2023-12-07

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https://dx.doi.org/10.1163/18750796-20222830

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WORLD MYCOTOXIN JOURNAL 16 (2023) 301–315

World
Mycotoxin
Journal
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RESEARCH ARTICLE

Effectiveness of complementary sorting methods in reducing aflatoxin contamination in groundnuts

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Received 23 December 2022 | Accepted 26 July 2023 | Published online 12 October 2023 |
Published in print 7 December 2023

Abstract

Aflatoxin contamination of staple foods remains a public health concern in many tropical and sub-tropical countries. In sub-Saharan Africa, groundnuts are a significant source of aflatoxin (AF) in vulnerable populations such as infants and young children. However, there are limited scalable and affordable technological interventions to reduce the risk of aflatoxin ingestion in low and middle-income contexts. This study compared the effectiveness of complementary sorting methods in reducing AF contamination, time taken, and percentage loss of groundnuts. The study also evaluated bulk density and kernel weight as proxies for AF. Groundnuts were sampled from 19 bags at a medium-scale enterprise in Tanzania (Halisi) that processes cereal-based blended flours for complementary feeding. The samples were subjected to six sorting methods: (1) size (S) sorting, which yielded large and small fractions (n = 38); (2) density (D) sorting, which yielded heavy and light fractions (n = 38), (3) visual (V) sorting, (4) the combination of size and visual (SV), (5) the combination of density and visual (DV), and (6) the combination of size, density, and visual (SDV) which yielded grades 1, 2, 3, and 4 (n = 76). Samples of unsorted groundnuts and grades from all six sorting regimes (n = 418) were analysed for total aflatoxin by enzyme-linked immunosorbent assay. Analysis of variance (ANOVA) at a 5% significance level was used to compare AF reduction efficiency. Aflatoxin levels were reduced by 99% for the highest grade (G1) by the SDV sorting method. The SDV sorting method was the most effective in reducing AF contamination by removing 14% outsort (Grade 4) from 1 kg groundnut within 22 min. Bulk density and 100 kernels weight were inversely associated with AF, indicating their value as AF proxies. Scaling up such low-cost sorting methods can significantly reduce AF along the value chain and improve food safety.

Keywords

aflatoxin – sorting methods – aflatoxin reduction – groundnut contamination

1 Introduction

After soya beans, groundnuts (*Arachis hypogaea*) are the second most important commodity for the production of oil seeds, the thirteenth-most important crop for human consumption, and the third-most essential crop for the production of edible vegetable oil globally (Taphee *et al*., 2015; Upadhyaya and Dwivedi, 2015). The groundnut seed is high in nutrients and contains substantial amounts of high-quality vegetable oil

Published with license by Koninklijke Brill NV | DOI: 10.1163/18750796-20222830
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(48-50%), protein (26-28%), minerals (Ca, P, Mg, Zn, and Fe), dietary fibre, and vitamins (E, K, and B complex) (Pasupuleti and Nigam, 2013). Around the globe, groundnuts are grown in more than a hundred different tropical, subtropical, and warm temperate regions (Upadhyaya *et al.*, 2012). Despite having minimal input, low output, and limited market access, groundnut farming in East Africa is still essential for low-income communities due to its nutritional benefits (Carr, 2001).

Tanzania produced 690,000 tons of groundnuts 2020, accounting for 4% of Africa's production and 1.29% of the world's total output (Hatibu *et al.*, 2022). Tanzania's primary producing areas for groundnuts are Mtwara, Songwe, Dodoma, Shinyanga, Geita, Singida, Kigoma, and Tabora. About 14 million people in these areas and across the nation benefit directly and indirectly from groundnut production (Akpo *et al.*, 2021; URT, 2017).

The high nutritive value of groundnuts makes them an ideal substrate for microbial and fungal growth. Species within *Aspergillus* section *Flavi* species, such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, are reported to spoil groundnuts and contaminate them with mycotoxins, including aflatoxins (Mupunga *et al.*, 2017). Four important forms of aflatoxin (AF) exist; aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2). The most common and toxic of the four types is AFB_1 (Mughal *et al.*, 2017; Nugraha *et al.*, 2018; Shan, 2020). Ingestion of a high dose of AF can cause acute aflatoxicosis. In contrast, long-term low to moderate doses intake can result in liver cancer, hepatotoxicity, immune suppression, low birth weight, and child stunting (Bray *et al.*, 2018; Gong *et al.*, 2016; Pierron *et al.*, 2016). The International Agency for Research on Cancer (IARC) classified AF as a class 1 liver carcinogenic agent due to evidence of carcinogenicity in humans (IARC, 1993). Globally, up to 28.2% of annual liver cancer cases are linked to aflatoxin exposure (Kimanya *et al.*, 2021). Additionally, adverse economic effects result because the market value of the affected crops declines due to AF contamination. According to studies by Gbashi *et al.* (2018) and N'dede *et al.* (2012), AF-contaminated products are rejected by the regulatory authority at the exit, provided a lower price, and consequently forced into an alternate usage (such as feed). They also suffer more significant sorting losses.

Aflatoxin usually contaminates food crops in the field, harvest, storage, and during processing (Turner *et al.*, 2009; Winter and Pereg, 2019). In sub-Saharan Africa, aflatoxin contamination is common due to favourable agro-climatic conditions, virulent fungi, poor agronomic practices, and substandard post-harvest practices and processing (Milićević *et al.*, 2019). Aflatoxin contamination of crops costs Africa USD 750 million each year (Gbashi *et al.*, 2018). Globally mycotoxins contaminate up to 25% of human food staples (Moretti *et al.*, 2017; Turner *et al.*, 2012). Crops susceptible to mycotoxins include oilseeds, cereals, dried root crops, fruits, and coffee (Kimanya *et al.*, 2018). Lack of food safety capacity, limited awareness, and regulation across the value chains, especially for groundnuts and maize, expose humans and animals to contamination (Gbashi *et al.*, 2018). In 1981, Ngindu *et al.* (1982) reported an acute aflatoxicosis outbreak in Kenya. In 2001 12 people in the Meru district of Kenya died from consuming AF-contaminated maize that had been stored improperly (Shephard, 2003). In 2004, another 125 deaths were reported in Kenya due to an outbreak of acute aflatoxicosis caused by maize consumption (Probst *et al.*, 2007). A rapid epidemiological survey conducted in two districts (Chemba and Kondoa Dc) of Dodoma, Tanzania 2016 also reported an outbreak of acute aflatoxicosis. Among 68 cases reported, 20 died due to ingesting aflatoxin-contaminated maize (stiff porridge) (Kamala *et al.*, 2018).

A popular topic among researchers has been searching for an efficient method to lower AF to a permissible regulatory limit. Comparatively to AF degradation by chemicals, physical processes, including visual, screening, density, roasting, dehulling, winnowing, and decortication, can reduce AF contamination while keeping the quality and making the kernels harmless to humans and animals.

This study was conducted in the context of the larger Mycotoxins Mitigation Trial (MMT), a cluster randomised control trial in Kongwa District, Tanzania. The blending of cereal and groundnuts in preparation of porridge flour for infants and young children (IYC) is common in Kongwa (Kassim *et al.*, 2023; Mollay *et al.*, 2021; Phillips *et al.*, 2021). The most common ratio of maize to groundnuts was 4:1 (Mollay *et al.*, 2021). The MMT provided low-AF blended flour as an intervention to reduce AF exposure in IYC. Initially, the low-AF flour was processed in partnership with a small-medium scale processor. Visual sorting of groundnuts (*Pendo* variety) to reduce AF was done. This turned out to be very laborious and time-consuming. Size screening was introduced before visual sorting to improve speed and efficiency. Previous maize sorting efforts by MMT investigators found that density sorting using a simple technical device (DropSort) (Nelson, 2016) was effective at separating maize into two fractions (heavy and

FIGURE 1 Grading of groundnuts during visual sorting at Halisi product limited.

light) based on density, whereby the heavier fraction was significantly lower in fumonisin (Ngure *et al.*, 2021; Stafstrom *et al.*, 2021). groundnuts' size and density sorting has been tested in a laboratory setting (Aoun *et al.*, 2020). However, the effectiveness of these methods has not been systematically explored as part of routine food processing in a small- to medium-scale enterprise.

This study aimed to test whether combining multiple sorting strategies would reduce AF more effectively than visual sorting alone. The time needed to sort and the percentage of groundnuts lost were measured for each or a combination of methods. The physical characteristics of groundnuts connected to AF that may serve as substitutes to increase sorting effectiveness were also investigated.

2 Materials and methods

Sampling scheme

The groundnut samples were collected from Halisi Product Limited (Halisi), a small-medium enterprise (SME) food processor in Arusha, Tanzania. The groundnut samples with moisture content <8% (tested using Dickey-John moisture tester, Handheld-GAC Mini-Series) were stored in propylene bags in a well-ventilated and fumigated warehouse.

200 bags of 100 kg each were procured in two batches of 100 bags each. 19 bags were randomly selected from the two batches for the sorting experiment. Ten bags came from the first batch and 9 from the second. For this case, one bag of 100 kg was considered as the sampling unit. To ensure each part of the bag had an equal chance of being drawn as a sample, the bag was emptied onto a tarpaulin carpet (8 × 4 square feet), spread into a rectangular layout and subdivided into quadrants. One kg of small sub-samples was drawn by hand from each quadrant 20-25 times and mixed to make a 4 kg aggregated sample. The sampling was done in triplicate for each sorting experiment.

Sorting methods

Visual sorting

Three trained graders were given different samples (two graders given six samples each and one given seven interchangeably in each sorting method, n = 19) of 4 kg each in triplicate and tasked to sort the groundnuts into four grades (G1, G2, G3, and G4) (Figure 1). Grade

FIGURE 2 Images showing the physical characteristics of grades obtained through visual sorting. (A) Sorted grade 1, (B) grade 2, (C) grade 3 and (D) grade 4.

one (G1) was identified as full smooth (non-shrivelled) kernels with uniform natural tan-brown colour and no discolouration (Figure 2A). Grade two (G2) were large, low-grade shrivelled kernels with a typical colour and no discolouration (Figure 2B). Grade three (G3) were large and small, highly shrivelled kernels with typical colour and discolouration (Figure 2C). Grade four (G4) kernels were considered unsafe for human and animal consumption or loss, as they were discoloured, had dark spots, mould (whitish, greenish), any unnatural colour, insect damage, or germinated (Figure 2D).

Each sample was sorted by a different grader and double-checked by a second-grader to reduce grader bias. The time each grader took to sort 1 kg of groundnuts was recorded to determine the time taken for each sample in triplicate. The weight of each grade was recorded at the end of each sorting activity. The same grades from the triplicates were combined, mixed, and sub-samples (700 g) from each grade were taken to the laboratory for aflatoxin analysis.

Size sorting

Size sorting using a robust screen (0.75 cm to 0.79 cm) was done for 4 kg groundnut samples collected in triplicate (n = 19) (Figure 3). Four kg samples were subjected to the screen to separate small (S) and large (L) groundnut kernels. The time used and the percentage weight of S and L fractions were recorded. The same fraction (S or L) from each triplicate was combined and mixed to obtain two sub-samples (n = 19) of 700 g each for AF testing.

Density sorting

Density sorting was done for 19 samples in triplicates to yield heavier (H) (0.314-0.376 g) and light (L) (0.255-0.344 g) fractions. Density sorting was conducted using a DropSort device (The Widget Factory, Ithaca, NY, USA) with a Grizzly G0710 1 hp blower, flow rate of 537 feet^3/min (Grizzly Industrial®, Bellingham, WA, USA) (Stafstrom *et al.*, 2021) (Figure 4) developed by John Fuchs (Nelson, 2016). The device creates a vacuum that separates small, light, and low-density groundnut

FIGURE 3 Sorting on the screening device at Halisi product limited.

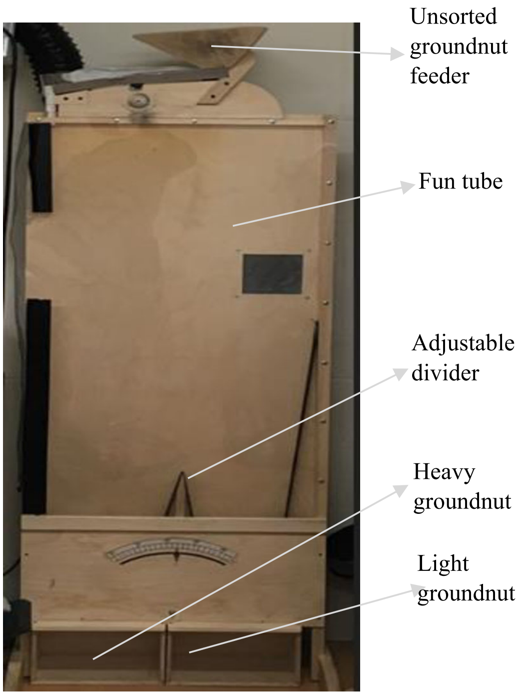

FIGURE 4 DropSort device used for density sorting of groundnuts at Halisi.

kernels from heavier ones as the groundnuts are fed through a feeder that allows a consistent stream of groundnuts to fall through (Stafstrom *et al.*, 2021).

One kg of groundnut was fed into the feeder, and heavier groundnuts fell disproportionately into the accept bin while the lighter kernels fell into the reject bin. The bins were separated by a lever that was adjusted after several calibration experiments for each batch of groundnuts. The H fraction was run through the DropSort device an extra two times to ensure the L fraction was separated. The time spent running each sample was recorded. Each fraction's weight, bulk density (BD), and hundred kernel weight (100 KW) were also recorded.

Combined size and visual sorting

From 19 samples, four kg in triplicate were submitted to size screening, as explained in the section 'Size sorting' to separate into large (L) and small (S) groundnut kernels. The two fractions (L and S) were subjected to visual sorting to produce Large (G1, G2, G3 and G4) and Small (G1, G3 and G4) based on grades characteristics explained in the section 'Visual sorting'. The amount of time spent per kg groundnuts and the percentage of each grade's weight were noted. For the AF test, identical grades of large and small groundnut from various replicates of the same sample were merged.

Combined density and visual sorting

Groundnut samples (n = 19) collected in triplicate were subjected to a DropSort device (Figure 4) to yield Heavier (H) and Light (L) kernels. This was followed by visual sorting to yield H (G1, G2, G3 and G4) and L (G1, G2, G3, and G4). The total amount of time needed to separate 1 kg by density and then visually was recorded. Additionally, the weights of the final four grades were noted. Similar grades from heavy and light of the same sample were merged for the AF test.

Combined size, density, and visual sorting

Four kg in triplicate were submitted to size screening, as explained in the section on 'Size sorting', to separate into large (L) and small (S) groundnut kernels (n = 19). The two fractions (L and S) were subjected to density

TABLE 1 Summary of sample size for aflatoxin (AF) test from each sorting experiment (n = 19)[1]

SN	Experiments	Grades per experiment	Subtotal in 19 bags
1	Visual sorting	4 (Grades 1, 2, 3, and 4)	76
2	Density sorting	2 (Heavy and light)	38
3	Size screening	2 (Large and small)	38
4	Size screening + visual sorting	4 (Grades 1, 2, 3, and 4)	76
5	Density + visual sorting	4 (Grades 1, 2, 3, and 4)	76
6	Size screening + density + visual	4 (Grades 1, 2, 3, and 4)	76
	Total	20	380

[1] Unsorted groundnuts (n = 38) were also included to determine the initial AF status before sorting.

sorting to yield four fractions (Large heavy (Lh), large light (Ll), Small heavy (Sh) and small light (Sl)). This was followed by visual sorting to get grades from each fraction based on characteristics explained in the section on 'Visual sorting'. (Lh-G1, G2, G3, and G4), (Ll-G1, G2, G3, and G4), (Sh-G1, G3, and G4) and (Sl-G1, G3, and G4). The total time spent per kg groundnuts and each grade's weight percentage were noted. For the AF test, identical grades of Lh, Ll, Sh, and Sl from various replicates of the same sample were merged.

A total of 418 sub-samples (380 grades (Table 1), and 38 unsorted groundnuts) were collected from the study site and shipped to the Nelson Mandela African Institution of Science and Technology Laboratory (NM-AIST Lab), Arusha, Tanzania for AF analysis. All samples were kept in a −40 °C freezer prior to analysis. To confirm the tested results, 40 samples (9.6%) were randomly selected and re-tested. The AF results from the two batches were also compared.

Total aflatoxin analysis

Total aflatoxin was analysed using commercially available low matrix enzyme-linked immunosorbent assay (ELISA) kits (Helica Biosystems Inc., Santa Ana, CA, USA). 250 g of groundnut sample were milled using a laboratory scale grinder (500A Multi-function/Herb Grinder, China PR) to fine instant coffee particle size (50% passes through a 20-mesh screen). Aflatoxin was extracted from grounded groundnut samples using 80% methanol at a ratio of 1:5 between sample and solvent, respectively, and analysed following the manufacturer's protocol.

The solid-phase direct competitive aflatoxin ELISA kit consists of a 96-well microplate coated with an antibody that had been optimised to cross-react with the four subtypes of aflatoxin such that B_1, B_2, G_1, and G_2. The lower and upper limits of quantification (LOQ) of methods (0-20 μg/kg) were provided, and the samples with total aflatoxin levels higher than the upper LOQ were diluted and retested.

Two quality control reference materials (QCRM) with low AF (mean = 4 μg/kg, standard deviation (SD) = 1.41) and high AF (mean = 14 μg/kg with SD = 1.41) concentrations within the detection range of the kit, were also included in each ELISA plate. The results for each plate were considered valid when the low and high AF QCRM samples fell within the range. Samples were reanalysed on a new ELISA plate if the low or high AF QCRM samples fell out of range.

The ELISA AF standards, QCRMs, and samples were run in duplicates on each ELISA plate. Optical densities (OD) of the reactions for aflatoxin were quantified using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) with an absorbance filter of 450 nm, paired with Gen5™ software (BioTek 194 Instruments, Inc.). A standard curve was constructed based on OD values, expressed as a percentage, concerning the AF standards. Test values were interpreted vis-à-vis standards included in each experiment. Approximately 10% of randomly selected samples were re-tested using ELISA to check for consistency.

Statistical analysis

Data analysis was performed using JAMOVI software (version 2.2.2) (Olumade and Uzairue, 2021). AF data was first transformed by natural logarithm before any statistical analyses since they were not normally distributed. The raw data for AF results were adjusted by adding an integer 1 to avoid infinite values where the concentration was 0 μg/kg (Aoun *et al.*, 2020). Pairwise Pearson's correlations were used to evaluate the association between hundred kernel weight (100 KW), bulk density (BD), and AF level. Tukey's honest significance test was used to compare the effects of each sorting method on mean AF levels, meantime used, and mean percentage weight loss (grade 4). For pairwise compar-

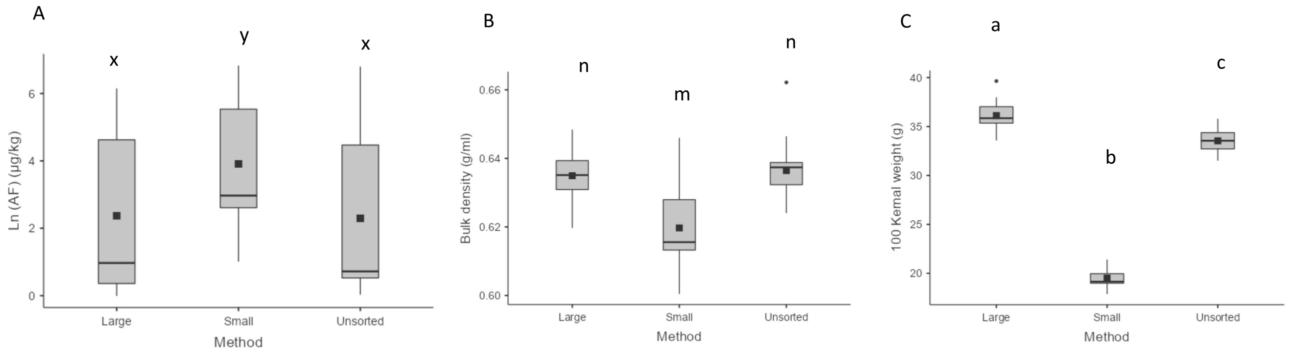

FIGURE 5 Effect of size sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi, harvested initially from Kongwa, Tanzania, in 2019 and 2020. Each fraction's aflatoxin means the median is presented on the boxplot (n = 19). The letters on the boxplots represent the results of Tukey's test; treatments with different letters are significantly different at a 95% level of confidence.

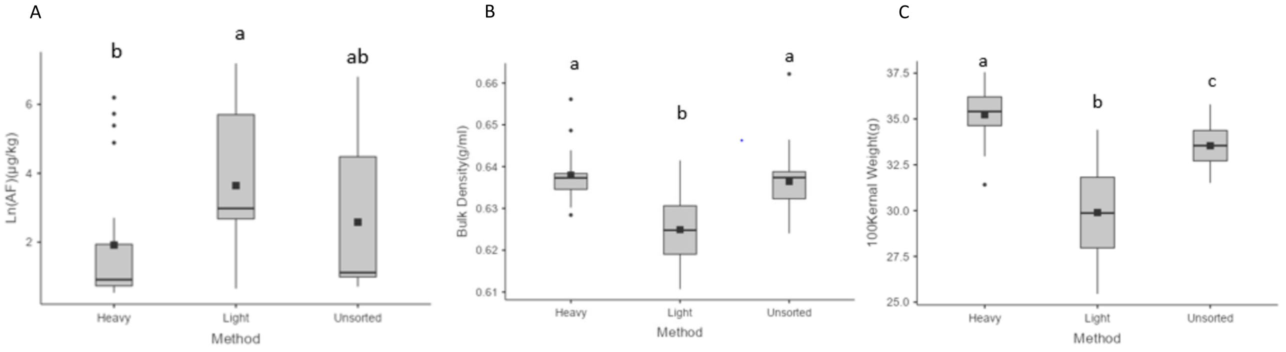

FIGURE 6 Effect of density sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi and originally harvested from Kongwa, Tanzania, in 2019 and 2020. The letters on the boxplots represent the results of Tukey's test; treatments with different letters are significantly different at a 95% level of confidence.

isons, a two-tailed t-test was used. The P-value of ≤0.05 at a 95% confidence interval (95% CI) was considered statistically significant.

The percentage AF reduction was obtained by taking the difference in mean AF level between unsorted and sorted fractions divided by unsorted mean AF, multiplied by a hundred.

3 Results

Effect of size screening on aflatoxin levels, BD and 100 KW

Small groundnut kernels had higher mean aflatoxin (geometric mean (GM) 49.95 µg/kg) compared to large kernels (10.71 µg/kg), though the difference was marginal ($P = 0.058$). There was no significant difference in mean aflatoxin between unsorted and large kernels ($P > 0.05$) (Figure 5A).

Small kernels had significantly lower BD and 100 KW compared to the unsorted and large kernels ($P < 0.05$) (Figures 5B and 5C). A significant difference in 100 KW between large and un-sorted kernels was also observed (Figure 5C). There was no significant difference in BD between large and unsorted kernels (Figure 5B).

Effect of density sorting on aflatoxin levels, BD and 100 KW

Density sorting resulted in lower mean AF for the heavier fraction (GM = 4.35 µg/kg) compared to the light fraction (33.45 µg/kg) ($P < 0.05$). There was no significant difference in mean AF between the H fraction and unsorted and the L fraction and unsorted groundnuts (Figure 6A). The L fraction had a significantly lower BD and 100 KW than the unsorted and H fraction ($P < 0.05$) (Figure 6B and 6C). H fraction also showed a significantly higher 100 KW than unsorted (Figure 6C). No significant difference in BD between H fraction and unsorted was observed (Figure 6B).

Effect of visual sorting on aflatoxin levels, BD, and 100 KW

Groundnuts were distinguished into four categories: G1, G2, G3 and G4, based on characteristics explained in

TABLE 2 Pairs of grades that were significantly different in aflatoxin (AF) level, bulk density (BD), and 100 KW[1]

Sorting methods[2]	Pairs of grades with significant differences in mean AF	Pairs of grades with significant differences in BD	Pairs of grades with significant differences in 100 KW
V	unsorted-G1, unsorted-G4, G1-G2, G1-G4, G2-G4, and G3-G4	unsorted-G4, G1-G4, G2-G4, and G3-G4	unsorted-G1, unsorted-G2, unsorted-G3, unsorted-G4, G1-G2, G1-G3, G1-G4, G2-G3, G2-G4, and G3-G4
SV	unsorted-G1, unsorted-G4, G1-G4, G2-G4, and G3-G4	unsorted-G4, G1-G4, G2-G4, and G3-G4	G1-G2, G1-G3, G1-G4, unsorted-G1, G2-G3, G2-G4, unsorted-G3, and unsorted-G4
DV	unsorted-G1, unsorted-G4, G1-G2, G1-G3, G1-G4, G2-G4, and G3-G4	unsorted-G4, G1-G4, G2-G4, and G3-G4	unsorted-G1, unsorted-G2, unsorted-G3, unsorted-G4, G1-G2, G1-G3, G1-G4, G2-G3, G2-G4, and G3-G4
SDV	unsorted-G1, unsorted-G4, G1-G2, G1-G3, G1-G4, G2-G4, and G3-G4	unsorted-G4, G1-G4, G2-G4, and G3-G4	unsorted-G1, unsorted-G2, unsorted-G3, unsorted-G4 G1-G2, G1-G3, G1-G4, G2-G3, and G2-G4

1 Summary to show pairwise grades with a significant difference at $P < 0.05$ (G1 = grade 1, G2 = grade2, G3 = grade 3, and G4 = grade 4).

2 V = visual sorting; D = density sorting; S = size sorting.

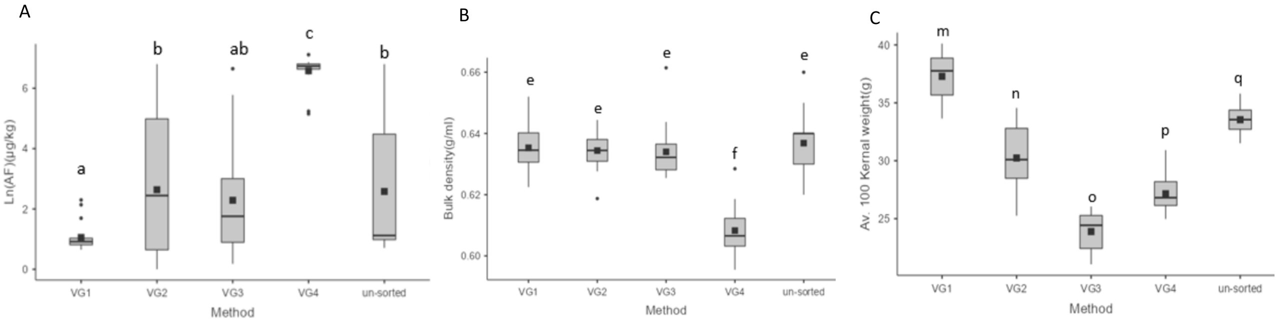

FIGURE 7 Effect of visual sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi. The box plots present the mean and median of the four grades (Visual grade (VG) 1, 2, 3, and 4) separated by the methods. The letters on the boxplots represent mean comparison by Tukey's test treatments. Different letters show that the means significantly differ at 95% confidence.

the Materials and methods and Figure 2A-D. G1 groundnuts, which were cleaner, resulted in significantly lower AF concentration (GM 1.76 µg/kg) than all the other fractions, including G4 grades (GM 720.54 µg/kg) and unsorted ones (GM 13.20 µg/kg) (Table 2 and Figure 7A).

The G4 fraction resulted in significantly lower BD than unsorted groundnut, G1, G2, and G3 fractions (Figure 7B). As for 100 KW, both G3 and G4 samples resulted in significantly lower values compared to other fractions, while G1 sorted groundnuts had the significantly highest value of 100 KW (Figure 7C and Table 2).

Effect of size + visual sorting on aflatoxin levels, BD, and 100 KW

Combining size and visual sorting effectively separated the groundnuts by aflatoxin level between the four grades (G1, G2, G3, and G4). Grade 1 had the lowest aflatoxin level (GM 1.31 µg/kg), while G4 had the highest level of aflatoxin (GM 572.49 µg/kg) (Table 2 and Figure 8A). Post hoc comparisons revealed a significantly lowest BD in G4 than the rest of the fractions (Figure 8b). As for 100 KW, both G3 and G4 samples resulted in significantly lower values compared to other fractions, while G1 sorted groundnuts had the significantly highest value of 100 KW (Table 2 and Figure 8C).

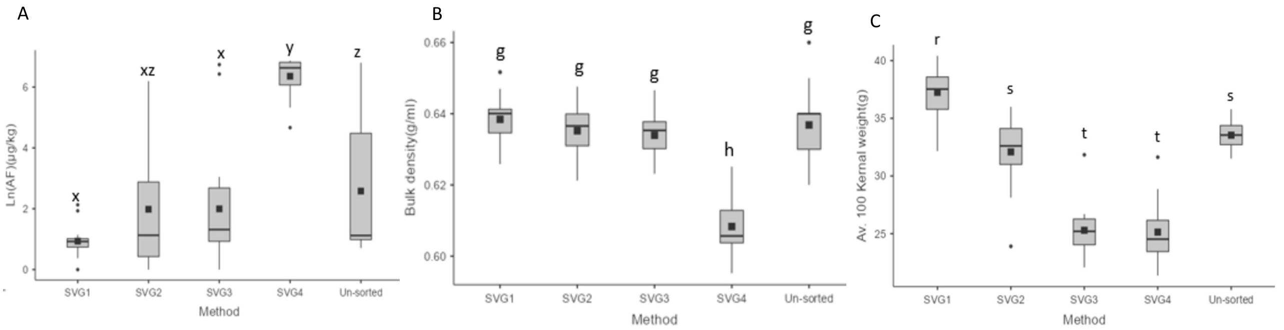

FIGURE 8 Effect of the combination of size and visual sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi. The box plots present the mean and median of the four grades (Size visual grade (SVG) 1, 2, 3, and 4) separated by the methods. The letters on the boxplots represent mean comparison by Tukey's test treatments. Different letters show that the means significantly differ at 95% confidence.

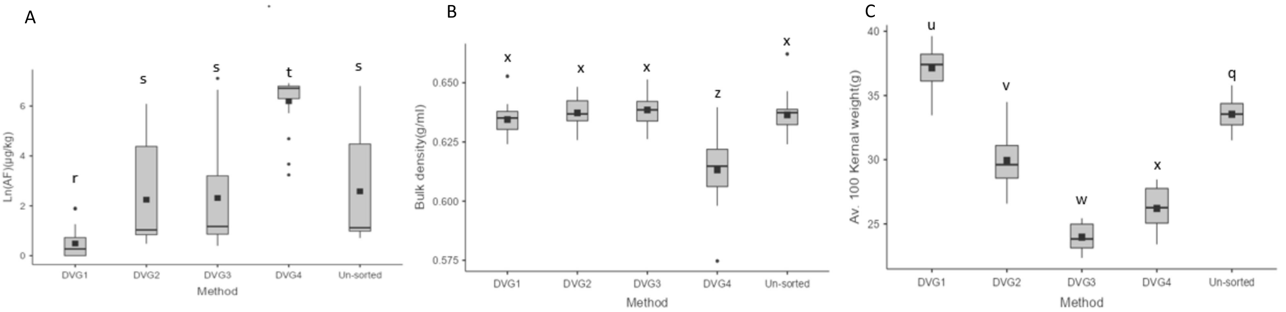

FIGURE 9 Effect of the combination of density and visual sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi. The box plots present the mean and median of the four grades (Density visual grade (DVG) 1, 2, 3, and 4) separated by the methods. The boxplots' letters denote mean comparisons using Tukey's test treatments. At a 95% confidence level, different letters indicate that the means are considerably different.

Effect of density + visual sorting on aflatoxin levels, BD, and 100 KW

Significant differences in mean AF between grades were observed (Table 2 and Figure 9A). The G1 fraction, which was clean, had the lowest aflatoxin level (GM 0.29 µg/kg), while G4 had the highest level of AF (GM 487.85 µg/kg). BD for the G4 fraction was much lower than for the unsorted G1, G2, and G3 fractions (Figure 9B). Compared to the other fractions, the 100 KW values for the G3 and G4 samples were noticeably lower, and the 100 KW value for the G1 sorted groundnuts was noticeably higher (Figure 9C and Table 2).

Effect of size + density + visual sorting on aflatoxin levels, BD, and 100 KW

The combination of size, density, and visual sorting methods separated G1, G2, G3 and G4 grades based on the characteristics above. Differences in mean AF levels between grades were significant. The average amount of aflatoxin contamination decreased from 13.2 g/kg in unsorted to 0.13 g/kg in G1. The mean AF level in G4 was highly elevated (GM 620.17 µg/kg) (Table 2 and Figure 10A). Post hoc comparisons showed that the BD in G4 was much lower than that in the other fractions (Figure 10B). The G1 sorted groundnuts exhibited the significantly greatest value of 100 KW, whereas both G3 and G4 samples had significantly lower values for 100 KW in comparison to other fractions (Figure 10C and Table 2)

Association between BD, 100 KW, and aflatoxin level (n = 418)

Pearson correlations were used to evaluate the relationship between BD, 100 KW, and AF. The relationship between 100 KW, BD, and AF levels was significantly inverse (r = −0.451, $P < 0.01$) (Table 3). Lower AF levels were related to higher BD and 100 KW. Between BD and 100 KW, there was a highly significant positive connection (r = 0.243, $P < 0.05$).

The average time used and percentage loss (G4) by each method

It took an average of 28.1 min (1,686 s) and 18.8 min (1,128 s) to sort 1 kg of groundnuts by visual and size-visual sorting, respectively. The combination of density and visual sorting took 21.9 min (1,314 s), whereas size, density, and visual sorting took 22.3 min (1,338 s). A sig-

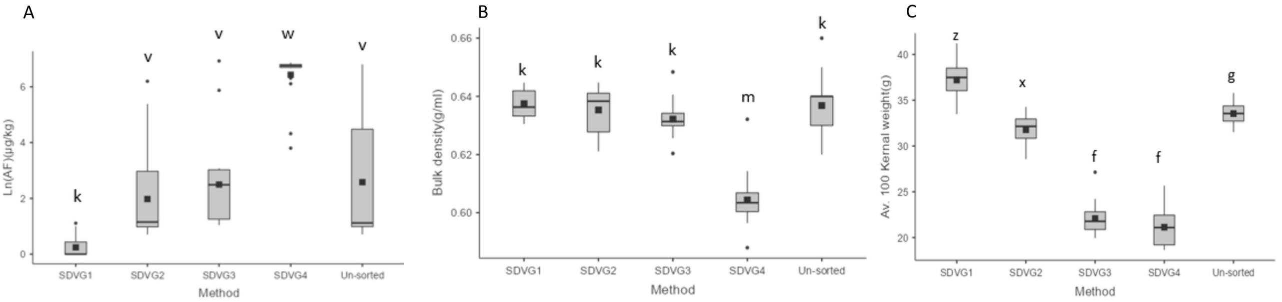

FIGURE 10 Effect of the combination of size + density + visual sorting on (A) aflatoxin (AF) concentration, (B) bulk density (BD), and (C) 100 KW of groundnut collected at Halisi. The box plots present the mean and median of the four grades (Size density visual grade (SDVG) 1, 2, 3, and 4) separated by the methods. The boxplots' letters denote mean comparisons using Tukey's test treatments. At a 95% confidence level, different letters indicate that the means are considerably different.

TABLE 3 Correlation matrix to show an association between aflatoxin (AF) level, bulk density and 100 KW (n = 418)

	Test	LN (AF) (µg/kg)	Bulk density (g/ml)
Bulk density (g/ml)	Pearson's r	−0.245	–
	P-value	<0.001	–
Av. 100 KWt(g)	Pearson's r	−0.451	0.243
	P-value	<0.001	< 0.001

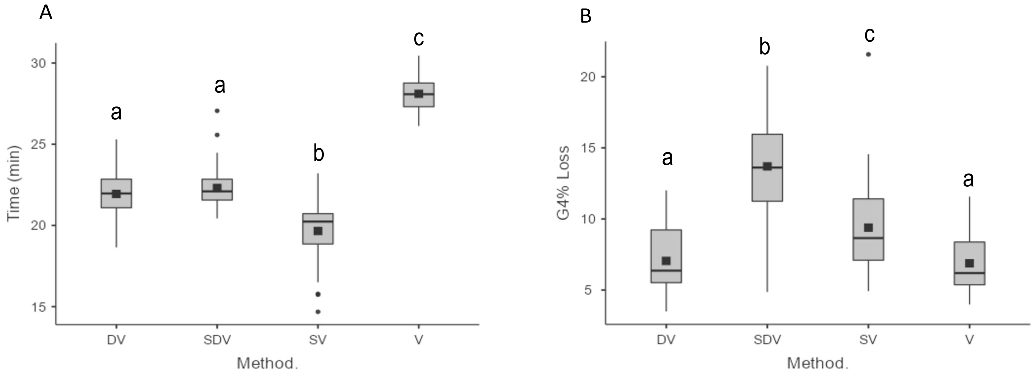

FIGURE 11 (A) Time taken by visual (V), size visual (SV), density visual (DV), and size density visual (SDV) methods to sort 1 kg of groundnut. (B) Percentage loss/grade 4(G4) reduced during sorting. Determined by Tukey's test, similar letters on boxplots indicate no significant difference.

nificant variation in sorting times for 1 kg of groundnuts was observed between DV-SV, DV-V, SDV-SV, SDV-V, and SV-V but not the DV-SDV method (Figure 11A). A significant difference in percentage loss (G4) was observed between DV-SDV, DV-SV, SDV-SV, SDV-V, and SV-V methods ($P < 0.05$), but not between DV-V methods (Figure 11B).

4 Discussion

During the low-AF complementary feeding flour processing for the MMT project, visual sorting of groundnuts into three grades resulted in 27-30% loss (Ngure, unpublished data). Due to time and resource constraints, there were no further sorting efforts to salvage such losses for alternative uses. In this study, visual sorting was done to separate groundnut into four grades to mitigate such losses and add value in processing low-AF food. We hypothesised that separation of AF levels through such grading would minimise losses since grades with relatively lower AF <10 µg/kg could potentially be used for human consumption, 10-100 µg/kg animal feed, and >100 µg/kg channelled to an alternative use such as the production of fuel pellets.

Visual sorting grains and nuts to reduce mycotoxins is common in low-income contexts (Aoun *et al.*, 2020; Xu *et al.*, 2017). It is characterised by removing insect-damaged, discoloured, rotten, and mouldy kernels (Afolabi *et al.*, 2006; Kabak *et al.*, 2006; Mutiga *et al.*, 2014). Visual sorting has been effective in reducing AF in maize and groundnuts worldwide (Aoun *et al.*, 2020; Wild *et al.*, 2015; Xu *et al.*, 2017), but it has been reported to be ineffective in reducing AF in maize in Kenya (Mutiga *et al.*, 2014).

In this study, visual sorting of raw unsorted groundnuts reduced AF contamination by 59% in G1. About 30 min were spent by graders during the visual sorting method to separate/grade 1 kg of groundnut into the four grades, which was longer than any other method with 7% groundnut loss. The difference in mean AF between unsorted groundnuts and G1 shows the effectiveness of visual sorting to lower AF to below the regulatory limit. However, we discovered that specific kernels with a healthy appearance had high AF contents. This indicates that determining grain AF levels is not always as simple as looking at the kernel. Visual sorting may not be as effective for grain contaminated with AF since *A. flavus* and *A. parasiticus* enter groundnut kernels in distinct ways (Aoun *et al.*, 2020). More studies on the biology of the colonisation of these species and the effectiveness of kernel sorting based on visual sorting are required. Furthermore, AF concentration in groundnuts classified as G2 and G3 was not significantly different, showing that visual sorting based on the degree of shrivelling alone may not distinguish different AF contamination levels in different grades (Aoun *et al.*, 2020). Consistent with our hypothesis, Grade 4 (720.54 μg/kg) was the worst in terms of physical attributes and had the highest AF contamination.

The combination of size and visual sorting was observed to lower the AF contamination of unsorted groundnut by 61% in G1. A clear and significant difference was observed between G4 (572.49 μg/kg) and G1 (1.31 μg/kg). The combination of groundnut size and visual sorting reduced processing time from 28 min for 1 kg of groundnut to 18 min. This was in line with the observations made during the processing of low AF food at Halisi (Ngure, unpublished data).

The combination of density using DropSort and the visual sorting method reduced AF contamination of unsorted by 81% (in G1) compared to 59% by visual sorting. This suggested that the method could reduce AF in groundnut by stratifying the sample based on kernel weight. Although there was a similar amount of loss (7%) with visual sorting, it took the least time (21 min for 1 kg of groundnuts). Previous findings on density sorting of maize using a similar 'DropSort' device reduced fumonisin by separating the kernels by bulk density and kernel weight, such that the lighter fraction was more contaminated than, the heavier fraction (Aoun *et al.*, 2020; Ngure *et al.*, 2021). The toxin concentrations in the light fractions may be a good proxy of the toxin concentration in the unsorted grain lot. A grain lot is highly heterogeneous, and most aflatoxin is typically concentrated in a small and light portion of the kernels (Stasiewicz *et al.*, 2017; Whitaker, 2003). Density sorting can reduce sampling variance by generating more homogeneous grain fractions in terms of mass, volume, size (kernel width and depth), shape (roundness), and toxicity. As a result, density sorting can be useful for breeders and grain purchasers in estimating mycotoxin in grain lots and for mycotoxin resistance phenotyping.

The combination of size, density, and visual (SDV) reduced AF levels of unsorted groundnuts by 99% in G1. It was the best approach for minimising AF and saving time; SDV needed just 22 min to clean 1 kg of groundnuts, although there was a 14% loss. It was successfully stratifying grades by AF level, G1 (0.13 μg/kg), G2 (5.31 μg/kg), G3 (10.07 μg/kg), and G4 (620.17 μg/kg), in contrast to visual sorting, G1 (1.7 μg/kg), G2 (7.24 μg/kg), G3 (6.89 μg/kg), and G4 (720.54 μg/kg). The novel SDV, which involved the techniques above, increases the likelihood of removing contaminated small and light groundnut fractions, which might elude visual sorting. It is affordable and can be adopted by small-scale farmers and small and medium enterprises (SMEs) in low-and-middle-income settings. The SDV approach involves a 14% loss, which may limit small-scale farmers from using it out of fear of losing money or drive-up prices to make up for the loss.

Generally, according to the methods above, the AF concentrations in G1, G2, and G3 fall into the acceptable regulatory limit (<10 g/kg), which is safe for human consumption. The hypothesis that sorting is done through the four grades (G1, G2, G3, and G4) increases the chances of minimising losses. The study has shown that combining two or more sorting algorithms increases efficiency from 59% AF reduction by V to 99% by SDV (Figure 12). By combining density and visual sorting, fumonisin reduction to an undetected level was observed by Aoun *et al.* (2020). Likewise, combining size and density sorting lowered the fumonisin to below Codex's (4 mg/kg) regulatory limit (Aoun *et al.*, 2020).

Aflatoxin levels were negatively correlated with 100 KW and BD. Light fractions had higher AF levels than those heavy fractions. This corroborates the find-

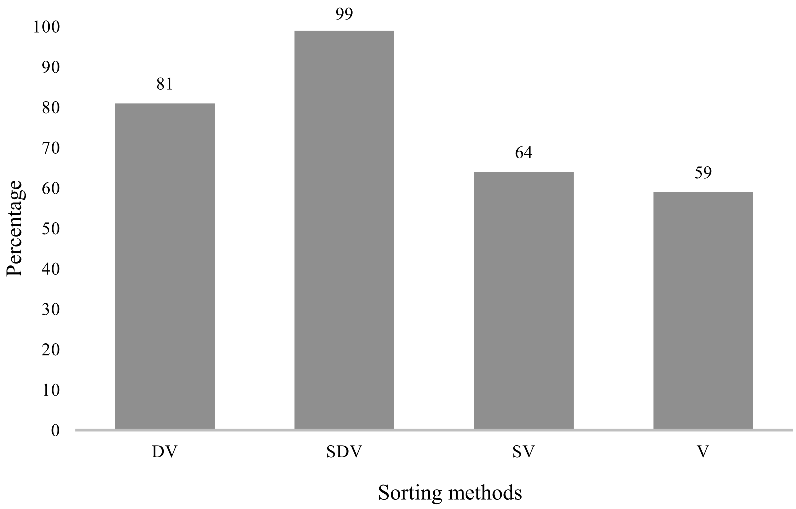

FIGURE 12 Percentage of aflatoxin reduction by visual method (V), density visual (DV), size visual SV), and size density visual method (SDV) from groundnut samples. The percentage of AF each method could reduce from unsorted grain is indicated by the number at the top of the histogram bar.

ings of Aoun *et al.* (2020) and Morales *et al.* (2018), who found a negative association between 100 KW/BD and aflatoxin in groundnut and maize (Aoun *et al.*, 2020; Morales *et al.*, 2018). 100 KW and BD can be used as proxy indicators of the health of groundnuts and toxicity. These physical attributes are necessary for screening and informing procurement decisions. Unsorted and bulk groundnuts with a high proportion of shrivelled and light kernels will likely have high AF levels and render visual sorting impractical. The lower the proportion of the light and small fraction, the better. The association between toxicity and weight/size suggests that early fungal infection may reduce groundnut growth. Fungal infection can also influence kernel mass and density as microbial growth requires utilising the kernel's resources and retards kernel growth and development (Aoun *et al.*, 2020).

The study involves using groundnuts from farmers, which were relatively lower in AF. Further studies may consider a wide range of AF contaminated groundnut from vulnerable groundnut varieties like *mnanje 2009*, *naliendele 2009*, *mangaka 2009*, *masasi 2009*, *nachingwea 2009*, *nachi 2015*, and *Narinut 2015* (commonly grown in Tanzania) (Akpo *et al.*, 2021) from the market.

5 Conclusions

Visual sorting of groundnuts is a widely adopted method, mainly in low-income countries. We found visual sorting relatively effective in reducing aflatoxin levels compared to size and density sorting alone. Complementing visual with size and density sorting (SDV) offers a more efficient alternative in reducing aflatoxin in groundnut, saving on time and losses in resource-limited areas where groundnut is considered a rich source of essential nutrients. If SDV is scaled up and adopted, it can significantly reduce AF contamination and exposure through groundnuts. However, precise outsort management and disposal strategies are required to prevent these from returning to the food system.

Unsorted groundnut with a lower percentage of lighter (low BD and 100 KW) and shrivelled kernels might be helpful to judge good quality groundnuts even before procurement and sorting to minimise costs.

Further research on scaling up these sorting methods and safe out-sort management is recommended. Cost-benefit analysis of all sorting methods will also provide insights into the scalability of these methods.

Acknowledgements

Authors acknowledge the Principal Investigators of the MMT project, Prof. Rebecca Stoltzfus of Cornell University and Goshen College, and Prof. Rebecca Nelson of Cornell University USA, who led the acquisition of funds and contributed to the design of this study; Co-Investigator Dr Neema Kassim of Nelson Mandela African Institution of Science and Technology (NM-AIST); and team members; Prof. Paul C. Turner of Maryland University USA, and Dr Erica Phillips of Cornell University USA for their support to the implementation of the study. Many thanks also to William Mgongo and Rosemary Kayanda for their support in data collection; and William Stafstrom from Cornel University and Joachim Dotto from Sokoine University of Agriculture (SUA), Tanzania, for their practical comments during the implementation of the study.

Authors' contribution

John Mshanga (JM), Edna Makule (EM), and Francis Ngure (FN) designed the study. JM: Participated in conceptualisation, methodology, investigation, analysis, visualisation, and writing of initial and subsequent drafts of the manuscript. EM: review. FN: conceptualisation, methodology, supporting implementation, and critical review of the manuscript. All authors have agreed to the publication of the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

Data used in this manuscript are available upon formal request to the corresponding author.

Funding

This study is part of the Mycotoxin Mitigation Trial (MMT) project funded by the Bill and Melinda Gates Foundation through Grant Number OPP1155626, registered under Cornell University, USA.

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