use. For this experiment, both naturally contaminated (non-sterilised control group) and *A. flavus* inoculated unshelled peanuts were used.

Fungal isolate and inoculum production

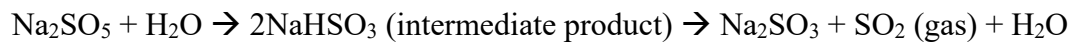
The aflatoxin-producing *A. flavus* isolate (P40-2), previously isolated from Ethiopian peanut soil samples, was used to prepare the *A. flavus*-inoculated peanut samples. This isolate was characterised using a polyphasic approach (Table S1) following Frisvad et al. (2019) and is preserved at -20°C in a glycerol-water solution (30:70, v/v) in the mycotheque of the Magan Centre of Applied Mycology, Cranfield University.

For inoculum preparation, the isolate was subcultured (3 µl centrally inoculated) on Malt Extract Agar (MEA, Oxoid Ltd., Fisher Scientific, Loughborough, UK) supplemented with 0.05% chloramphenicol at 30°C for 7 days of growth in the dark. At the end of the incubation period, the surface of the fungal colony was gently scraped with a sterile spatula, and spores were transferred to sterile distilled water containing Tween80 (0.005%). The fungal spore concentration was adjusted to 1×10^5 spores/ml using a Thoma haemocytometer counting chamber and 1 ml of the determined concentration was used as inoculum of *A. flavus* and added to the unshelled peanuts.

SO₂-releasing bags

Slow-release laminated SO₂ pads (Tessara PTY, Ltd, Cape Town, South Africa) were used in this experiment to evaluate their effectiveness in controlling fungal growth and mycotoxin

production. The pads were converted into sealable mini-bags (11 cm × 15 cm) and stored at 4°C until use. These pads are made of sodium metabisulphite (NaMBS; Na₂SO₅) encapsulated in a wax matrix. Water vapour that originates from commodity respiration reacts with NaMBS to release SO₂, which acts as a fumigator, according to the following reaction:



The SO₂ pads used in this experiment followed a dual-release principle, characterised by an initial rapid phase in which concentrations exceeding 100 ppm were released within the first 24 hours, followed by a sustained phase with lower emissions of approximately 3–5 ppm maintained for around 60 days. Plastic sealable bags of the same size served as controls.

Peanut water activity adjustment and experimental design

A water adsorption curve (Figure S1) was initially developed by adding different volumes (0 – 1.8 ml) of distilled water to 6 g of sub-samples (x 3 replicates) of unshelled peanuts in 50 ml Duran glass bottles. These were shaken vigorously and stored overnight at 4°C to allow water adsorption. Samples were then placed at room temperature to equilibrate for 30 minutes, and the *a_w* was determined using the portable HygroPalm HP23-A device (RoTronic, Bassersdorf, Switzerland). Water adsorption curve was determined by plotting the amount of added water (ml) against the *a_w* values measured. This curve was used to determine the exact amount of water needed to reach the target *a_w* levels set for the experiments. Per each *a_w*, 600 g of peanuts were adjusted to target levels of 0.85 and 0.95 by adding sterile water based on the adsorption curve. Half of them (300 g) were kept as naturally contaminated and the other half (300 g) were inoculated with *A. flavus* and then split into plastic bags (3 replicates, 50 g each) and SO₂-releasing bags (3 replicates, 50 g each) and incubated at 25°C for 15 days (Figure S2). For the *A. flavus*–inoculated peanuts, the total amount of added water was reduced by 1 ml to account for the volume of the *A. flavus* inoculum (1 × 10⁵ spores ml⁻¹). The samples were kept at 4°C

overnight for equilibration. 0.85 and 0.95 a_w were chosen to simulate moderately and highly unsafe conditions of storage which are conducive for aflatoxin production.

Quantification of the fungal community and *Aspergillus flavus* populations

The efficacy of SO₂ releasing bags was tested on total fungal populations following the serial dilution method described by Pitt and Hocking (2009). Two grams of unshelled peanuts from each of the three replicates, were weighed and transferred into sterile stomacher bags containing 18 ml of sterile 0.1% peptone water to obtain initial 10⁻¹ dilution. After 30 minutes of soaking, samples were placed into the stomacher blender (Lab-Blender 400; Seward Medical, UK) and homogenised for 4 minutes. Afterwards, 10-fold serial dilutions were prepared and 100 µl were plated with a sterile Drigalski spreader in triplicates on Dichloran Rose-Bengal Chloramphenicol (DRBC, Oxoid Ltd., Fisher Scientific, Loughborough, UK). Inoculated plates were incubated at 25°C for 5 days after which the Colony Forming Units (CFUs) were determined. Total colonies growing on the plates in a range of 5 to 50 colonies (Al-Jaza et al., 2022) were counted using a colony counter (Gallenkamp CNW 325 010X Colony Counter Lab) and their numbers expressed as Log₁₀ of CFUs per grams of dry mass of sample (Log₁₀ CFU g⁻¹ dry mass). Moreover, *A. flavus* colonies were specifically enumerated, alongside the total fungal counts, by comparing their morphological characteristics with those of the reference isolate (P40-2) used in this study.

Multi-mycotoxins analysis by LC-MS/MS

Naturally contaminated and *A. flavus*-inoculated unshelled peanuts were stored at -20°C after the experiment and then dried at 60°C for 24 hours in an oven (Gen Lab Ltd., Model: OU/100/F). Each treatment and its biological replicates were individually ground into a fine powder using a Waring commercial blender at maximum speed for 30–35 seconds and stored at -20°C until analysis.

Mycotoxin extraction followed the method reported by Crosta et al. (2025). In total, 72 samples were analysed, including three technical replicates per treatment. For LC-MS/MS analysis, 5 µl of each sample was injected into the sampling port of the qTRAP 6500+ LC-MS/MS system (SCIEX instrument, Foster city, CA, USA) equipped with an electrospray ionisation (ESI). Data acquisition was conducted with Analyst® Data Acquisition version 1.6.3, and quantification through MultiQuant™ version 3.0.3. Quantification was performed using a standard curve from standard stock solutions of aflatoxins B₁, B₂, G₁, G₂ (AFB₁, AFB₂, AFG₁, AFG₂) and cyclopiazonic acid (CPA) provided by Romer labs, Austria. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on (Malachová *et al.*, 2014) and they are included in table S2.

Data analysis

Statistical analyses were performed using Statistica (version 14; SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA) was conducted to evaluate the effect of storage bag type (plastic bags vs SO₂-releasing bags), water activity (0.85 vs 0.95 a_w), and contamination status (natural contamination vs inoculation), as well as their interactions on total fungal count, *A. flavus* count and mycotoxin production. Significant differences between means were determined using Tukey's HSD post-hoc test ($p \leq 0.05$). Graphs were generated using GraphPad Prism (version 8.0.2; GraphPad Software, Inc., San Diego CA, USA).

Results and discussion

Fungal spoilage of peanuts is initiated during the pre-harvest phase, if favourable conditions (e.g. insect damage, pods breakage, abundant rainfall) are encountered. Occurrence of toxigenic fungal species may lead to the production of mycotoxins that, if no mitigation strategies are applied, will accumulate during post-harvest processing and storage resulting in mycotoxins contamination exceeding the legislative limits (Cervini et al., 2022).

204 A combination of good agricultural practices, pre-harvest and post-harvest controlling
 205 strategies would mitigate fungal growth and mycotoxins occurrence in peanuts. In the present
 206 study we have shown, for the first time, that NaMBS immobilised storage bags which release
 207 SO₂ may provide a possible solution for storage of unshelled peanuts. Indeed, such food
 208 preservation treatment has been mainly used to control post-harvest decay by *B. cinerea* rot in
 209 grapes during post-harvest stages. Usually, the NaMBS immobilised sheets are placed over,
 210 but not touching the grapes during shipping to allow release of SO₂ and to avoid or limit
 211 damage. The amount of SO₂ released is affected by the temperature; therefore, the efficiency
 212 of these sheets depends on good temperature chain management (Ahmed et al., 2018; Habib et
 213 al., 2021).

214 Total fungal count and *A. flavus* count were significantly affected ($p < 0.001$) by a_w , storage
 215 bag type, contamination status and their three-way interaction (Table S3). In naturally
 216 contaminated peanuts stored in SO₂-releasing bags, total fungal growth was completely
 217 inhibited at both a_w levels. In contrast, naturally contaminated peanuts stored in plastic bags
 218 exhibited measurable fungal growth, with significantly higher counts at 0.95 a_w ($\log_{10} 7.43 \pm$
 219 0.11 CFU g⁻¹) compared with 0.85 a_w ($\log_{10} 2.95 \pm 0.08$ CFU g⁻¹). In *A. flavus*-inoculated
 220 peanuts, storage in SO₂-releasing bags resulted in a significant reduction in total fungal counts
 221 compared with plastic bags with the efficacy varying according to a_w (Figure 1A).

222 A similar pattern was observed for *A. flavus* counts. In naturally contaminated peanuts, *A.*
 223 *flavus* growth was significantly inhibited when samples were stored in SO₂-releasing bags,
 224 while in plastic bags *A. flavus* was detected only at 0.95 a_w ($\log_{10} 6.52 \pm 0.74$ CFU g⁻¹). In
 225 inoculated peanuts, SO₂-releasing bags significantly reduced viable *A. flavus* counts compared
 226 with plastic bags, with higher counts consistently observed at the higher a_w level (Figure 1B).
 227 These data suggested that at high fungal loads, favoured by high a_w conditions (0.95 a_w), the
 228 immobilised preservative bag system, may result in reduction of fungal populations with *A.*

flavus being more resilient to it at higher a_w (0.95 a_w) than at moderately dry conditions (0.85 a_w). This finding was also supported by visual observations: no visible fungal growth was detected in peanuts stored in SO₂-releasing bags, whereas control samples showed moderate spoilage at 0.85 and heavy spoilage at 0.95 a_w (Figure 2). Water activity and the type of storage bags significantly influenced mycotoxin production ($p < 0.001$, Table S4). With respect to mycotoxins, in our study AFB₁, AFB₂ and CPA were mainly detected at 0.95 a_w in both naturally contaminated and *A. flavus*-inoculated peanuts while no mycotoxins were detected at 0.85 a_w , in control samples, at 25°C (Table 1). The inhibitory effect of low a_w on mycotoxin production in peanuts at 25°C has been previously documented. Garcia-Cela et al. (2020), in a study investigating CO₂ production as an indicator of *A. flavus* colonisation and associated AFs and CPA contamination in shelled peanuts, reported that AFB₁, AFB₂ and CPA were not detected at 0.90 a_w after 7 days of incubation at 25°C, whereas they were detected at 0.95 a_w . Liu et al. (2017) also found that AFB₁ was absent at 0.85 a_w but it could be detected at water activities above 0.90. Also, Abdel-Hadi et al. (2010) observed higher AFB₁ at 0.90-0.95 a_w but couldn't detect any at 0.85 a_w , consistent with our findings. Previously, Passone et al. (2010) found that AFB₁ was produced at 0.85-0.88 a_w but only after 1-month storage. The application of storage bags which released SO₂ reduced the levels of AFs and CPA below the LOD and LOQ in both naturally contaminated peanuts and *A. flavus*-inoculated samples (Table 1). No previous studies have examined the use of SO₂ for minimising mycotoxins contamination of raw commodities such as unshelled peanuts. In a study by Al-Jaza et al. (2022) slow release SO₂ laminated sheets (40 and 70% NaMBS) were tested to control *A. flavus* spoilage and AFB₁ contamination in *A. flavus*-inoculated whole red stored chillies. They showed that AFB₁ was completely inhibited using both concentrations of NaMBS immobilised on the laminated sheets.

Conclusions

The results of this study suggest that slow-releasing SO₂ bags may represent a promising mitigation strategy for reducing fungal spoilage and mycotoxin contamination during the storage of unshelled peanuts. To confirm their effectiveness, further pilot-scale trials using larger quantities of peanuts are now warranted. These studies should also include shelled peanuts, as they are the preferred form of storage in many African countries.

In addition, fundamental research is needed to assess the safety of this intervention, particularly the potential formation of secondary metabolites resulting from interactions between SO₂ and mycotoxins, which appear to be effectively suppressed by this treatment. It is also essential to evaluate the impact of SO₂ application on the nutritional quality and palatability of peanuts to ensure that this approach supports both food safety and nutrition security in relevant contexts.

Dedication

The authors dedicate this paper to the memory of Prof. Naresh Magan, who initiated and led this work at Cranfield University before his untimely passing.

Authors contribution declaration

Carla Cervini: writing – original draft, supervision, conceptualization, methodology, writing – review and editing, data curation, formal analysis, investigation; Vaibhav Verma: writing – original draft, investigation; Carol Verheecke-Vaessen: funding acquisition, writing – original draft, supervision, methodology, formal analysis; Angel Medina: funding acquisition, resources, methodology; Naresh Magan: funding acquisition, conceptualization, methodology.

Funding

274 This work was supported by the EWA-BELT (Grant agreement No 862848) funded by Horizon
275 2020 European Union Funding for Research & Innovation and NutriNuts funded by Innovate
276 UK (project reference 105663).

277 **Conflict of interest**

278 The authors declare that there is no conflict of interest.

279 **Data availability statement**

280 All data is incorporated into the article.

281

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Figure 1. Effect of SO₂-releasing bags on A) total fungal population (log₁₀ CFU g⁻¹ dry peanuts) and B) *A. flavus* counts (log₁₀ CFU g⁻¹ dry peanuts) at 0.85 and 0.95 a_w in naturally contaminated and *A. flavus*-inoculated unshelled peanuts assessed on DRBC after 5 days of incubation at 25°C. Different letters within each panel indicate significant differences (Tukey test, p ≤ 0.05).

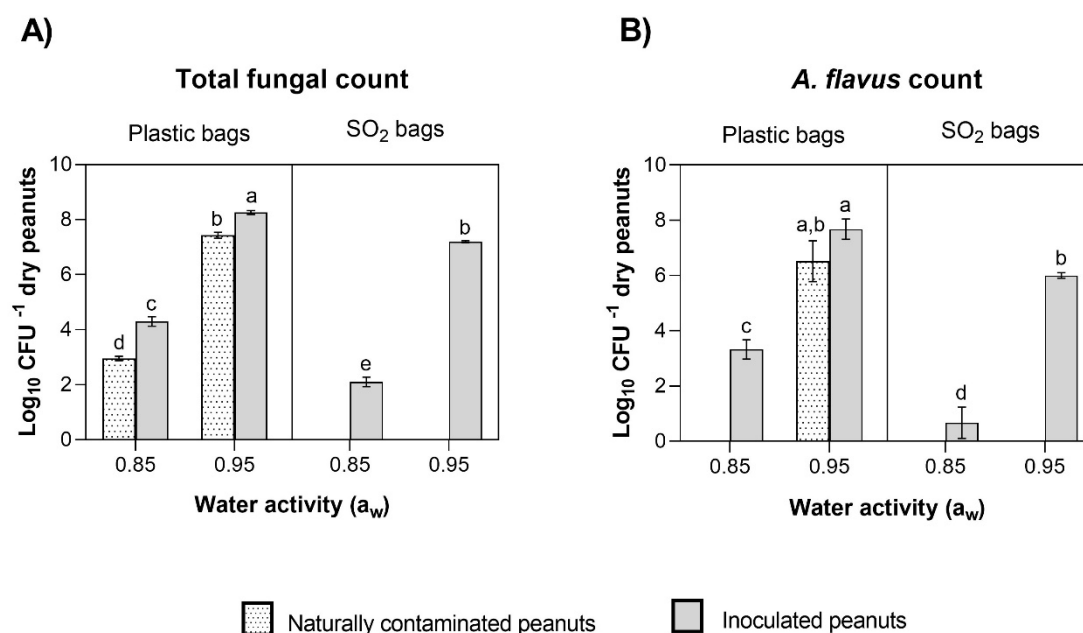
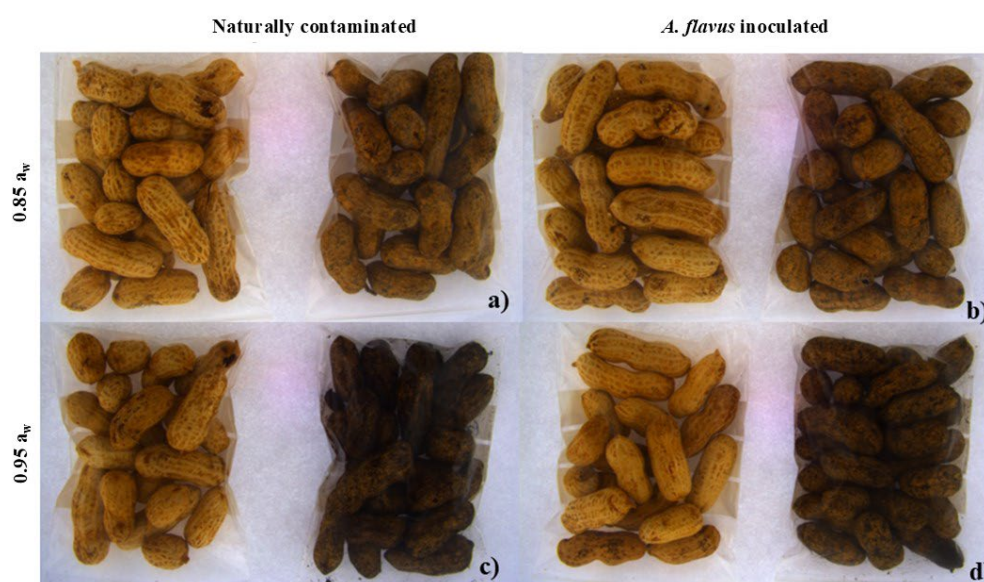


Figure 2. Visual appearance of unshelled peanuts after 15 days of incubation at 25°C. The pictures represent: 0.85 a_w (a) naturally contaminated and (b) *A. flavus*-inoculated; 0.95 a_w (c) naturally contaminated and (d) *A. flavus*-inoculated peanuts. In each panel, peanuts stored in SO₂-releasing bags are shown on the left, and those stored in plastic bags (control) on the right.



433 **Table 1.** Mean values (ng g⁻¹) of aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂) and cyclopiazonic acid (CPA) in naturally contaminated and *A. flavus*
 434 inoculated unshelled peanuts. Different letters indicate significant difference (Tukey test, p ≤ 0.05) within each mycotoxin.

Contamination	a _w	AFB ₁ (ng g ⁻¹)		AFB ₂ (ng g ⁻¹)		CPA (ng g ⁻¹)	
		Plastic bags	SO ₂ bags	Plastic bags	SO ₂ bags	Plastic bags	SO ₂ bags
Naturally contaminated peanuts	0.85	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	0.95	94.94 ± 54.81 ^b	< LOD	83.70 ± 16.04 ^a	< LOD	112.71 ± 65.12 ^b	< LOD
<i>A. flavus</i> inoculated peanuts	0.85	1.58 ± 1.37 ^b	< LOD	< LOD	< LOD	< LOD	< LOD
	0.95	585.4 ± 186.39 ^a	< LOD	113.53 ± 33.83 ^a	< LOD	699.29 ± 283.15 ^a	< LOD

435 LOD: Limit of detection.

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First evidence of SO₂-releasing bags controlling fungal growth, aflatoxins and cyclopiazonic acid contamination in unshelled peanuts

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2026-03

Oxford University Press (OUP)

<https://doi.org/10.1093/lambio/ovag029>

Cervini C, Verma V, Verheecke-Vaessen C, et al., (2026) First evidence of SO₂-releasing bags controlling fungal growth, aflatoxins and cyclopiazonic acid contamination in unshelled peanuts. Letters in Applied Microbiology, Volume 79, Issue 3, March 2026, Article number ovag029