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Aflatoxin evaluation and integrated management strategies to minimize toxin contamination in maize and peanuts

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ABSTRACT

Commercial varieties of maize and peanuts are highly prone to *Aspergillus flavus* infection and aflatoxin contamination at pre and postharvest stages. In a screening of 600 samples each of maize and peanuts from Tamil Nadu, India, for the mycotoxin aflatoxin B1 (AFB1), 28 and 32.67 % of the maize and peanut samples tested positive, where 6 and 9.5 % of both pre and postharvest maize and peanut samples exceeded the minimum threshold level of 20 μg/kg. The highest level of AFB1 was recorded as 147.23 and 162.72 μg/kg in maize and peanut and in postharvest samples. Additionally, a high number of positive samples in postharvest and Rabi season crops indicates the influence of moisture on *A. flavus* proliferation and toxin production. Effective pre-harvest management practices include the integrated use of biocontrol agents such as *Trichoderma viride* and *Bacillus subtilis*, while postharvest measures involve drying of harvest produce (with moisture content below 9 %), treatment with *Andrographis paniculata* extracts and storage in jute bags. Further, our findings emphasize the integration of pre and postharvest management strategies to mitigate *A. flavus* infection and aflatoxin contamination in maize and peanuts, thereby ensuring food security for the growing human population.

1. Introduction

Maize (*Zea mays* L.), referred to as the "queen of cereals" due to its remarkable genetic yield potential among cereal crops, holds great economic significance as a human food and animal feed. It serves as a versatile agricultural crop in 166 countries worldwide, encompassing tropical, subtropical, and temperate regions, ranging from sea level to 3000 m elevations. Approximately 1162 million tons of maize are produced globally, and the crop is cultivated on 201 million hectares [1]. Peanut (*Arachis hypogaea* L.), also known as groundnut, is the world's fourth most essential source of edible oil and the third most valuable source of vegetable protein [2,3]. Beyond its agricultural significance, peanuts play a crucial role in human nutrition due to their high levels of protein, fats, energy, and essential minerals. Peanut seeds typically contain 18.92 to 30.53 % crude protein, as well as oil content ranging from 33.6 to 54.95 % [4]. Further, it is known for its remarkable health benefits, improving low-density lipoprotein (LDL) levels that can diminish the risk of cardiovascular diseases [5].

While maize and peanuts are celebrated for their productivity and as a valuable calorific source, products from these crops are vulnerable to toxin contamination due to infection by toxigenic fungi, such as *Aspergillus*, which produces aflatoxins. Since *Aspergillus* infects the crop as

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Table 1
Aflatoxin B1 content in maize and peanut samples assessed by indirect competitive enzyme-linked immunosorbent assay (ELISA).

Sample	Season	No. of samples analyzed	No. of samples with aflatoxin B1 (μg/kg)				
			0	1–20	20–50	50–100	>100
Preharvest maize	Kharif 2020	50	46	3	1	0	0
	Rabi 2020	50	38	9	3	0	0
	Kharif 2021	50	48	2	0	0	0
	Rabi 2021	50	41	7	2	0	0
	Kharif 2022	50	48	2	0	0	0
	Rabi 2022	50	42	6	2	0	0
	Total	300	263	29	8	0	0
Postharvest maize	Kharif 2020	50	30	16	3	1	0
	Rabi 2020	50	23	20	4	2	1
	Kharif 2021	50	31	15	3	1	0
	Rabi 2021	50	24	21	3	1	1
	Kharif 2022	50	35	12	2	1	0
	Rabi 2022	50	26	19	4	1	0
	Total	300	169	103	19	7	2
Preharvest peanut	Kharif 2020	50	44	5	1	0	0
	Rabi 2020	50	35	10	5	0	0
	Kharif 2021	50	46	3	1	0	0
	Rabi 2021	50	38	9	3	0	0
	Kharif 2022	50	46	3	1	0	0
	Rabi 2022	50	40	7	3	0	0
	Total	300	249	37	14	0	0
Postharvest peanut	Kharif 2020	50	29	14	5	1	1
	Rabi 2020	50	15	24	7	3	1
	Kharif 2021	50	30	14	5	1	0
	Rabi 2021	50	22	19	6	2	1
	Kharif 2022	50	34	13	3	0	0
	Rabi 2022	50	25	18	5	1	1
	Total	300	155	102	31	8	4

well as its produce, toxin contamination occurs at any stage in the production chain [6]. The impact of mycotoxin contamination is profound, often resulting in the loss of heavily contaminated crops. Global reports highlight widespread contamination of dietary staples with aflatoxins [7,8]. Further, as an essential component in animal feed, contaminated crops also cause health issues in livestock and the presence of toxic substances in animal products. Notably, in the densely populated Indian subcontinent, 43 % of maize samples from food and feed sources tested positive for aflatoxins, with the highest level of aflatoxin B1 (AFB1) recorded at 245 μg/kg [9,10]. Similarly, 21 % of peanut samples exceeded the tolerance limit of 20 μg/kg [11].

Given the carcinogenic nature of aflatoxins, substantial efforts have been taken to eliminate *A. flavus* and its toxic byproducts in human food and livestock feeds. Cultural practices, such as adjustment of sowing and harvesting dates, have proven effective in minimizing aflatoxin contamination during preharvest stages. However, suboptimal storage conditions during postharvest facilitate the invasion of the fungi into grains, leading to the accumulation of aflatoxins. While fungicides reduce the incidence of *A. flavus*, concerns about their residual effect on human health and the environment necessitate the search for safer control options [12].

Subsequently, various biological, physical, and chemical detoxification methods have been employed to reduce aflatoxin content in foods and feeds [13–16]. These methods predominantly rely on the degradation, modification, and adsorption of aflatoxins. Microbial detoxification involving species like *Flavobacterium aurantiacum*, *Mucor* sp., *Rhizopus* spp., *Corynebacterium rubrum*, *Phoma* sp., *Rhodococcus erythropolis*, *Bacillus licheniformis* and *Mycobacterium fluoranthenivorans* have exhibited promising outcomes [17–19]. Physical detoxification using hydrated sodium calcium aluminosilicate has also shown potential to minimise toxin levels [20,21]. Nevertheless, these methods are associated with their own limitations, requiring a delicate balance between effectiveness and preserving the safety and nutritional value of the treated products.

Extracts from plants, particularly medicinal herbs, have demonstrated inhibitory effects on *A. flavus* growth and aflatoxin production [22–27]. Moreover, the use of herbal extracts has also shown potential for aflatoxin degradation and remains safe and cost-effective [28,29]. Additionally, storage conditions and structures can significantly influence aflatoxin production, emphasizing the importance of proper pre and postharvest management practices in reducing aflatoxin contamination [30–33].

Hence, considering the need for a robust management strategy to mitigate aflatoxin contamination, the current study aimed to undertake a comprehensive survey to assess the current level of aflatoxin contamination and identify effective pre and postharvest management strategies to minimize aflatoxin contamination in maize and peanut production and supply chain. These efforts ultimately lead to ensuring a secure food supply to meet human needs.

2. Materials and methods

2.1. Survey to access A. flavus incidence

A comprehensive three-year survey spanning 2020 to 2022 was conducted to assess aflatoxin contamination in maize and peanut samples across the seven agro-climatic zones within the Tamil Nadu state of India. These zones consists of 17 districts, namely, Coimbatore, Cuddalore, Dharmapuri, Dindigul, Erode, Kallakurichi, Madurai, Namakkal, Perambalur, Salem, Theni, Tiruchirappalli, Tiruppur, Tiruvannamalai, Thoothukudi, Viluppuram and Virudhunagar. A total of 1200 samples, 600 from each of the crops (peanut and maize), were collected from seed sources, farms, storage facilities (public and private) and retail to represent pre and postharvest stages (Table 1). Samples (~1 kg of grains/seeds per sample) were collected from Kharif (sown in July and harvested in September or October) and Rabi (Sown in November and harvested in April or May) season crops.

Samples were processed at the Postharvest Pathology laboratory of Tamil Nadu Agricultural University (TNAU), Coimbatore. Samples with high moisture content were dried in a hot air oven at 45 °C for 48 h, to reach a moisture content of ~13 and 10 % for maize and peanut,

respectively. Maize grains were ground using a coffee mill, while peanuts were milled using a blender. The coffee mill and blender were sterilized with 70 % ethanol between samples to prevent cross-contamination. All samples were divided into two halves, one for microbial and the other for aflatoxin analysis. Samples for microbial analysis were stored in hermetic bags at 4 °C, while those for aflatoxin analysis at −20 °C.

2.2. *A. flavus* isolation and spore suspension preparation

Isolation of single *Aspergillus* strain from the randomly selected maize and peanut samples with detectable AFB1 was performed using potato dextrose agar (PDA) medium and hyphal tip isolation. Strains belonging to *A. flavus* were identified using taxonomic keys and species descriptions in Okayo et al. [34]. All the *A. flavus* isolates were screened for their potential to produce aflatoxins in potato dextrose broth. Cultures were grown in 250 ml conical flasks with 100 ml of potato dextrose broth at room temperature (28 ± 2 °C) for 10 days. After incubation, the culture filtrate was collected and subjected to chloroform extraction, followed by quantification of aflatoxin content using the enzyme-linked immunosorbent assay (ELISA) method. Isolates producing detectable levels of aflatoxin were classified as virulent and were maintained on PDA at 4 °C. Isolates with the highest production of aflatoxin were selected from both maize and peanut to use as inoculum for postharvest management studies using plant products and packaging techniques.

To prepare spore suspension, *Aspergillus* cultures were grown on PDA for seven days at 30 ± 2 °C. Asexual spores (conidia) were harvested using 0.1 % Tween-80 solution, counted with a hemocytometer and adjusted to 10^5 conidia/ml using water. Spore suspensions were stored at 4 °C and used within one week of preparation for analysis or inoculation.

2.3. Analysis of AFB1 content using ELISA

AFB1 content in samples was quantified using the ELISA technique in which polyclonal antibodies against AFB1 conjugated to Bovine Serum Albumin (AFB1-BSA) were produced in a New Zealand white inbred rabbit, as described in Reddy et al. [35]. The rabbit was immunized with an emulsion of 80 μg AFB1-BSA (Sigma, USA) in 0.1 M phosphate buffer (pH 7.0), prepared with Freund's complete adjuvant, followed by additional injections with Freund's incomplete adjuvant. Samples (2 g) were ground in a coffee grinder and mixed with a solvent comprising 70 % methanol, 30 % water, and 0.5 g KCl. This mixture was incubated on a rotary shaker for 30 min at room temperature (28 ± 2 °C), followed by filtration through Whatman No. 41 filter paper. The filtrate was then used for AFB1 content determination using the indirect competitive ELISA method. Microtiter plates were coated with AFB1-BSA (100 μg/ml) in carbonate coating buffer. Subsequent steps included washing with phosphate-buffered saline (PBS) containing Tween-20 (PBST), treatment with PBST-BSA, addition of sample extract or AFB1 standard, incubation with AFB1-BSA antiserum (1 : 6000 dilution), followed by alkaline phosphatase-labeled goat anti-rabbit IgG conjugate. The plates were developed using p-nitrophenyl phosphate and read at 405 nm in an ELISA reader to calculate AFB1 concentration in the samples.

2.4. Field experiments to test management practices

For maize, two field experiments were conducted at the Department of Millets, TNAU, Coimbatore, India, during 2021 and 2022 Rabi seasons, when the epidemics of *A. flavus* are prominent. The susceptible maize cultivar COH(M)-6 was utilized in these trials. The experiments were organized in a randomized block design (RBD), featuring six treatments and three replications. The treatments consist of T1: seed treatment with metalaxyl (@ 2 g/kg of seed) + basal application of *Trichoderma viride* (commercial talc powder formulation from TNAU with *T. viride* strain ITCC. 6914 concentrations above 2 x 10^6 colony forming units [CFU]/g) @ 2.5 kg/ha mixed with 50 kg of farmyard manure (FYM) before planting, T2: cob borer (*Helicoverpa armigera*) management - carbaryl 10 % D (@ 25 kg/ha) and 15 days later carbaryl 50 WP (1 kg/ha), T3: Exposing drought during maturity stage (66 – 95 days) where no irrigation from 66 to 95th day, T4: Harvest before seeds become hard and dry, T5: integrated disease management (IDM) practices, combining all above four treatments and T6: control without any treatments. The *A. flavus* population in the rhizosphere soil, seed infection, and AFB1 contamination in maize were assessed in all the treatments.

For peanuts, two field experiments were conducted, one at the TNAU Agricultural Research Station, Bhavanisagar, and the other at the TNAU main campus, Coimbatore, Tamil Nadu, India, in 2021 and 2022 Rabi seasons. The trials were conducted in drought and normal environmental conditions as peanut is cultivated both as an irrigated and rainfed crop in Tamil Nadu. VRI-2*A,* an *A. flavus* susceptible cultivar, was used, and the trials were laid out in a randomized block design with nine treatments, three replications and a plot size of 5 × 4 m with 30 × 15 cm spacing. The treatments consist of T1: *B. subtilis* application as a seed treatment (commercial formulation from TNAU @ 10 g/kg) and soil application (commercial formulation 2.5 kg/ha applied as a mixture with 150 kg FYM) at 30, 60, and 90 days after sowing (DAS), T2: seed treatment of *B. subtilis* + basal application of *T. viride* (commercial formulation 2.5 kg/ha applied with FYM) + soil application of *B. subtilis* (commercial formulation 2.5 kg/ha applied as a mixture with 150 kg FYM) at 30, 60 and 90 DAS, T3: seed treatment of *B. subtilis* + basal application of *T. viride* + soil application of *T. viride* at 60 and 90 DAS, T4: gypsum (calcium sulphate) application @ 400 kg/ha at 45 DAS, T5: application of carbofuran 3G @ 33 kg/ha at 30 DAS, T6: spraying 0.5 % potassium chloride during flowering and pod development stages, T7: IDM integration of all the above practices, T8: farmers' practice which includes the use of gypsum and carbofuran chemicals without any biocontrol agents, and T9: untreated control (no application of the above-said materials). The population of *A. flavus* in the rhizosphere soil, *A. flavus* infection and AFB1 contamination in maize kernels and peanut pods were evaluated in addition to kernel and pod yield.

2.5. Assessment of *A. flavus* population in rhizosphere soil

The assessment of *A. flavus* populations at the initial stage (before planting) and at 30, 60, and 90 days after sowing in both the treatment and control plots were determined by the dilution plate technique. Soil samples were collected with an auger at a depth of 5 cm [36]. Four g of soil samples were added to 100 ml of sterile distilled water in a 250 ml conical flask and kept on a rotary shaker at 150 rpm for 10 min. From this suspension, a series of dilutions were made, and 10^{-3} dilutions were used for population assessment. One ml of the soil suspension was mixed with 20 ml of sterilized PDA medium and poured into sterile 9 mm diameter Petri plates. The plates were incubated at room temperature (28 ± 2 °C) for 5 – 7 days, and *A. flavus* colonies that developed were counted.

2.6. Assessment of *A. flavus* infection in maize and peanut samples

To estimate *A. flavus* infection rates, plants were harvested from each experimental plot, and the kernels (maize) / pods (peanut) were removed and dried in shade for 3 – 4 days. A random sample of 100 kernels / pods from each plot was selected and surface sterilized by soaking in a 0.1 % aqueous solution of mercuric chloride for 3 min, followed by rinsing with sterile distilled water. The sterilized kernels were placed on sterile filter paper in 10 cm-diameter Petri dishes and incubated at 25 °C for 6 days under high humidity conditions. Sterile distilled water (1 – 2 ml) was added daily during the first 5 days to maintain moisture. At the end of the incubation period, kernels showing visible fungal growth characteristics of *A. flavus* were counted. The percentage of infection was determined using the formula: (Number of

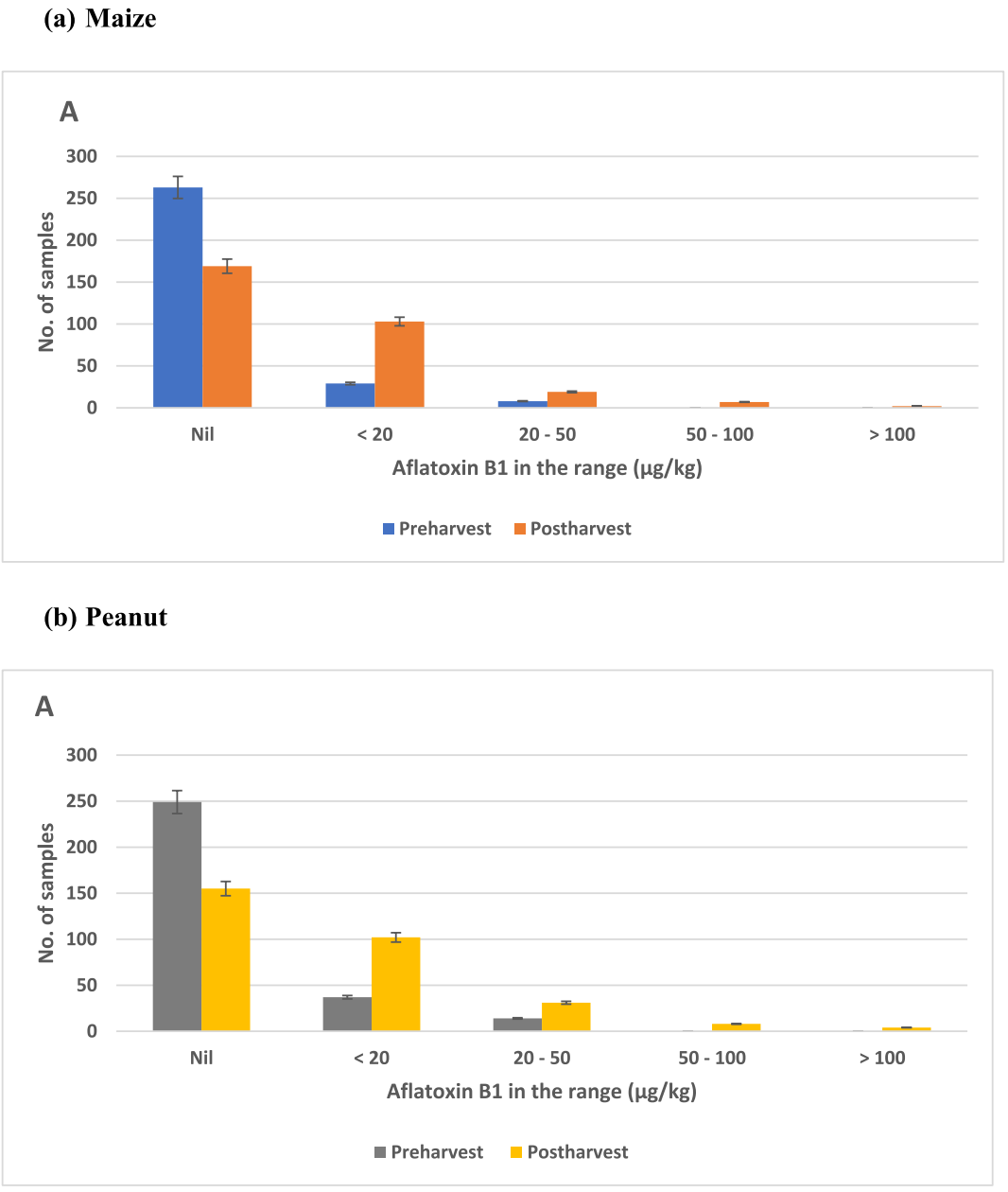

Fig. 1. Aflatoxin B1 content in preharvest versus postharvest samples of maize (a) and peanut (b).

Infected Kernels or Pods / Total Kernels or Pods Tested) × 100 [37].

2.7. Aflatoxin detoxification using medicinal plants

Based on earlier findings, medicinal plants, including *Adhatoda vasica*, *Andrographis paniculata*, *Trachyspermum ammi*, and Zimmu (an interspecific hybrid of *Allium cepa* and *A. sativum*) were used for the management of fungal infections in postharvest samples. These medicinal plants were collected from the herbal garden at the Horticulture College and Research Institute, TNAU, Coimbatore, India. Aqueous extracts of medicinal plants were prepared by grinding one g of leaves or seeds with three ml of sterile distilled water. The homogenate was centrifuged at 14000 × *g* for 15 min, and the supernatant was used for further studies. Five hundred µl of plant extract was mixed with 50 ng of aflatoxin in a microcentrifuge tube and kept at 37 °C for 24 h in an incubator. After incubation, the aflatoxin in the mixture was extracted with 300 µl of chloroform. The chloroform fraction was evaporated on a heat block at 60 °C, and the residue was dissolved in 10 µl of methanol and analyzed by thin-layer chromatography (TLC). Ten µl of the chloroform extract was separated on a 0.25 mm silica gel G TLC plate (Merck). The plate was developed using the solvent chloroform: acetone: water (88 : 12 : 1). The chromatogram was viewed under UV light (365 nm).

2.8. Postharvest management of aflatoxin using plant products and packaging techniques

Normal and infected kernels were treated with plant products from Zimmu, *T. ammi*, *A. vasica* and *A. paniculata* at a rate of 30 g/kg sample (3 %) in a completely randomized design (CRD) with nil treatment as a control. Plant powders were thoroughly mixed with the kernels/pods. Subsequently, the influence of different packaging materials such as jute, polypropylene woven bags and cloth bags on *A. flavus* infection and aflatoxin production were also tested along with the various plant products where kernels / pods stored in open condition served as control. Five kg of uninfected kernels / pods were mixed with *A. flavus*

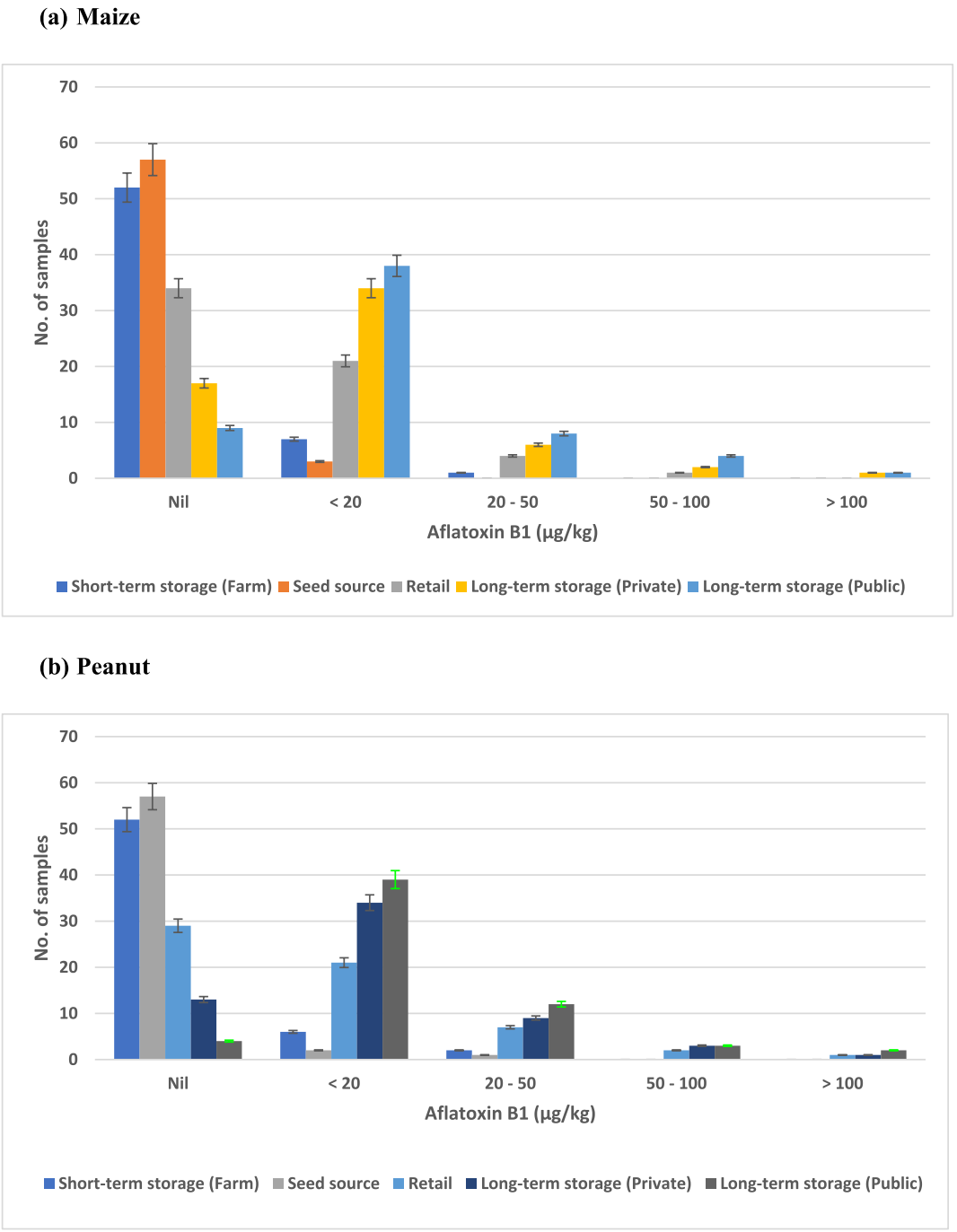

Fig. 2. Aflatoxin B1 content in maize (a) and peanut (b) samples at various stages of production and supply chain.

(isolate from maize and peanut sample with highest toxin production) spore suspension (10^5 conidia/ml). The open ends of the bags were tied with nylon twines, and the bags were placed on laboratory racks. Representative kernels from each bag were drawn after five months in storage, and aflatoxin content was estimated by the ELISA method as described earlier.

2.9. Statistical analysis

The aflatoxin contamination levels in maize and peanut samples were grouped into 5 categories (i.) no aflatoxin, (ii) aflatoxin below the Indian threshold (20 µg/kg), (iii) 20 – 50 µg/kg, (iv.) 50 – 100 µg/kg, (v.) > 100 µg/kg. The analysis of variance (ANOVA) for the values for aflatoxin contamination levels was carried out using the general linear model (GLM) for unbalanced data where crop type was used as a compounding factor.

The field experiments were conducted using RBD with multiple treatments and replicates to minimize the impact of variability across blocks. The statistical analysis was performed using Tukey's test to compare the means of different treatments. This test was chosen as it effectively identifies significant differences between treatment means while accounting for the experimental design's inherent variability. The significance level (p-value) was set at 0.05 for all analyses.

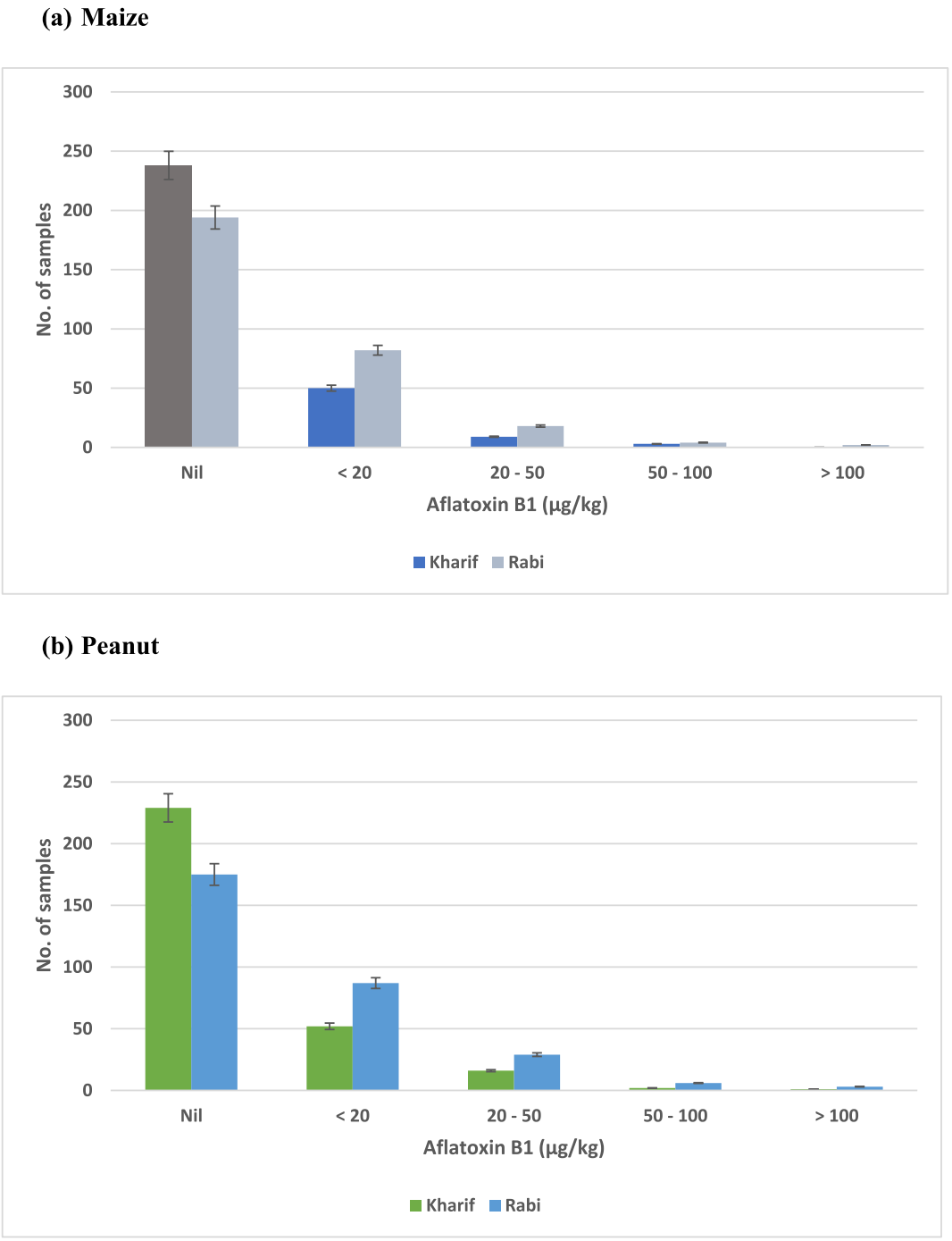

Fig. 3. Aflatoxin B1 content in Kharif versus Rabi season samples of maize (a) and peanut (b).

3. Results

3.1. Aflatoxin contamination in maize and peanut samples

In maize, 28 % of the samples exhibited aflatoxin contamination with toxin levels ranging from 0.5 to 147.23 µg/kg. Notably, 12.33 and 43.67 % of pre and postharvest samples were positive for AFB1 and 6 % exceeded the 20 µg/kg threshold. A three-fold difference is observed between the two sample sets for all three years however, 2020 had the highest number of positive samples compared to 2021 and 2022 (Table 1). A similar trend was also observed for peanuts, but interestingly more samples (32.67 %) were positive for AFB1, with the toxin levels ranging from 0.83 to 162.72 µg/kg. Specifically, 17 and 48 % of pre and postharvest peanut samples were contaminated with AFB1, with 9 % exceeding the critical threshold of 20 µg/kg (Table 1). These results confirm that the postharvest stage is the most vulnerable period for *Aspergillus* spp. infection and aflatoxin production in both these crops (Fig. 1). Within the postharvest lot, samples procured from long-term storage units had a higher number and levels of contamination than seed source and short-term storage lot of the farm (Fig. 2). Further, the number of contaminated samples with low as well as high aflatoxin levels was higher in the Rabi season across all three years (Table 1; Fig. 3).

Table 2
Aflatoxin (AFB1) productivity by *Aspergillus flavus* isolates.

District	*A. flavus* isolate of maize	Aflatoxin B1 (ng/ml) maize	*A. flavus* isolate of peanut	Aflatoxin B1 (ng/ml) peanut
Coimbatore	AFM1	2.75	AFG1	2.21
Coimbatore	AFM2	61.28	AFG2	0.85
Coimbatore	AFM3	11.77	AFG3	77.02
Coimbatore	AFM4	0.0	AFG4	0.62
Cuddalore	AFM5	0.0	AFG5	0.32
Cuddalore	AFM6	8.25	AFG6	10.54
Cuddalore	AFM7	82.25	AFG7	11.27
Dindigul	AFM8	0.0	AFG8	13.8
Dindigul	AFM9	11.23	AFG9	0.0
Dindigul	AFM10	0.0	AFG10	0.0
Erode	AFM11	71.23	AFG11	14.17
Erode	AFM12	0.0	AFG12	116.02
Erode	AFM13	0.0	AFG13	0.0
Kallakurichi	AFM14	9.98	AFG14	0.0
Kallakurichi	AFM15	10.65	AFG15	59.48
Kallakurichi	AFM16	12.33	AFG16	0.0
Madurai	AFM17	0.0	AFG17	52.08
Madurai	AFM18	0.0	AFG18	60.79
Madurai	AFM19	12.7	AFG19	2.21
Namakkal	AFM20	0.0	AFG20	0.85
Namakkal	AFM21	0.0	AFG21	77.02
Namakkal	AFM22	54.9	AFG22	0.62
Perambalur	AFM23	0.0	AFG23	0.32
Perambalur	AFM24	47.5	AFG24	10.54
Salem	AFM26	1.25	AFG26	1.83
Salem	AFM27	61.76	AFG27	67.07
Salem	AFM28	12.25	AFG28	13.24
Dharmapuri	AFM29	0.0	AFG29	0.0
Dharmapuri	AFM30	0.0	AFG30	13.24
Dharmapuri	AFM31	8.73	AFG31	0.0
Theni	AFM32	82.73	AFG32	7.73
Theni	AFM33	3.23	AFG33	106.04
Tiruppur	AFM34	11.71	AFG34	2.23
Tiruppur	AFM35	6.88	AFG35	12.7
Thoothukudi	AFM36	71.71	AFG36	5.88
Thoothukudi	AFM37	8.68	AFG37	95.02
Thoothukudi	AFM38	0.0	AFG38	7.68
Viluppuram	AFM39	10.46	AFG39	0.0
Viluppuram	AFM40	11.13	AFG40	10.6
Viluppuram	AFM41	12.81	AFG41	1.72
Tiruvannamalai	AFM42	81.18	AFG42	11.29
Tiruvannamalai	AFM43	0.0	AFG43	13.8
Tiruvannamalai	AFM44	13.18	AFG44	104.49
Tiruchirappalli	AFM45	0.0	AFG45	0.0
Tiruchirappalli	AFM46	0.0	AFG46	17.28
Tiruchirappalli	AFM47	55.38	AFG47	0.0
Viruthunagar	AFM48	0.0	AFG48	0.0
Viruthunagar	AFM49	47.98	AFG49	59.48
Viruthunagar	AFM50	56.69	AFG50	0.0

3.2. AFB1 productivity by A. flavus isolates

Hundred fungal isolated from the randomly selected 50 maize and peanut samples, with detectable aflatoxin, were identified as *A. flavus* based on the greenish colony with radical spread, raised, floccose and rough centre. Under the microscope, the colonies were biseriate, radiating philiades, subglobose or globose vesicles and conidia with thin walls and rough texture. Within the subset, 38 and 32 isolates from peanut and maize were toxigenic (producing aflatoxin). For isolates from maize, the AFB1 production ranged from 0.85 to 82.73 ng/ml, with the highest aflatoxin level from isolate AFM32. For peanuts, the AFB1 level ranged from 0.32 to 116.02 ng/ml, with highest production in isolate AFG12 (Table 2).

3.3. Aflatoxin management in maize

Among the various treatments other than IDM to mitigate aflatoxin contamination, the combination of seed treatment with metalaxyl (2 g/kg of seed) and basal application of *T. viride* (2.5 kg/ha) mixed with 50 kg of farmyard manure (FYM) before planting was notably effective at reducing aflatoxin contamination to 3.75 μg/kg compared to that in the control group at 25.43 μg/kg. Additionally, cob borer management reduced AFB1 contamination from 25.43 to 13.76 μg/kg. However, exposing the crop to drought during the maturity stage (66 – 95 days) led to an increase in aflatoxin contamination, reaching 27.66 μg/kg. Next, based on drought exposure analysis, it is apparent that crops exposed to drought stress will increase contamination. Furthermore, harvesting maize crops before seeds become hard and dry, referred to as the premature stage, increased aflatoxin contamination from 25.43 to 26.57 μg/kg, indicating that soft kernels serve as infection sites for *A. flavus*. Field trials revealed varying yields, with the exposure of crops to drought during the maturity stage (66 – 95 days) resulting in the lowest yield at 4718 kg/ha. In contrast, harvesting crops before physiological maturity (4815 kg/ha) had a slightly higher yield, while fields where IDM practices were implemented had the highest yield, at 5435.6 kg/ha and reduced *A. flavus* infection (Table 3; Fig. 4).

Table 3
Effect of cultivation practices on *Aspergillus flavus* infection and yield of maize under field conditions.

Treatments	*A. flavus* infection (%)	Kernel yield (kg/ha)	100 seed weight (g)
Seed treatment of metalaxyl @ 2 g/kg of seed + basal application of *Trichoderma viride* @ 2.5 kg/ha mixed with 50 kg of farmyard manure (FYM) before planting	10 ± 1.64^{bc}	5415.6 ± 128.53^{a}	21.76 ± 0.78^{a}
Cob borer (*Helicoverpa armigera*) management - carbaryl 10 % D @25 kg/ha and 15 days later carbaryl 50 WP @1 kg/ha	15 ± 1.50^{b}	5365.0 ± 100.81^{a}	20.65 ± 1.21^{ab}
Exposing drought during maturity stage (66 – 95 days) where no irrigation from 66 to 95th day	29.5 ± 5.39^{a}	4718.3 ± 102.64^{b}	21.5 ± 0.88^{a}
Harvest before seeds become hard and dry	28.33 ± 1.72^{a}	4815.0 ± 124.28^{b}	19.28 ± 0.74^{b}
Integrated disease management (IDM) combining all the above four practices	3.5 ± 1.12^{c}	5435.6 ± 127.15^{a}	21.76 ± 0.65^{a}
Control	27.3 ± 4.02^{a}	4975.5 ± 128.17^{b}	20.14 ± 1.10^{ab}

Note: In each column, figure followed by same letter do not differ significantly at 5 % level by Tukey's test.

3.4. Aflatoxin management in peanut

In peanuts, drought stress significantly impacts aflatoxin contamination in late maturity stages, with maximum AFB1 content reaching 61.23 and 68.4 μg/kg in normal and drought-imposed field conditions. However, seed treatment and soil application of *B. subtilis*, either by itself or in combination with *T. viride* displayed significant reductions in aflatoxin contamination compared to the control. Additionally, the application of gypsum (calcium sulphate) resulted in reduced AFB1 content of 13.20 and 15.8 μg/kg in normal and drought conditions, respectively, compared to the control (26.23 and 28.4 μg/kg). Similarly, spraying 0.5 % potassium chloride during flowering and pod development stages reduced aflatoxin contamination to 15.60 and 16.2 μg/kg in normal and drought-imposed plots, compared to the control (26.23 and

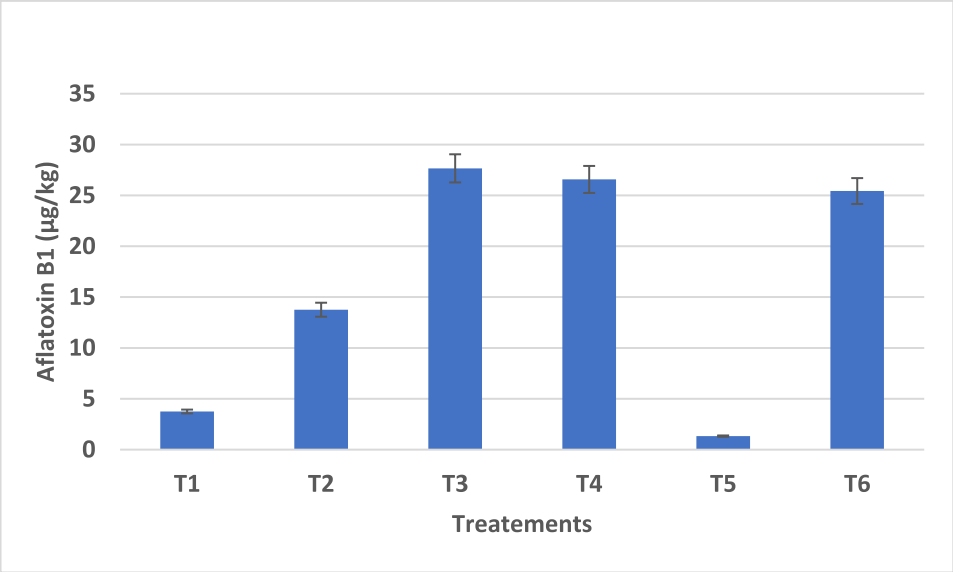

Fig. 4. Effect of cultivation practices on the management of aflatoxin contamination in maize under field conditions: Treatment (T) 1 - Seed treatment of Metalaxyl @ 2 g/kg of seed + basal application of *Trichoderma viride* @ 2.5 kg/ha mixed with 50 kg of FYM before planting; T2 - Cob borer (*Helicoverpa armigera*) management - Carbaryl 10 % D 25 kg/ha and 15 days later Carbaryl 50 WP 1.0 kg/ha; T3 - Exposing drought during the maturity stage (66 – 95 days) - no irrigation from 66 to 95th day; T4 - Harvest before seeds become hard and dry; T5 - Integrated disease management (IDM) combining all above 4 treatments; T6 - Control.

Table 4
Effect of good agriculture practices for the management of aflatoxin contamination in peanut under drought and normal field conditions.

Treatments	Normal condition		Drought condition	
	Aspergillus flavus infection (%)	Pod yield (kg/ha)	*A. flavus* infection (%)	Pod yield (kg/ha)
Seed treatment (@ 10 g/kg) and soil application (@ 2.5 kg/ha) of *Bacillus subtilis* (Bbv57) at 30, 60 and 90 days after sowing (DAS)	9.5 ± 2.15^{def}	1920.5 ± 145.47^{c}	10.18 ± 0.45^{d}	1919.1 ± 45.35^{b}
Seed treatment of *B. subtilis* + basal application of 2.5 kg/ha of *Trichoderma viride* + soil application of 2.5 kg/ha of *B. subtilis* at 30, 60 and 90 DAS	8 ± 2.37^{ef}	1928.3 ± 80.24^{bc}	10.53 ± 1.66^{d}	1921.6 ± 131.30^{b}
Seed treatment of *B. subtilis* + basal application of *T. viride* + soil application of *T. viride* at 60 and 90 DAS	10.7 ± 0.03^{de}	1991.6 ± 85.78^{abc}	10.88 ± 1.51^{d}	1975.1 ± 174.94^{ab}
Gypsum (calcium sulphate) application @ 400 kg/ha on 45th DAS	21 ± 4.96^{bc}	2223.3 ± 142.90^{ab}	23 ± 1.62^{bc}	2211.6 ± 109.53^{ab}
Carbofuran 3 G @ 33 kg/ha	16 ± 0.71^{cd}	2015.0 ± 123.39^{abc}	19 ± 2.4^{c}	2006.6 ± 123.60^{ab}
Spraying 0.5 % potassium chloride during flowering and pod development stages	23 ± 3.52^{bc}	1950.0 ± 101.27^{bc}	25 ± 1.91^{b}	1945 ± 113.75^{ab}
Integrated disease management (IDM) integration of all the above treatments	2 ± 0.45^{f}	2245.6 ± 105.49^{a}	2.9 ± 0.37^{e}	2233.5 ± 54.19^{a}
Farmers practice use of gypsum and carbofuran but no biocontrol application in soil	25 ± 0.90^{b}	2114.3 ± 104.93^{abc}	28 ± 2.16^{b}	2100.3 ± 156.70^{ab}
Control	33 ± 4.81^{a}	1475.0 ± 52.62^{d}	39 ± 3.47^{a}	1470.1 ± 65.06^{c}

Note: In each column, figure followed by same letter do not differ significantly at 5 % level by Tukey's test.

28.4 µg/kg). Another significant factor influencing aflatoxin contamination in field conditions is insect pressure. The experimental plots experienced 2.6 % pod infection by nematodes and borers. Notably, nematode (*Meloidogyne arenaria*) infestation can exacerbate aflatoxin contamination when plants face drought stress during pod maturation. The application of Carbofuran resulted in a significant reduction in aflatoxin contamination in both normal (10.57 µg/kg) and drought (11.11 µg/kg) conditions, compared to the control (26.23 to 28.4 µg/kg).

In normal and drought-imposed conditions, fields with IDM practices recorded the highest pod yield of 2245.6 and 2233.5 kg/ha. In contrast, control plots exhibited the lowest pod yield of 1495 and 1470 kg/ha in normal and drought-imposed fields. In terms of *A. flavus* infection, fields with IDM practice demonstrated the lowest infection rates of 2 and 2.9 % in normal and drought-imposed fields, respectively. Conversely, control plots showed the highest infection rates of 33 and 39 % in normal and drought-imposed fields. Generally, *A. flavus* infection was more prevalent in drought-imposed fields compared to normal conditions (Table 4; Fig. 5).

3.5. Population of *A. flavus* in rhizosphere soil

Notably, in maize fields where biocontrol agents and IDM practices were implemented, there was a significant reduction in the population density of *A. flavus* in rhizosphere soil. The baseline *A. flavus* population in untreated control plots was 23.85×10^3 CFU/g at the time of sowing, which was significantly reduced to 3.56×10^3 CFU/g in biocontrol-treated fields and 3.25×10^3 CFU/g in fields with IDM practices at harvest as shown in (a) of Fig. 6. Statistical analysis confirmed that these reductions were significant at $p \leq 0.05$. For peanuts, at the time of sowing, untreated control soil contained *A. flavus* populations of 3.66 and 3.77 x 10^3 CFU/g in normal and drought-imposed fields,

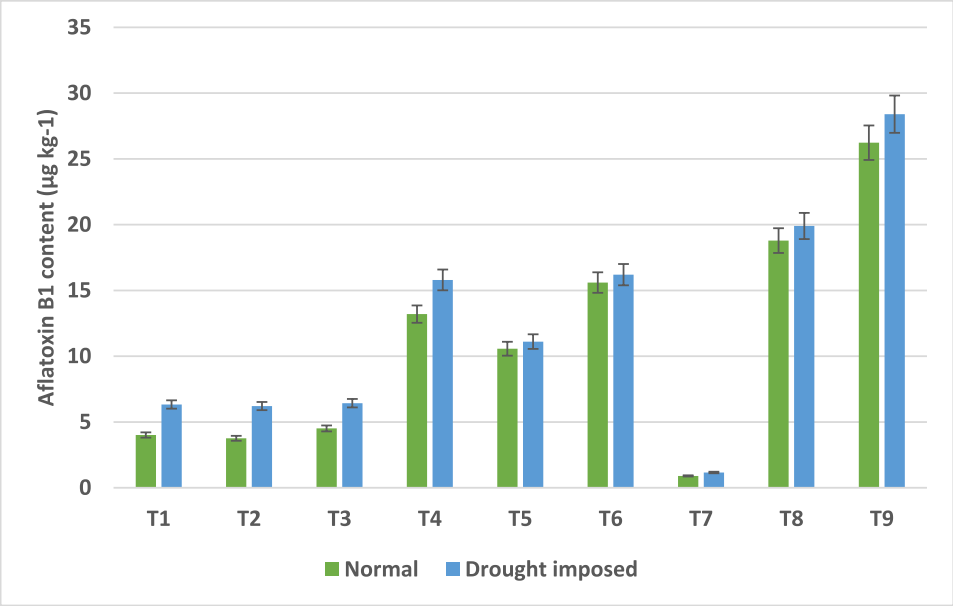

Fig. 5. Effect of cultivation practices for the management of aflatoxin contamination in peanut under field conditions: Treatment (T)1 - Seed treatment (@ 10 g/kg) and soil application (@ 2.5 kg/ha) of *Bacillus subtilis* (Bbv57) at 30, 60 and 90 days after sowing (DAS); T2- Seed treatment of *B. subtilis* + basal application of 2.5 kg/ha of *Trichoderma viride* (Tv) + soil application of 2.5 kg/ha of *B. subtilis* at 30, 60 and 90 DAS; T3- Seed treatment of *B. subtilis* + basal application of Tv + soil application of Tv at 60 and 90 DAS; T4 - Gypsum (calcium sulphate) application @ 400 kg/ha at 45th DAS; T5 - Carbofuran 3 G @ 33 kg/ha; T6 - Spraying 0.5 % potassium chloride during flowering and pod development stages; T7 - Integrated disease management (IDM) integration of all above treatments, T8 - Farmers practice use of gypsum and carbofuran chemicals without any biocontrol; T9 - Control.

respectively. In contrast, fields treated with biocontrol agents had *A. flavus* populations of 3.56 and 3 x 10^3 CFU/g in normal and drought-imposed fields, respectively, at the time of harvest shown in (b) of Fig. 6. The results indicated variations in *A. flavus* populations across different treatments and time points, providing insights into the effectiveness of the integrated agricultural practices in managing *A. flavus*.

3.6. Detoxification of aflatoxins using medicinal plants

The laboratory analysis focused on the aqueous extracts from the leaves or seeds of four medicinal herbs to evaluate their potential to detoxify AFB1, utilizing TLC technique. Among the four plant extracts, the leaf extract of *A. paniculata* demonstrated the most significant degradation of AFB1 after a 24-h incubation at 37 °C. Quantification of AFB1 in the mixture revealed a greater than 96.8 % loss of AFB1, indicating that the aqueous extract of *A. paniculata* was most effective at degrading the toxin, followed by the *A. vasica* extract (93.8 %), *T. ammi* extract (76.6 %), and Zimmu (71.8 %) shown in Fig. 7.

3.7. Postharvest aflatoxin management via plant products and packing for maize and peanut

Maize kernels stored in jute and fertilizer bags offered complete protection against *A. flavus* contamination for up to 6 months under normal conditions (with a moisture level of 9 %), regardless of treatment with the plant product shown in Fig. 8. However, traces of aflatoxin contamination (0.5 µg/kg AFB1) were observed in the control samples after six months of storage in cloth bags, and contamination was detected in the samples stored in open room conditions from the second month onward. Notably, samples treated with *A. paniculata* showed aflatoxin contamination four months after treatment. Under artificially inoculated conditions, no aflatoxin contamination was observed in plant product-treated samples for up to four months, except for samples stored in fertilizer bags, where contamination was observed after two months. In contrast, control samples under both natural and artificial inoculated conditions were found to be contaminated with aflatoxin. Under natural conditions, the aflatoxin levels in maize control samples reached 25.43 µg/kg by the end of the storage period. In peanut control samples, the aflatoxin levels were 26.23 µg/kg under normal conditions and 28.4 µg/kg under drought-imposed conditions. In artificially inoculated control samples, the aflatoxin levels in maize reached 50.27 µg/kg, while in peanuts, the levels were 48.6 µg/kg in normal conditions and 52.3 µg/kg under drought-imposed conditions. Extracts from *A. vasica* were found to be the second most effective product under normal conditions, as all packing methods were free from aflatoxin contamination except for samples placed in open conditions. Under artificially inoculated conditions, aflatoxin contamination was found in the fertilizer bag packing and, in the samples, stored in open conditions. Aqueous extracts of *T. ammi* seeds effectively prevented aflatoxin contamination in maize with different packing materials under normal conditions, with aflatoxin contamination only present in samples stored in open conditions. Under artificially inoculated conditions, the jute bag packing material was free of aflatoxin contamination, and the other packing materials were not contaminated for up to four months. Zimmu plant extracts demonstrated the absence of aflatoxin contamination under normal conditions for up to six months, except for samples placed in open conditions. Under artificially inoculated conditions, aflatoxin contamination was observed in fertilizer bags and samples placed in open conditions, with no contamination in jute bags for up to two months.

These findings highlight the potential of plant products and appropriate packing materials for managing aflatoxin contamination in maize during postharvest storage. Jute bags, followed by fertilizer bags, were effective at preventing contamination, while plant extracts from *A. paniculata*, *A. vasica*, *T. ammi*, and Zimmu demonstrated varying degrees of efficacy at reducing aflatoxin contamination in maize kernels under different storage conditions.

For peanuts, the plant products were applied to peanut kernels at varying moisture content levels of 6.5 % (natural) and 9 % (artificially inoculated conditions) and packed in different materials shown in Fig. 9. Packaging with jute bags and fertilizer bags provided complete protection from fungal contamination for up to five months, whether the kernels were treated with a plant product, in normal conditions. Cloth

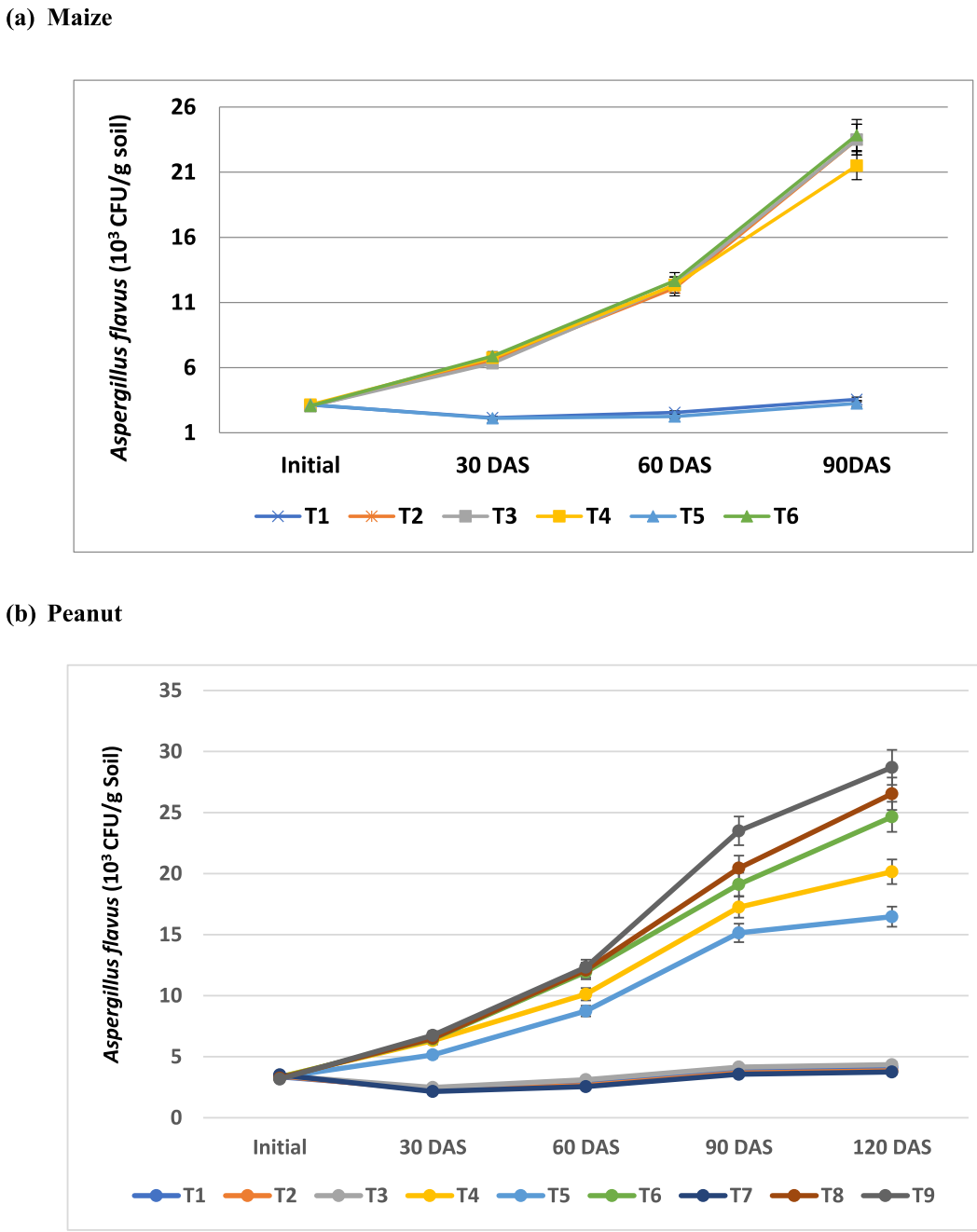

Fig. 6. Soil population of *Aspergillus flavus* at different crop growth stages in the experimental plots. (a) *A. flavus* population in maize field and (b) *A. flavus* population in peanut field. In peanut, T1, T2, T3 and T7 clustered as they had nearly similar *A. flavus* content at each sampling point. CFU: colony forming units, DAS: days after sowing and T: treatments.

bags showed traces of aflatoxin contamination in control samples, and aflatoxin contamination was found in samples stored in open conditions. The *A. vasica*, *A. paniculata*, and Zimmu-treated samples were free from aflatoxin contamination when packed in jute and cloth bags in artificially inoculated conditions. However, samples placed in fertilizer bags and open conditions were contaminated with aflatoxin. Among the different packing materials, jute bags effectively prevented aflatoxin contamination when treated with plant products. In artificially inoculated conditions, traces of infection (below 1 μg/kg, which are negligible and well below the European Union Commission's allowable threshold of 4 μg/kg) were observed in untreated control samples, but they were free from contamination under natural conditions.

4. Discussion

4.1. Aflatoxin contamination in maize and peanut samples

Aflatoxin contamination of maize and peanut in regional markets is a pressing public health concern. Our findings reveal 28 and 32.67 % of maize and peanuts samples, respectively were contaminated in Tamil Nadu, the southernmost state in India, where these two crops play a vital role in human diet and animal feed. These findings align with previous reports on the feed samples from northern India, where AFB1 contents ranged between 0.412 and 0.514 ppm [38–41]. Growth and population increase of *Aspergillus* spp. and subsequent aflatoxin production in maize and peanuts are dependent on environmental factors such as temperature, humidity/moisture, insect damage, and handling during

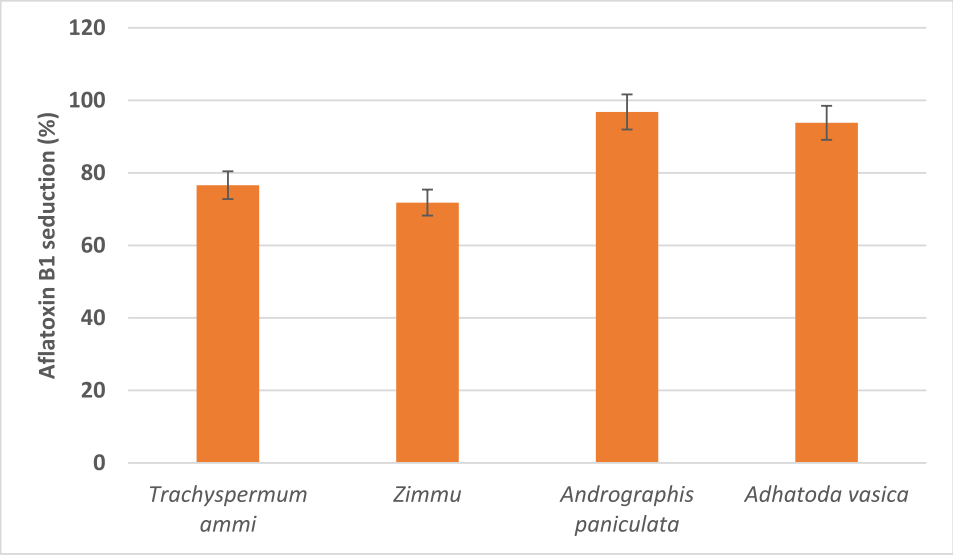

Fig. 7. Detoxification of Aflatoxin B1 by four medicinal plant extracts.

harvesting and storage [42,43]. In support of these findings, an increasing number of maize and peanut samples from Rabi season and long-term storage units were positive for AFB1, indicating the role of moisture and high frequency of rainfall favouring *A. flavus* proliferation and toxin contamination.

4.2. Aflatoxin production by A. flavus isolates

Toxigenic isolates of *A. flavus* produced AFB1 in quantities ranging from 0.85 to 82.73 ng/ml for maize and from 0.32 to 116.02 ng/ml for peanuts, while atoxigenic isolates did not produce detectable levels of AFB1. Out of 100 isolates used in the study, 30 % of the samples were atoxigenic. We recovered a large proportion of contaminated samples similar to the earlier study in Ghana, where the widespread occurrence of toxigenic *A. flavus* isolates observed from maize and peanut samples [44]. Within India, a significant diversity in aflatoxin production was also found among different *A. flavus* isolates from maize [45].

4.3. Preharvest management practices to minimize aflatoxin in maize and peanut

Implementing effective preharvest management practices is a key strategy for mitigating aflatoxin contamination in crops [46]. Among the various treatments tested, the combination of seed treatment with metalaxyl (2 g/kg of seed) and basal application of *T. viride* (2.5 kg/ha) mixed with 50 kg of FYM before planting was particularly effective at reducing aflatoxin contamination. This approach introduces fungal competitors and aligns with the concept that the presence of other fungi can reduce aflatoxin production by *Aspergillus* spp. [47]. The antagonistic effect of fungal species, such as *T. viride* and *Fusarium* sp., on aflatoxin production has also been reported by Narendrakumar and Dhanapani [48], further supporting our findings. Insect management, specifically cob borer control, significantly reduced AFB1 contamination as insect-feeding activity was associated with fungal infection of maize grains and subsequent mycotoxin production [49,50]. Exposing crops to drought during the maturity stage increased aflatoxin contamination which is consistent with the role of adequate moisture in preventing aflatoxin contamination in maize [51]. For peanuts, we tested cultivation practices under normal and drought-imposed conditions where IDM and seed treatment and soil application of biocontrol agents *B. subtilis* and *T. viride* are the best management strategies, as noticed earlier [49,50]. Since damage to peanut pods, either by nematodes or borers, can create opportunities for fungal infection [51,52], insecticides have been found to reduce damage to peanut pods, indirectly reducing aflatoxin levels as observed earlier [53].

4.4. Detoxification of aflatoxins by medicinal plants

Among the Indian medicinal herbs, *A. paniculata* leaf extract demonstrated the most effective degradation of AFB1, with >96.8 and 97.8 % reduction of aflatoxin. The other plant extracts from *Adathoda*, *T. ammi*, and Zimmu also showed varying degrees of AFB1 degradation. These plant extracts suppress the growth of toxigenic fungi and reduce toxin production through antimicrobial and antioxidant activities [54].

4.5. Postharvest management practices to minimize aflatoxin in maize and peanut

In India, low levels of awareness and inadequate postharvest management practices among farmers have been linked to increased *A. flavus* infection in maize and peanut produces [55]. Specifically, farmers and supply chain agents are unaware of the importance of maintaining low moisture levels of < 10 %, which, if exceeded, leads to colonization by aflatoxigenic species and subsequent aflatoxin contamination [56–58]. In our experiments, the storage of maize and peanuts in jute bags effectively minimized aflatoxin contamination compared to other packaging materials. Further, the treatment of maize and peanut produce with *Andrographis* and *Adathoda* extract before storage in jute or other packaging materials such as fertilizer bags has minimized aflatoxin contamination.

5. Conclusion

Aflatoxin contamination in maize and peanut plants is a significant global challenge that affects food safety and public health. Through a comprehensive survey, our study reports a wide presence of aflatoxin contamination at various stages of maize and peanut production and supply chain in the southern part of India. Integrated pre and postharvest management strategies were devised through the integration of seed treatment and soil application of biocontrol agents, packaging types, and treatment of harvest products with plant extracts, which will ensure a continuous supply of healthy maize and peanut products to the human population worldwide.

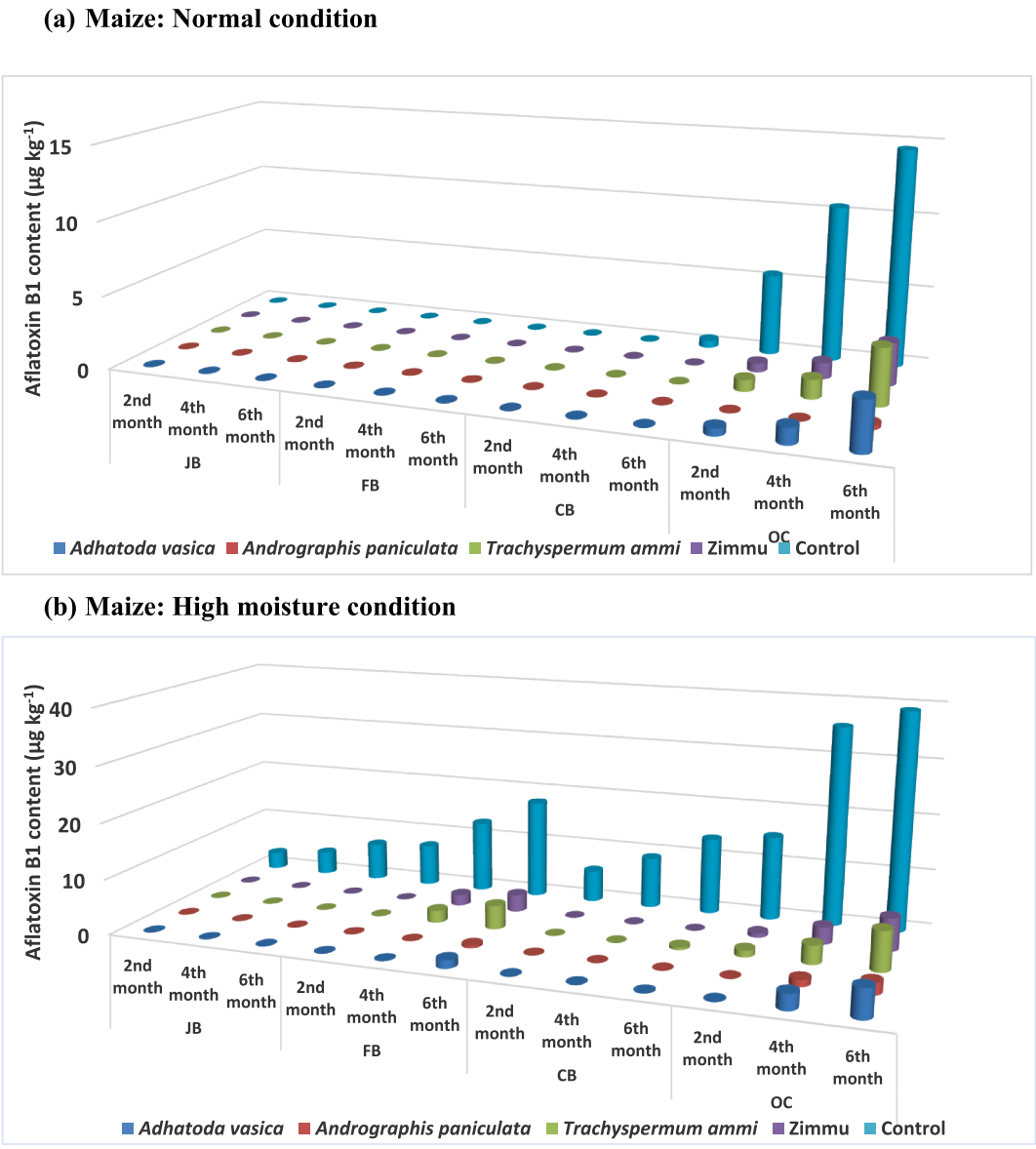

Fig. 8. Postharvest management of aflatoxin contamination in maize by packing and protection methods. (a) Normal condition (grains with moisture content 9 %) and (b) High moisture condition (grains with moisture content 11 %) inoculated with *Aspergillus flavus* spore suspension (10^5 conidia/ml). Packaging material consists of JB: Jute Bag, FB: Fertilizer Bag, CB: Cloth Bag and OC: Open Condition.

CRediT authorship contribution statement

Muthusamy Karthikeyan: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shanmuga Priya Dhanabalan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bavish Shanmugavel:** Data curation, Formal analysis, Writing – review & editing. **Shajith Basha Jaffer:** Writing – review & editing, Formal analysis, Data curation. **Subbaiyan Marimuthu:** Writing – review & editing, Formal analysis, Data curation. **Krishnan Radhika:** Writing – review & editing, Formal analysis, Data curation. **Elakkiya Nagappan:** Data curation, Formal analysis, Writing – review & editing. **Iruthayasamy Johnson:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sambasivam Periyannan:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Consent for publication

Not applicable.

Availability of data and materials

Seeds of plant materials used in this study are available from the corresponding author by request.

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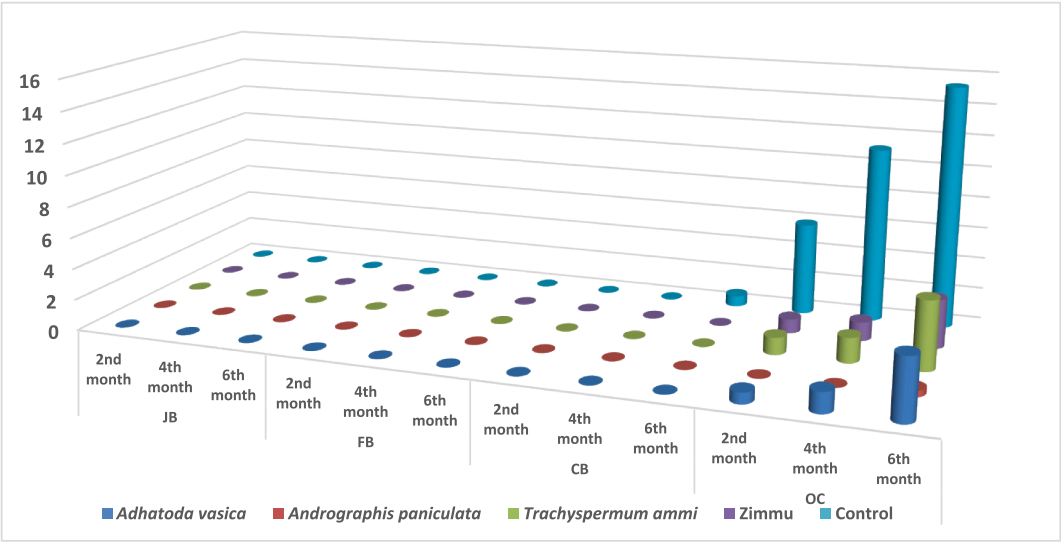

(a) Peanut: High moisture condition

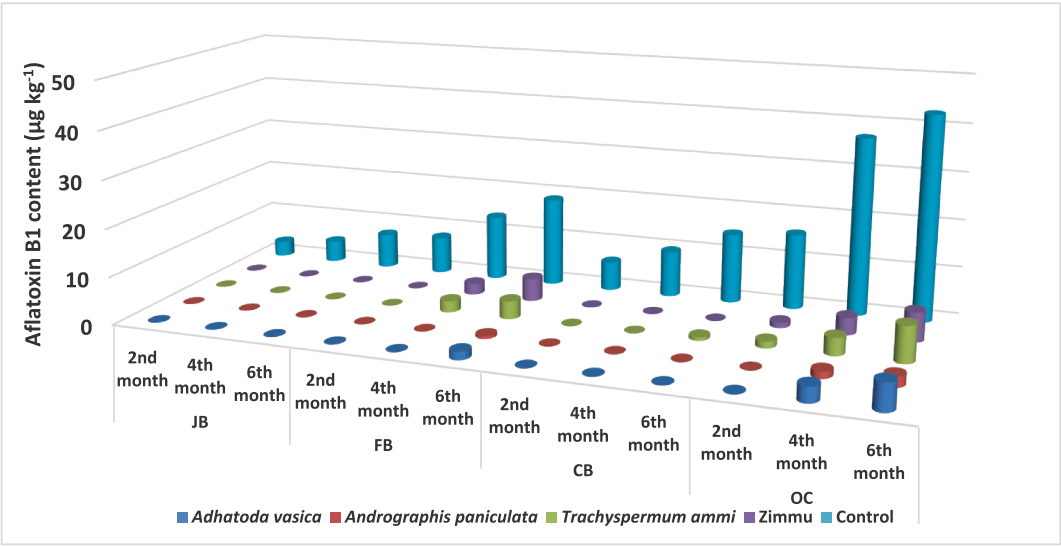

Fig. 9. Postharvest management of aflatoxin contamination in peanut by packing and protection methods. (a) Normal condition (pods with moisture content 6.5 %) and (b) High moisture condition (pods with moisture content 9 %) inoculated with *Aspergillus flavus* spore suspension (10^5 conidia/ml). Packaging material consists of JB: Jute Bag, FB: Fertilizer Bag, CB: Cloth Bag and OC: Open Condition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

[1] FAOSTAT, Crops Statistics - Concepts Definitions and Classifications, FAO Statistics Division ESS, Rome, 2021.
[2] G. Govindaraj, G.D. Satish Kumar, M.S. Basu, Benefits of improved groundnut technologies to resource-poor farmers: a participatory approach, Agric. Econ. Res. Rev. 22 (2009) 355–360, https://doi.org/10.22004/ag.econ.57478.
[3] H.D. Upadhyaya, L.J. Reddy, C.L. Gowda, S. Singh, Identification of diverse groundnut germplasm: sources of early maturity in a core collection, Field Crops Res. 97 (2–3) (2006) 261–271, https://doi.org/10.1016/j.fcr.2005.10.010.
[4] J.Y. Asibuo, R. Akromah, O. Safo-Kantanka, H.K. Adu-Dapaah, S. Ohemeng-Dapaah, A. Agyeman, Chemical composition of groundnut *Arachis hypogaea* (L) landraces, Afr. J. Biotechnol. 7 (13) (2008) 2203–2208, https://doi.org/10.5897/AJB08.113.
[5] K. Suchoszek-Lukaniuk, A. Jaromin, M. Korycinska, A. Kozubek, Health benefits of peanut (*Arachis hypogaea* L,) seeds and peanut oil consumption, in: V.R. Preedy, R. R. Watson, V.B. Patel (Eds.), Nuts and Seeds in Health and Disease Prevention, 2011, pp. 873–880, https://doi.org/10.1016/B978-0-12-375688-6.10103-3.
[6] L.L. Unnevehr, D. Grace, Aflatoxins: finding solutions for improved food safety. 2020 Vision Focus, International Food Policy Research Institute, USA, 2013, https://doi.org/10.2499/9780896296763.
[7] B.R. Rushing, M.I. Selim, Aflatoxin B1: a review on metabolism toxicity occurrence in food occupational exposure and detoxification methods, Food Chem. Toxicol. 124 (2019) 81–100, https://doi.org/10.1016/j.fct.2018.11.047.

[8] C.P. Wild, P.C. Turner, The toxicology of aflatoxins as a basis for public health decisions, Mutagenesis 17 (6) (2002) 471–481, https://doi.org/10.1093/mutage/17.6.471.

[9] A. Vijayasamundeeswari, M. Mohankumar, M. Karthikeyan, S. Vijayanandraj, V. Paranidharan, R. Velazhahan, Prevalence of aflatoxin B1 contamination in pre and postharvest maize kernels food products poultry and livestock feeds in Tamil Nadu, Indian, J. Plant Prot. 49 (2) (2009) 221–224, https://doi.org/10.2478/v10045-009-0033-1.

[10] F. Waliyar, S.V. Reddy, K. Subramaniam, T.Y. Reddy, K.R. Devi, P.Q. Craufurd, T. R. Wheeler, Importance of mycotoxins in food and feed in India, Asp, Appl. Biol. 68 (2003) 147–154. https://oar.icrisat.org/1320/1/AspectsAppBiol68_147-154-2003.pdf.

[11] S. Vasanthi, R.V. Bhat, Mycotoxins in foods - occurrence health & economic significance & food control measures, Indian J. Med. Res. 108 (1998) 212–224.

[12] D. Ferrigo, M. Mondin, C. Scopel, E.D. Maso, M. Stefenatti, A. Raiola, R. Causin, Effects of a prothioconazole- and tebuconazole-based fungicide on *Aspergillus flavous* development under laboratory and field conditions, Europ. J. Plant Pathol 155 (2019) 151–161, https://doi.org/10.1007/s10658-019-01757-4.

[13] R. Mann, H.J. Rehm, Degradation products from aflatoxin B1 by *Corynebacterium rubrum Aspergillus niger Trichoderma viride* and Mucorambigus, Eur. J. Appl. Microbiol. 2 (1976) 297–306, https://doi.org/10.1007/BF01278613.

[14] A. Nazhand, A. Durazzo, M. Lucarini, E.B. Souto, A. Santini, Characteristics occurrence detection and detoxification of aflatoxins in foods and feeds, Foods 9 (5) (2020) 644, https://doi.org/10.3390/foods9050644.

[15] H. Rastegar, S. Shoeibi, H. Yazdanpanah, M. Amirahmadi, A.M. Khaneghah, B. Campagnollo, A.S. Santana, Removal of aflatoxin B1 by roasting with lemon juice and/or citric acid in contaminated pistachio nuts, Food Con. 71 (2017) 279–284, https://doi.org/10.1016/j.foodcont.2016.06.045.

[16] K.F. Wochner, M.C.C. Moreira, D.L. Kalschne, E. Colla, D.A. Drunkler, Detoxification of Aflatoxin B1 and M1 by *Lactobacillus acidophilus* and prebiotics in whole cow's milk, J. Food Saf. 39 (5) (2019) e12670, https://doi.org/10.1111/jfs.12670.

[17] J.W. Dorner, R.J. Cole, W.J. Connick, D.J. Daigle, M.R. McGuire, B.S. Shasha, Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts, Biol. Control 26 (3) (2003) 318–324, https://doi.org/10.1016/S1049-9644(02)00139-1.

[18] A. Sadeghi, M. Ebrahimi, M. Raeisi, Z. Nematollahi, Biological control of foodborne pathogens and aflatoxins by selected probiotic LAB isolated from rice bran sourdough, Biol. Con. 130 (2019) 70–79, https://doi.org/10.1016/j.biocontrol.2018.09.017.

[19] F.A. Sadiq, B. Yan, F. Tian, J. Zhao, H. Zhang, W. Chen, Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: a comprehensive review, Compr. Rev. Food Sci. Food Saf. 18 (5) (2019) 1403–1436, https://doi.org/10.1111/1541-4337.12481.

[20] K. Markov, B. Mihaljevic, A.M. Domijan, J. Pleadin, F. Delas, J. Frece, Inactivation of aflatoxigenic fungi and the reduction of aflatoxin B1 in vitro and in situ using gamma irradiation, Food Con. 54 (2015) 79–85, https://doi.org/10.1016/j.foodcont.2015.01.036.

[21] T.D. Phillips, L.F. Kubena, R.B. Harvey, D.R. Taylor, N.D. Heidelbaugh, Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin, Poul. Sci. 67 (2) (1988) 243–247, https://doi.org/10.3382/ps.0670243.

[22] C. Hontanaya, G. Meca, F.B. Luciano, J. Manes, G. Font, Inhibition of aflatoxin B1 B2 G1 and G2 production by *Aspergillus parasiticus* in nuts using yellow and oriental mustard flours, Food Con 47 (2015) 154–160, https://doi.org/10.1016/j.foodcont.2014.07.008.

[23] P.A. Paranagama, K.H.T. Abeysekera, K.P. Abeywickrama, L. Nugaliyadde, Fungicidal and anti-aflatoxigenic effects of the essential oil of Cymbopogon citrates (DC) Staps, (lemongrass) against *Aspergillus flavus* link isolated from stored rice, Lett. Appl. Microbiol. 37 (1) (2003) 86–90, https://doi.org/10.1046/j.1472-765x.2003.01351.x.

[24] R. Sandosskumar, M. Karthikeyan, S. Mathiyazhagan, M. Mohankumar, G. Chandrasekar, R. Velazhahan, Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B 1 by the medicinal plant zimmu *Allium sativum* L,× *Allium cepa* L, World J. Microbiol. Biotechnol. 23 (2007) 1007–1014, https://doi.org/10.1007/s11274-006-9327-x.

[25] M. Yin, W. Cheng, Inhibitory of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices, J. Food Prot. 61 (1) (1998) 123–125, https://doi.org/10.4315/0362-028x-61.1.123.

[26] A. Durazzo, E. D'Addezio L Camilli, R. Piccinelli, A. Turrini, From plant compounds to botanicals and back: a current snapshot, Molecules 23 (8) (2018) 1844, https://doi.org/10.3390/molecules23081844.

[27] S.S. Hajare, S.N. Hajare, A. Sharma, Aflatoxin inactivation using aqueous extract of ajowan *Trachyspermum ammi* seeds, J. Food Sci. 70 (1) (2005) 29–34, https://doi.org/10.1111/j.1365-2621.2005.tb09016.x.

[28] B. Ponzilacqua, G.E. Rottinghaus, B.R. Landers, C.A.F. Oliveira, Effects of medicinal herb and Brazilian traditional plant extracts on in vitro mycotoxin decontamination, Food Con 100 (2019) 24–27, https://doi.org/10.1016/j.foodcont.2019.01.009.

[29] D. Sapcota, R. Islam, A.K. Medhi, Efficacy of *Andrographis paniculata* in ameliorating aflatoxicosis in broilers, Indian J. Vet. 82 (5) (2005) 529–532.

[30] R. Velazhahan, S. Vijayanandraj, A. Vijayasamundeeswari, V. Paranidharan, R. Samiyappan, Detoxification of aflatoxins by seed extracts of the medicinal plant *Trachyspermum ammi* L, *Sprague* ex Turrill–structural analysis and biological toxicity of degradation product of aflatoxin G1, Food Con 21 (5) (2010) 719–725, https://doi.org/10.1016/j.foodcont.2009.10.014.

[31] R. Velazhahan, Bioprospecting of medicinal plants for detoxification of aflatoxins, Int. J. Nutr. Pharmacol. Neurol. Dis. 7 (3) (2017) 60–63.

[32] S. Vijayanandraj, R. Brinda, K. Kannan, R. Adhithya, S. Vinothini, Detoxification of aflatoxin B1 by an aqueous extract from leaves of *Adhatoda vasica* Nees, Microbiol. Res. 169 (4) (2014) 294–300, https://doi.org/10.1016/j.micres.2013.07.008.

[33] G. Vaamonde, A. Patriarca, V.E.F. Pinto, Effect of water activity and temperature on production of aflatoxin and cyclopiazonic acid by *Aspergillus flavus* in peanuts, in: Advances in Food Mycology, Springer US, Boston MA, 2006, pp. 225–235, https://doi.org/10.1007/0-387-28391-9_15.

[34] R.O. Okayo, D.O. Andika, M.M. Dida, G.O. K'Otuto, D.M. Gichimu, Morphological and molecular characterization of toxigenic *Aspergillus flavus* from groundnut kernels in Kenya, Intl. J. Microbiol (2020) 8854718, https://doi.org/10.1155/2020/8854718.

[35] S.V. Reddy, D. Kiran Mayi, U. UmaReddy, K. Thirumala Devi, D.V.R. Reddy, Aflatoxins B_1 in different grades of chillies (Capsicum annum L,) in India as determined by indirect competitive-ELISA, Food Addit. Cont. 18 (6) (2001) 553–558, https://doi.org/10.1080/02652030119491.

[36] S. Nahdi, Drought stress and preharvest seed invasion of selected groundnut genotypes by *Aspergillus flavus* and aflatoxin contamination, Indian Phytopathol. 49 (1) (1996) 52–56.

[37] F. Waliyar, H. Hassan, S. Bonkoungou, Sources of resistance to *Aspergillus flavus* and aflatoxin contamination in groundnut genotypes in West Africa, Plant Dis. 78 (7) (1994) 704–708.

[38] T.K. Dutta, P. Das, Isolation of aflatoxigenic strains of *Aspergillus* and detection of aflatoxin B 1 from feeds in India, Mycopathologia 151 (2001) 29–33, https://doi.org/10.1023/a:1010960402254.

[39] A.S. Dhavan, M.R. Choudary, Incidence of aflatoxins in animal feedstuffs: a decade's scenario in India, J. AOAC Int. 78 (3) (1995) 693–697, https://doi.org/10.1093/jaoac/78.3.693.

[40] P.S. Reddy, C.V. Reddy, V.R. Reddy, P.V. Rao, Occurrence of aflatoxin in some feed ingredients in three geographical regions of Andhra Pradesh, Indian J. Ani. Sci. 54 (1984) 235–238.

[41] T. Singh, R.P.S. Tyagi, B. Ram, Occurrence of aflatoxin B1 in animal and poultry feeds, Indian J. Dairy Sci. 37 (1984) 167–169, https://doi.org/10.5829/idosi.aejts.2015.7.4.10172.

[42] K. Hell, K.F. Cardwell, M. Setamou, F. Schulthess, Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin, Afr. Entomol. 8 (2) (2000) 169–177.

[43] A.O. Oyebanji, B.J. Efiuvwevwere, Growth of spoilage mold and aflatoxin B1 production in naturally contaminated or artificially inoculated maize as influenced by moisture content under ambient tropical condition, Int. Biodeterior, Biodegradation 44 (1999) 209–217.

[44] D. Agbetiameh, A. Ortega-Beltran, R.T. Awuah, J. Atenkeng, P.J. Cotty, R. Bandyopadhyay, Prevalence of Aflatoxin contamination in maize and groundnut in Ghana: population structure, distribution, and toxigenicity of the causal agents, Plant Dis. 102 (4) (2018) 764–772, https://doi.org/10.1094/PDIS-05-17-0749-RE.

[45] M. Karthikeyan, R. Sandosskumar, S. Mathiyazhagan, M. Mohankumar, V. Valluvaparidasan, Genetic variability and aflatoxigenic potential of *Aspergillus flavus* isolates from maize, Arch. Phytopathol. Plant Prot 42 (1) (2009) 83–91, https://doi.org/10.1080/03235400600950961.

[46] J.A. Beti, T.W. Phillips, E.B. Smalley, Effects of maize weevils Coleoptera: Curculionidae on production of aflatoxin B1 by *Aspergillus flavus* in stored corn, J. Econ. Entomol. 88 (6) (1995) 1776–1782, https://doi.org/10.1093/jee/88.6.1776.

[47] W.J. Drepper, B.L. Renfro, Comparison of methods for inoculation of ears and stalks of maize with *Fusarium moniliforme*, Plant Dis. 74 (12) (1990) 952–956.

[48] R. Jones, H. Duncan, P. Hamilton, Planting date harvest date and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn, Phytopathol 71 (1981) 810–816, https://doi.org/10.1094/Phyto-71-810.

[49] V. Anjaiah, R.P. Thakur, N. Koedam, Evaluation of bacteria and Trichoderma for biocontrol of pre-harvest seed infection by *Aspergillus flavus* in groundnut, Biocon. Sci. Tech. 16 (4) (2006) 431–436, https://doi.org/10.1080/09583150500532337.

[50] K.R.N. Reddy, C.S. Reddy, K. Muralidharan, Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains, Food Con. 20 (2) (2009) 173–178, https://doi.org/10.1016/j.foodcont.2008.03.009.

[51] S.B. Sharma, F. Waliyar, P. Subramanyam, B.J. Ndunguru, Role of *Scutellonema clathricaudatum* in etiology of groundnut growth variability in Niger, Plant Soil 143 (1992) 133–139, https://doi.org/10.1007/BF00009138.

[52] F. Waliyar, B.J. Ndunguru, S.B. Sharma, A. Bationo, Effect of liming and carbofuran on groundnut yield in sandy soils in Niger, Fert. Res. 33 (1992) 203–208, https://doi.org/10.1007/BF01050875.

[53] R.E. Lynch, D.M. Wilson, Enhanced infection of peanut *Arachis hypogaea* L, seeds with *Aspergillus flavus* group fungi due to External scarification of peanut pods by the lesser cornstalk borer *Elasmopalpus lignosellus* (zeller), Peanut Sci. 18 (2) (1991) 110–116.

[54] G.K. Jayaprakasha, L. Jagan Mohan Rao, K.K. Sakariah, Improved HPLC method for the determination of curcumin demethoxycurcumin and bisdemethoxycurcumin, J. Agric. Food Chem. 50 (13) (2002) 3668–3672, https://doi.org/10.1021/jf025506a.

[55] G.D. Kumar, M.N. Popat, Farmers' perceptions knowledge and management of aflatoxins in groundnuts (*Arachis hypogaea* L,) in India, Crop Prot. 29 (12) (2010) 1534–1541, https://doi.org/10.1016/j.cropro.2010.08.019.

[56] A.N. Kaaya, W. Kyamuhangire, The effect of storage time and agroecological zone on mold incidence and aflatoxin contamination of maize from traders in Uganda,

Int. J. Food Microbiol. 110 (3) (2006) 217–223, https://doi.org/10.1016/j.ijfoodmicro.2006.04.004.

[57] Y.Q. Wen, L.L. Xu, C.H. Xue, X.M. Jiang, Effect of stored humidity and initial moisture content on the qualities and mycotoxin levels of maize germ and its processing products, Toxins 12 (9) (2020) 535, https://doi.org/10.3390/toxins12090535.

[58] E. Garcia-Cela, F.J. Gari Sanchez, M. Sulyok, C. Verheecke-Vaessen, A. Medina, R. Krska, N. Magan, Carbon dioxide production as an indicator of *Aspergillus flavus* colonisation and aflatoxins/cyclopiazonic acid contamination in shelled peanuts stored under different interacting abiotic factors, Fungal Biol. 124 (1) (2020) 1–7, https://doi.org/10.1016/j.funbio.2019.10.003.