

# Post-harvest interventions decrease aflatoxin and fumonisin contamination in maize and subsequent dietary exposure in Tanzanian infants: a cluster randomised-controlled trial

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## RESEARCH ARTICLE

### Abstract

A cluster randomised controlled trial was performed in three agro-ecological zones of Tanzania to evaluate the effectiveness of locally available post-harvest mitigation strategies in preventing and reducing aflatoxin and fumonisin contamination in maize. A total of 300 children, each from one household, were randomly selected from 30 villages (intervention: n=15). The mitigation strategies focused on hand sorting (prior to storage and use), drying maize on mat/raised platforms, proper sun drying, application of storage insecticides and de-hulling before milling. Maize sample was collected from each household at harvest (baseline) and six months after harvest. Maize intake by each child, estimated using the 24 h dietary recall technique and its body weight measured using standard procedures were taken at six months after harvest. Aflatoxins and fumonisins in the maize samples were determined using HPLC. Follow-up (six month after harvest) data were available for 261 of the 300 households (intervention: n=136). Mean concentration of aflatoxins, or fumonisins was significantly ( $P<0.05$ ) lower in the intervention than in the control group: intervention effects:  $\mu\text{g}/\text{kg}$  (95% confidence interval (CI)) -4.9 (-7.3,-2.5), and -405, (-647,-162), respectively. The difference corresponds to 83 and 70% for aflatoxins, and fumonisins, respectively. At the end of the intervention, aflatoxin and fumonisin estimated mean intakes were lower in the intervention than in the control group by 78 and 65%, respectively. Six months after harvest, prevalence of underweight in the intervention group was 6.7% lower ( $P=0.014$ ) than in the control group. Mean weight-for-age Z-score difference between the groups was 0.57 (95% CI; 0.16,-0.98;  $P=0.007$ ). Post-harvest practices are effective in preventing and reducing aflatoxin and fumonisin contamination in maize and subsequent dietary exposure to infants. The interventions may be applied in these and other communities with similar environmental conditions or agricultural practices that favour production of aflatoxin and fumonisins in food crops. The trial was registered at ClinicalTrials.gov identifier: NCT02438774.

**Keywords:** exposure assessment, infant growth, mycotoxins, weight-for-age Z-scores

## 1. Introduction

Maize is a staple crop for the majority of Tanzanians and is used as a main ingredient for complementary food. Unfortunately, the crop is susceptible to contamination with

mycotoxins. Mycotoxins are a group of toxic compounds produced by strains of some fungal species when they colonise crops in the field and a wide range of foods and feeds during storage (CAST, 2003). Crops that are susceptible to mycotoxin contamination include cereals,

oilseeds, pulses, dried root crops, dried fruits, and coffee (Leslie *et al.*, 2008). Certain mycotoxins, such as aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) (Fink-Gremmels, 2008) and fumonisin B<sub>1</sub> (FB<sub>1</sub>) (Magoha *et al.*, 2014a) when present in consumed feed/food, can be carried-over to milk.

The most frequently encountered mycotoxins in Tanzanian food crops are aflatoxins (AFs) and fumonisins (FBs) (Kamala *et al.*, 2015; Kimanya *et al.*, 2008). However, other types of mycotoxins such as zearalenone, deoxynivalenol, ochratoxins, and HT-2 toxin have also been reported in Tanzanian food (Kamala *et al.*, 2015; Kimanya *et al.*, 2014) with co-occurrence of AFs and FBs reported in over 45% of maize samples. This suggests that people in rural Tanzania who produce maize under subsistence farming systems and rely on it as the main source of food (Kamala *et al.*, 2017; Suleiman and Rosentrater, 2015) are at a high risk of exposure to AFs and FBs. The average daily per-capita consumption of maize for adults in rural Tanzania is estimated to be 405 g (Smith and Subandoro, 2012). It is also reported that people in that area relying on maize as staple food, also use it as complementary food for their children. Feeding children with maize based foods introduces them to the risk of mycotoxin exposures and subsequently mycotoxin adverse health effects. Several studies suggest that high dietary exposure to mycotoxins may be a contributing factor to the high rates of malnutrition observed in sub-Saharan African countries including Tanzania (Gong *et al.*, 2004; Kimanya *et al.*, 2010; Shirima *et al.*, 2015; Wu *et al.*, 2014). In sub-Saharan Africa, infants experience severe growth faltering during the period of introduction of complementary foods (Victora *et al.*, 2010). For example, in Tanzania, 34% of children less than five years of age are stunted, 14% are underweight and 5% are wasted (NBS, 2016). Growth retardation is important from a public health perspective, because it is associated with effects such as increased vulnerability to infectious diseases and cognitive impairments that last well beyond childhood years (Ezzati *et al.*, 2002).

AFs are mycotoxins produced by toxigenic species of the genus *Aspergillus*. Health risks like suppression of the immune system, mutagenicity effects, liver cancers (Hussein and Brasel, 2001) and outbreak of acute toxicity (Gieseke, 2004) are some of the consequences associated with consumption of aflatoxin contaminated food. AFB<sub>1</sub> is the most prevalent form of AFs and has been classified by the International Agency for Research on Cancer (IARC) in group 1: 'carcinogenic to humans' (IARC, 1993).

FBs are mycotoxins predominantly produced in maize and maize products by toxigenic species of the genus *Fusarium*. FBs exposure has been linked to carcinogenic effects (Voss *et al.*, 2002), neural tube defects (Felkner *et al.*, 2009) and outbreak of food-borne diseases in India

(Bhat *et al.*, 1997); and is classified by the IARC in group 2B: 'possibly carcinogenic to humans' (IARC, 2002). Studies have indicated that FB<sub>1</sub>, synergistically, promotes liver tumours initiated by AFB<sub>1</sub> (Gelderblom *et al.*, 2002). Similarly, a synergistic effect on the reduction of body weight gain in male Wistar rats (Pozzi *et al.*, 2001) and acute combinative toxicity in animal cells (McKean *et al.*, 2006) have been reported. Shirima *et al.* (2015) found a significant association between fumonisin exposure and impaired child growth in Tanzania.

Several post-harvest prevention strategies (Chulze, 2010), and the general guidelines published by Codex Alimentarius Commission (CAC, 2003) to prevent mycotoxin development in cereals are available. However, they require evaluation for effectiveness under local conditions and post-harvest practices.

Therefore, a post-harvest intervention package was developed and introduced at farmers' levels in Tanzania. The aim of this study was to evaluate the effectiveness of this mitigation strategy in reducing AFs and FBs contamination in maize and subsequent dietary exposure in infants. The primary hypotheses were that the developed post-harvest intervention package when introduced at farmers' levels will reduce/prevent AFs and FBs incidence and levels in maize. Because feeding children with maize based foods introduces them to the risk of mycotoxin exposures and subsequently mycotoxin adverse health effects such as malnutrition, the intervention effect on individual AFs and FBs intake and weight-for-age Z-score (WAZ) were also examined.

## 2. Materials and methods

### Study designs, setting and randomisation

An unmatched cluster randomised controlled trial was carried out in three main maize producing agro-ecological zones of Tanzania, namely the Northern highland, Eastern lowland and South-western highland (Figure 1). For each zone, one maize-producing district, primarily for home consumption, was purposively selected, i.e. Hanang' in the Northern highland, Kilosa in the Eastern lowland and Rungwe in the South-western highland. The districts are divided into divisions, wards and villages. The village was the unit of randomisation. The number of clusters (villages) needed was equally divided over the three agro-ecological zones, each zone with intervention and control groups. We selected 10 villages randomly for each of the three ecological zones from a list of eligible villages (Figure 1). Randomisation was achieved by obtaining computer generated random numbers (for each agro-ecological zone) and assigning random numbers to each treatment group. Randomisation was carried out by a researcher, not involved

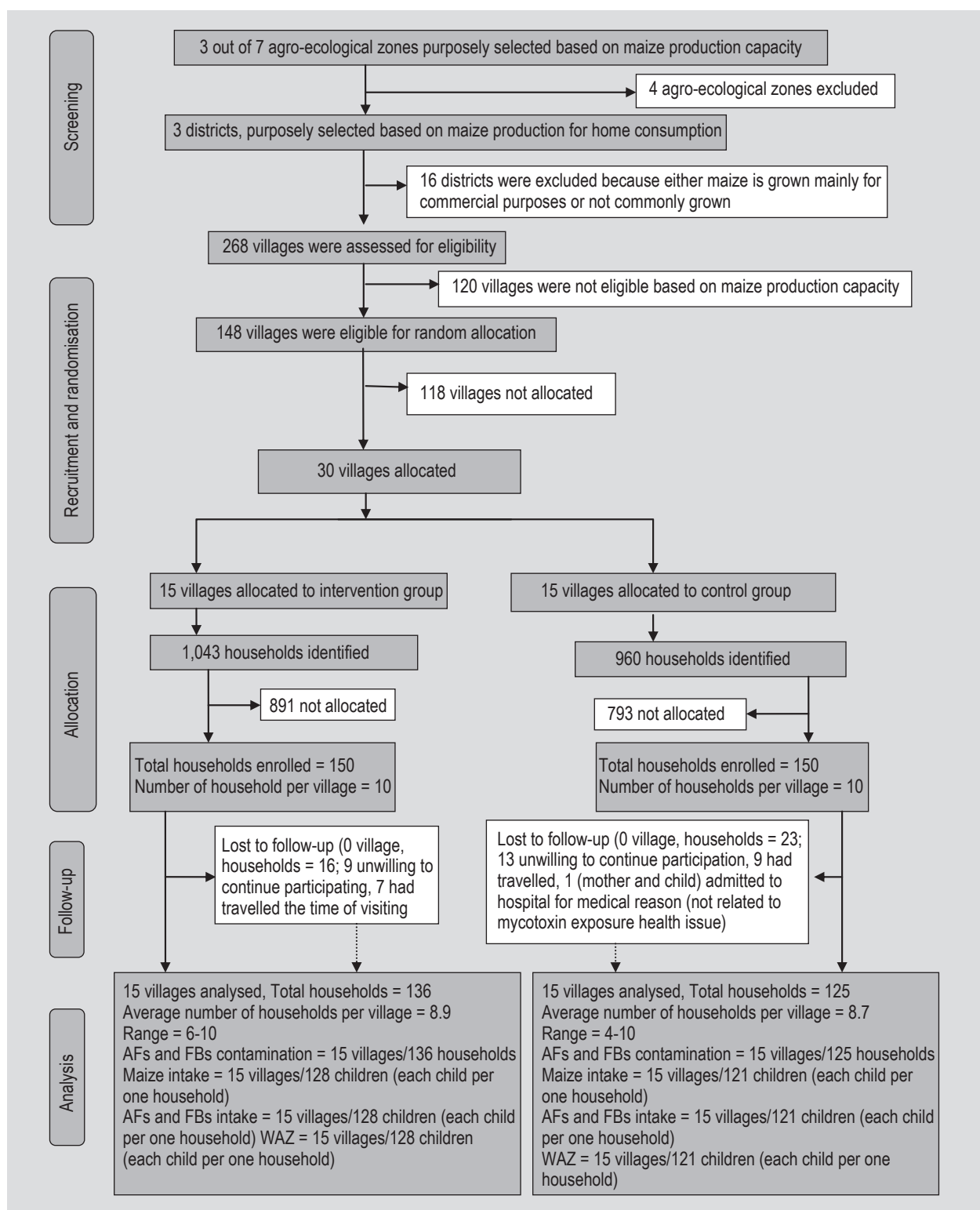


Figure 1. Participant flow chart of cluster randomised controlled trial.

in the field work. Fifteen villages were randomly allocated to control group (n=15 villages) or intervention group (n=15 villages).

#### Participants, recruitment and ethical considerations

A total of 300 (10 per village) infants aged 0-6 months (identified using their registration number and date of birth) were recruited from 2,001 households with eligible infants

identified in 30 randomly selected villages. Recruitment was done one month before the harvesting period (May 2013 for Hanang' and Kilosa, February 2014 for Rungwe). The criteria for inclusion of a household were having a breastfed infant aged 0-6 months (born to parents who are local residents) at the time of recruitment and being a maize producing household with capacity of storing maize for at least six months after harvest. A household with an infant having a known congenital malformation or chronic condition that could affect growth was excluded from the study. Ethical approval was obtained from the National Institute of Medical Research in Tanzania (NIMR/HQ/R. 8a/Vol. IX/1660). Permission to conduct the study was also obtained from the local authorities. The objectives of the study and its protocol were explained to the household heads/mothers of the eligible infants and their signed consent obtained. Agricultural extension (AE) workers, health officers, resident nurses and sociologists from all the participating villages were requested to guide farmers on application of the intervention strategies. They were informed of the nature and purpose of the study and accepted the responsibility. The study was registered at ClinicalTrials.gov, Identifier NCT02438774.

### **Intervention package and implementation**

The intervention package was developed using results obtained from an inventory of local risk factors for AFs and FBs contamination of maize in three agro-ecological zones (Kamala *et al.*, 2016) and the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC, 2003). The package was discussed in a meeting involving AE workers and health officers from all the participating villages. The control group received routine AE services on good practices for handling crops, which corresponds to the regular AE service for farmers at village level. The language of communication during the intervention delivery was Swahili, a written and spoken language in the study population. Farmers gathered to a nearby health facility or school in the particular village for informal meetings during which the mitigation strategies were introduced focusing on the following practices:

#### *Hand sorting*

Farmers were shown how to identify and separate damaged/infected maize kernels and cobs from healthy ones, prior to storage or use. Separating damaged/infected maize kernels and cobs from healthy ones reduces levels of already formed mycotoxins and helps to prevent spread of mould and production of mycotoxins during storage.

#### *Drying maize on mat/raised platforms*

With the aid of photos, AE workers demonstrated and encouraged the farmers to dry maize on the mats/sheets or raised platform. In these communities maize is usually spread on bare ground for sun drying, making it prone to humidity and fungal contamination from the soil.

#### *Adequate sun-drying*

Farmers were trained on how to judge the dryness of maize kernels by use of a 'table salt and bottle' technique. In this technique, for properly dried maize, the salt does not stick on the bottle wall after shaking for about 5 min. Prior to recommending the technique, it was validated at the Tanzania Food and Drugs Authority laboratory based on the method for determination of moisture content in whole maize grain (TBS, 2002). Improperly dried maize has moisture content above 13% and may favour growth of moulds and formation of mycotoxins in storage.

#### *Application of insecticides during storage*

Farmers were trained on how to mix insecticides with maize kernels in appropriate ratio and considering recommended insecticides for specific crops. Each household was provided with two packs of insecticides (Actelic powder or pirimiphos-methyl) recommended for protection of stored maize. The presence of insects in storage facilities affects mycotoxin formation by increasing humidity in stored grains via metabolic activity and spread fungal spores. Insect damage on maize can also increase the likelihood of fungal infestation.

#### *De-hulling of maize before milling*

Farmers were advised to de-hull maize before milling. Mycotoxins are likely to be more concentrated in the outer parts (pericarp and embryo) of the maize grain. Removal of these parts would result in a reduction of the mycotoxin level in maize.

### **Data collection**

#### *Outcomes and other measurements*

Data were collected using structured questionnaires prepared in English and translated into, and administered in Swahili. All filled questionnaires were manually checked for completeness and consistency. The data collection team was separate from the intervention support team and was blinded to the village allocation. Demographic information collected at baseline were infant characteristics (which include sex and date of birth) and maternal characteristics (which include age, marital status and level of education). Maize kernels or/flour samples were collected immediately

after harvest (June, 2013 in Hanang' and Kilosa, and March, 2014 in Rungwe) and at follow up (six months after harvest; November 2013 in Hanang' and Kilosa, and August 2014 in Rungwe) according to standards prescribed by the Tanzania Bureau of Standards (TBS, 2012). The child body weight and food consumption data used to estimate exposure as well as the child age used with body weight to calculate WAZ were collected at follow up (six months after harvest). Other information collected at follow up were age at first introduction of fluids/foods, breast feeding status and complementary feeding practices. The findings are reported according to the Consolidated Standards of Reporting Trials guidelines (Campbell *et al.*, 2012).

#### *Analysis of aflatoxins and fumonisins*

AFB<sub>1</sub>, aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) and aflatoxin G<sub>2</sub> (AFG<sub>2</sub>) contents of the maize were determined in accordance with the method described by Stroka *et al.* (2000). Total AFs in a sample was obtained by summing up the individual contents of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>. Determination of FB<sub>1</sub> and FB<sub>2</sub> was carried out based on the method described by Sydenham *et al.* (1992) with slight modification made by Samapundo *et al.* (2006). Total fumonisins content in a sample was determined by summing up the individual contents of FB<sub>1</sub> and FB<sub>2</sub>. The limits of detection (LODs) and limits of quantification (LOQs) of the analytical methods were previously described by Kamala *et al.* (2016).

#### *Weight for age Z-score estimation*

The WAZ was calculated using the WHO Anthro software (WHO, 2010). Children's body weight was taken according to standardised procedures (WHO, 2006) at the time of the complementary food survey. The age of children in months was calculated from difference between date of measurement and birth date. Children with WAZ below -2 standard deviation (SD) from the median of the WHO reference population were classified as underweight. We could not assess linear growth of the children because due to limitations in fieldwork logistics, we were unable to measure child length.

#### *Dietary assessment*

A repeated 24 h dietary recall (Gibson and Ferguson, 2008) was used to estimate the amount of maize consumed by the infants, at the end of the study. Only maize based meal consumed by a child was quantified for this purpose. Quantity consumed was estimated by recording the equivalent amount of food eaten as weighed by use of a digital food weighing scale. Two visits, with an interval of 1-2 weeks, were made to the home of each infant. In addition to the 24 h dietary recalls, a food frequency questionnaire was administered to assess the frequency

of consumption of maize-based food and other foods that were commonly consumed by the child, per week. Assessment of energy intake and nutrient adequacy was done by computing the number of meals consumed by each child and dietary diversity score (DDS), respectively, as recommended by PAHO/WHO (2003). The DDS is defined as the number of food groups consumed over a period of 24 h. The diet was classified into 8 food groups according to Dewey *et al.* (2006) which included: (1) grains, roots and tubers; (2) legumes and nuts; (3) dairy products; (4) flesh foods (meat, poultry, fish); (5) eggs; (6) vitamin A-rich fruits and vegetables; (7) other fruit and vegetables; and (8) fats and oil. A questionnaire that was previously used in communities in Tanzania with similar dietary habits to the study population was used for this purpose (Kimanya *et al.*, 2009, 2010). Adjustment of the moisture content of maize flour was done according to Kimanya *et al.* (2014). The amount of maize flour consumed by an infant from a thin (*uji*) or stiff (*ugali*) porridge was recorded in a database using the Lucille food intake software of Ghent University (Lucille, 2015), considering preparation techniques and the Tanzania food composition tables (Lukmanji *et al.*, 2008). To obtain the habitual maize based food intake of the child per day, the average maize based meal consumption of each child was multiplied with its weekly frequency of consumption divided by seven.

#### *Exposure assessment*

The dietary exposure of a child to AFs or FBs was assessed deterministically by combining the daily maize consumption by the child per kg body weight with the contamination in the maize. AFs intake was estimated as total AFs (AFB<sub>1</sub>+AFG<sub>1</sub>+AFB<sub>2</sub>+AFG<sub>2</sub>) and FBs intake, as total FBs (FB<sub>1</sub>+FB<sub>2</sub>). Contamination levels below LOD were considered as half LOD and those above LOD but below LOQ were considered as half LOQ. The daily maize intake per kg body weight per day (kg/kg bw/day) for each infant was calculated by dividing the child's daily maize intake (kg/day) by its body weight (kg). Thus, the mycotoxin intake (ng or µg/kg bw/day) was calculated by multiplying daily maize intake by mycotoxin contaminant in maize (µg/kg). Because AFs is a genotoxic carcinogen, WHO/FAO Joint Expert Committee on Food Additive and Contaminants (JECFA) cannot set a tolerable daily intake for it. Therefore, the mycotoxin intake was compared to 0.017 ng/kg bw/day obtained by dividing the BMDL<sub>10</sub> (benchmark dose level associated with a 10% extra risk of adverse effect) of 170 ng/kg bw/day (derived from the animal study) by a Margins of Exposure (MOE) of 10,000 considered a cut-off point of low public health concern (EFSA, 2005). FBs estimated intake was compared with the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day (WHO, 2011) above which were considered of public health concern. Effectiveness of the intervention was judged in terms of the

difference in mean intake and percent children exceeding the limit of public health concern for AFs or FBs.

### Sample size calculation, data management and statistical analysis

Data generated using questionnaires was coded and entered into Epidata 3.1 (Odense, Denmark). The primary study outcome measures were differences of the variation AFs or FBs contamination in maize over time, between the two groups. The secondary outcome measures included differences in proportions of children exceeding the health based guidance value of aflatoxins or fumonisins, mean AFs and FBs exposure and means of WAZ.

Sample size calculation for unmatched cluster randomised controlled trial was performed according to Hayes and Bennett (1999). On the basis of published information on FBs exposure in children from similar non-intervened population (Kimanya *et al.*, 2009), 30 clusters with 10 participants were necessary to detect a difference of 50% (15 to 7.5%) of the proportion of children exposed to FBs above the PMTDI of 2 µg/kg bw/day, using two sided significance levels of 0.05, variation in clusters means  $K_m=0.25$  and a power of 80%. To estimate the effect of the intervention of the primary outcome, the difference-in-differences approach was used. With this approach, we were able to estimate the difference in the over-time average change of AFs or FBs contamination between the two groups. Analysis of the primary outcomes was by intention-to-treat, and all missing households were analysed as part of the community in which they were enrolled (Armijo-Olivo *et al.*, 2009). Effects of intervention were analysed using multilevel mixed-effects models, with study group as fixed effect, and the village as a random effect to control for the cluster effect. Primary outcomes were adjusted for mother's education level and marital status and Akaike-Schwartz criteria were used to determine the optimal covariance structure (Burnham and Anderson, 2004). Intraclass correlation coefficients (ICCs) were computed from the multilevel linear regression models. For measurements for which only values at the end were available, the ICC coefficient was estimated from control group data at the end of the intervention. Beta coefficients of the intervention were used to assess the intervention effect. Detection of outliers was done by analysing the residuals and outliers were excluded using SD 2.5 as cut-off for dispersion of the residuals. Consequently, five and six data observations for FBs and AFs respectively were removed. To assess the association of AFs and FBs exposure with WAZ, separate multivariable regression models for each group were built with WAZ as the outcome variable and aflatoxin or fumonisin exposure as predictors. Since adjusting the models for baseline data (mother's level of education and marital status) did not modify the results unadjusted results are presented. Differences between dropout and remainder

groups were assessed using a t-test for continuous variables and a Chi-square test for categorical ones. Households' activities recommended by the intervention package were estimated as the total number of households who performed the practices divided by the total number of households in the intervention or control group. A Chi-square test was used to compare the proportion of farmers who performed the practices between intervention and control groups. The significance level for all analysis was set as 0.05. All data was processed with STATA version 12.0 (StataCorp, College Station, TX, USA).

## 3. Results

### Participants' characteristics

A total of 268 villages were assessed for eligibility; 120 villages were not eligible based on maize production capacity and 30 villages were allocated (Figure 1). A total of 2,001 households with eligible children were identified in the 30 selected villages. In each village 10 households were randomly selected (Figure 1). All villages completed the study and are included in the analysis. A total of 261 out of the 300 households completed the study. 14 (9%) of the 150 households in the intervention group and 25 (17%) of the 150 households in the control group were lost to follow-up (Figure 1). The drop out was significantly higher ( $P=0.03$ ) in the control compared to the intervention group. Those lost to follow-up were either not at home during the day of visit or not willing to continue to participate in the study. The baseline characteristics of the lost to follow-up (households and children) did not differ significantly compared to those who completed the trial. Baseline participant characteristics were comparable for all variables assessed (Table 1).

Table 1, further shows, that at the end of the intervention, 249 (95%) of the children received both complementary food and breast milk. About 12% of infants in the intervention and 10.5% in the control groups started to receive complementary foods as early as between the age of 0-2 months. More than half of the children in both groups were introduced to complementary food before the age of six months. The cereal, roots and tuber food group in the control and intervention groups was consumed by 100% of the children and consisted of over 90% of a maize based meal commonly prepared and consumed as thin or stiff porridge. Mean maize intake, number of meals consumed per day, dietary diversity score and other food groups consumed in addition to maize are indicated in Table 1.

### Primary outcomes: difference in aflatoxin and fumonisin contamination between intervention and control groups

Table 2 shows the results of the effect of the interventions on total AFs and total FBs concentration as mean concentrations and prevalence of samples above maximum

**Table 1. Participants' characteristics at baseline and end of the intervention.**

Variable	Intervention	Control
Baseline (n=300; clusters=30)		
Age of child in months, mean (SD) <sup>1</sup>	3.4 (2.1)	3.9 (2.1)
Female, %	44	46
Age of mother in years, mean (SD)	26.9 (6.5)	26.0 (5.9)
Education level of the mother		
Never attended, %	10	15
Primary education, %	75	71
Secondary education, %	15	14
Marital status		
Single, living with parents, %	23	24
Married, %	74	75
Widowed, %	1	1
Divorced, %	2	0
Number of children, mean (SD)	4 (2)	3 (2)
End (n=261; clusters=30)		
Children introduced to complementary foods	128	121
Age at introducing complementary food		
Mean, months, (SD)	4.8 (1.2)	4.6 (1.6)
0-2 months, %	12	10.5
<6 months, %	58	57
Maize intake		
Mean, g/day (SD)	56 (37)	59 (35)
Range, g/day	0.26-172	0.13-187
Other food group (%)		
Legumes	25	27
Vitamin A rich fruits and vegetables <sup>2</sup>	45	48
Dairy	27	23
Flesh food <sup>3</sup>	25	30
Other fruits and vegetables	8	8
Eggs	0.9	0
Dietary diversity score, mean (SD)	2.3 (0.9)	2.2 (0.9)
Number of meals per day, mean (SD)	2.4 (1)	2.6 (0.9)

<sup>1</sup> SD = standard deviation.

<sup>2</sup> Includes, pumpkin, carrots, yellow/orange sweet potatoes, ripe mango or papaya, passion fruit, any dark green leafy vegetables (spinach/amaranth/cassava), and other locally grown yellow/orange-colour fruits or vegetables.

<sup>3</sup> Includes, meat, poultry and fish.

limits. The intervention group had significantly lower total AFs and total FBs mean concentrations than the control group, intervention effects: 4.9 µg/kg ( $P<0.001$ ) for total AFs and 405.0 µg/kg ( $P=0.001$ ) for total FBs, corresponding to a relative difference of 83 and 70% for total AFs and total FBs, respectively. Similarly, the proportion of samples exceeding the maximum limit of 10 µg/kg set by the Tanzania Bureau of Standards for AFs contamination in maize (TBS, 2012) or

2,000 µg/kg set by Codex Alimentarius Commission for FBs in maize meal (CAC, 2014) in the intervention group was lower compared to that in the control group, intervention effect: 19% ( $P<0.001$ ) corresponding to a relative difference of 95% for total AFs and 11% ( $P<0.001$ ) corresponding to a relative difference of 72% for total FBs. Adjusting the models for baseline data did not modify the results.

### Secondary outcomes: difference in aflatoxins or fumonisins estimated intake and underweight between the intervention and control groups

Table 3 shows the differences in total AFs and total FBs estimated intake as well as prevalence of underweight among infants. The proportion of children exceeding PMTDI and the mean intake for FBs was significantly lower in the intervention compared to control group; group differences, 36% ( $<0.001$ ) for children above PMTDI and 3.9 µg/kg bw/day for FBs mean intake ( $P<0.001$ ) corresponding to a relative difference of 60% and 65% respectively. Similarly, mean intake for AFs in the intervention group was significantly lower in the intervention compared to control group; group differences 49 ng/kg bw/day ( $P<0.001$ ), equivalent to a relative difference of 78%. The proportion of children exceeding the health guidance value for AFs did not differ between the intervention and control groups. The prevalence of underweight was significantly lower in intervention group compared to the control group, after 6 months of intervention (group difference =6.7%;  $P=0.014$ ) and group mean differences in WAZ was 0.57 ( $P=0.007$ ). AFs and FBs intake were inversely associated with WAZ. AFs and FBs intake in the control group had a significant inverse association with WAZ ( $\beta=-0.041$ ; 95% CI: -0.067;-0.014;  $P=0.003$  for FBs intake and  $\beta=-0.007$  (-0.009;-0.0004),  $P=0.039$  for AFs intake). In the intervention group, AFs or FBs estimated intake were not significantly associated with WAZ ( $\beta=-0.032$ ; 95% CI: -0.049,-0.034;  $P=0.311$  for FBs, 0.008 (-0.016;-0.0005);  $P=0.068$  for AFs).

### Intervention compliance

Almost all households (96-100%) in the intervention group complied with the protocol (Table 4). The lowest compliance (72%) was observed for the maize de-hulling practice. In the control villages, occasionally, farmers practiced recommendations directed to the intervention group (Table 4).

## 4. Discussion

To our knowledge, this is the first randomised controlled trial intervention study to reduce or prevent AFs and FBs contamination in food crops and subsequent dietary exposure using local post-harvest practices, in rural areas. It reports the effectiveness of post-harvest measures (available in rural Tanzania) in preventing and reducing AFs and FBs

**Table 2. The difference in change (from baseline to end of intervention) of mean contamination and proportion of sample above maximum limit of aflatoxin and fumonisins in maize, between intervention and control groups (n=300, cluster=30).**

Parameter <sup>1</sup>	Mean $\pm$ standard deviation or % <sup>2</sup>		Differences <sup>3</sup>			Relative differences (%)
	$\Delta I$	$\Delta C$	Effect (95% CI)	P-value	ICC	
Total AFs, ( $\mu\text{g}/\text{kg}$ )	1.10 $\pm$ 0.70	5.9 $\pm$ 3.7	-4.9 (-7.3, -2.5)	<0.001	0.14	83
Total FBs, $\mu\text{g}/\text{kg}$	-745 $\pm$ 512	-317 $\pm$ 247	-405 (-647, -162)	0.001	0.10	70
Total AFs, >ML <sup>4</sup> , %	0.25	20	-19.3 (-28, -11)	<0.001	0.14	95
Total FBs >ML <sup>5</sup> , %	19	8	11 (10, 25)	<0.001	0.15	72

<sup>1</sup> AFs = aflatoxin B<sub>1</sub> + B<sub>2</sub> + G<sub>1</sub> + G<sub>2</sub>; FBs = fumonisin B<sub>1</sub> + B<sub>2</sub>.

<sup>2</sup>  $\Delta C$  = difference of aflatoxin or fumonisin contamination before and after the intervention in the control group;  $\Delta I$  = difference of the aflatoxin or fumonisin contamination before and after the intervention in the intervention group.

<sup>3</sup> CI = confidence interval; ICC = intraclass correlation.

<sup>4</sup> Maximum limit (ML) for AFs 10  $\mu\text{g}/\text{kg}$  for total AFs in maize according to Tanzania standard (TBS, 2012)

<sup>5</sup> Maximum limit for FBs 2,000  $\mu\text{g}/\text{kg}$  of maize meal according to codex standard (CAC, 2014).

**Table 3. The difference in end of study mycotoxin intake and weight-for-age-Z-score (n=261, cluster = 30) between the intervention and control groups.**

Variable <sup>1</sup>	Mean $\pm$ standard deviation or %		Differences <sup>2</sup>		
	Intervention	Control	Diff (95% CI)	P-value	ICC
AFs intake, ng/kg bw/day	14 (13)	63 (59)	-49 (-69, -25)	<0.001	0.12
FBs intake, $\mu\text{g}/\text{kg}$ bw/day	2.0 (3.2)	6.0 (9.4)	-3.9 (-5.8, -2.2)	<0.001	0.13
AFs intake, proportion > ETDI <sup>3</sup> , %	100	100	N/A	N/A	N/A
FBs intake, proportion > PMTDI <sup>4</sup> , %	26	60	-36 (-47, -24)	<0.001	0.45
WAZ	0.12 (1.10)	-0.47 (1.30)	0.57 (0.16, 0.98)	0.007	0.03
Proportion WAZ < -2 SD, %	2.8	9.0	6.7 (-12.6, -1.4)	0.014	0.05

<sup>1</sup> AFs = aflatoxin B<sub>1</sub> + B<sub>2</sub> + G<sub>1</sub> + G<sub>2</sub>; FBs = fumonisin B<sub>1</sub> + B<sub>2</sub>; WAZ = weight-for-age-Z-score; SD = standard deviation.

<sup>2</sup> CI = confidence interval; diff = difference of mean or percentage between intervention and control group; ICC = intraclass correlation; N/A = not applicable.

<sup>3</sup> ETDI = estimated tolerable daily intake; for AFs = 0.017 ng/kg bw/day equivalent to the margin of exposure of 10,000 considered as a cut-off point of low public health concern (EFSA, 2005).

<sup>4</sup> PMTDI = the provisional maximum tolerable daily intake; for FBs = 2  $\mu\text{g}/\text{kg}$  bw day (WHO, 2011).

**Table 4. The difference in proportions of farmers who applied various postharvest practices between the intervention (n=136, cluster=15) and control (n=125, clusters=15) groups.**

Practice	Intervention (%)	Control (%)	P-value
Drying on mat/raised platform	100	42	<0.001
Hand sorting	99	35	<0.001
Moisture content testing (salt and bottle technique)	96	0.02	<0.001
Insecticides use	100	15	<0.001
De-hulling of maize before milling	72	54	0.028

contamination of maize and thereby reducing the risk of exposures to the mycotoxins in infants.

The intervention effectively reduced or prevented AFs and FBs contamination in maize and subsequent dietary exposure to the toxins in infants. At the end of



the intervention it was observed that the intervention group had a significantly lower total AFs and FBs mean concentration relative to the control group. The observed difference of the mycotoxin mean concentration in the intervention is equivalent to 83 and 70% lower in total AFs and total FBs, respectively, of that in control group. AFs concentration level and prevalence was lowest at harvest stage in both groups, and increased during storage in the control group with little increase in the intervention group (Supplementary Table S1). The observed small increase for intervention group compared to control group at the end of the intervention is an indication that the intervention was able to restrict proliferation of *Aspergillus* spores and production of aflatoxins in storage since AFs are mainly produced during storage period. On the other hand, as a pre-harvest natural contaminant, FBs was highest at harvest period in both groups, the observed differences in FBs contamination is again an indication that the intervention was able to reduce FBs level formed pre-harvest or at harvest. FBs decrease was observed in both groups. However, the decrease was significantly higher in the intervention than in the control group. FBs decrease in both groups can be explained by the fact that the baseline data was obtained immediately when the maize was brought in from the field prior to any initial sorting and that 35% (Table 4) farmers in the control group reported that they sorted maize. The results are in accordance with the findings that, prevention strategies applied post-harvest, especially during the storage stage can only be effective for mycotoxins that are formed during this stage of the food chain (Magan and Aldred, 2007). These are also in order of the findings that pre-harvest natural contamination can only be minimised postharvest by application of processing techniques as the case for sorting which will minimise subsequent entry into the food chain (Magan and Aldred, 2007).

The obtained difference in estimated mean AFs or FBs intake between the two groups was consistent with observed difference in mean contamination of AFs and FBs in maize. The observed mean intake in the intervention group is equivalent to 78 and 65% lower in AFs and FBs intake, respectively of that in control group. This difference of AFs and FBs intake between the two groups has public health relevance given that our intervention targeted children whose ages are within the critically important first 1000 days. At this stage of life they have a lower body weight and lower ability to detoxify the toxins because the gut is not yet fully developed and is more permeable (Faustman *et al.*, 2000).

The observed mean difference in WAZ and prevalence of underweight between intervention and control groups was consistent with observed differences in AFs and FBs intake between the two groups. In addition, a significant negative inverse association between intake of AFs and FBs, and WAZ was observed for the control group. Findings from

this study are in line with findings reported in earlier studies in Tanzania (Kimanya *et al.*, 2010; Magoha *et al.*, 2014b; Shirima *et al.*, 2015) and other African countries (Gong *et al.*, 2002, 2004) which reported an inverse association of AFs or FBs exposures with child growth. WAZ is a composite index and is influenced by both the height-for-age of a child and its weight-for-height. It is therefore not possible to be certain whether the obtained prevalence of underweight was due to linear growth retardation, wasting or both. However, in the absence of significant wasting (weight-for-height) in a community, prevalence of 'weight-for age' reflects that of 'height-for age', in that both reflect the long term health and nutritional experience of the individual or population (WHO, 1995). According to the 2014 national nutrition survey in Tanzania, the rate of stunting, underweight and wasting in the studied areas ranges from 36.0 to 37.4%, 11.5 to 13.8% and 2.0 to 3.8%, respectively (TFNC, 2014). Given the low prevalence of wasting in the area, it is likely that the observed effect on WAZ is mostly attributable to improved linear growth.

The mechanisms underlying impaired growth and AFs exposure are still unclear but intestinal function damage, reduced immune function and alteration in the insulin-like growth factor axis caused by liver damage (Knipstein *et al.*, 2015), are suggested hypotheses. Similarly, the mechanism by which the FBs interfere with growth is not well understood. However, it has been shown that FBs inhibits ceramide synthase, which inhibits sphingolipid metabolism (Bouhet and Oswald, 2007). Complex sphingolipids are vital to cell membrane integrity and disturbance in this biosynthetic pathway could affect intestinal epithelial cell viability and proliferation, modify cytokine production, and modulate intestinal barrier function, affecting mucosal immunity (Bouhet *et al.*, 2006). The suggested mechanism for both AFs and FBs cause damage to the intestinal tract, leading to reduced absorptive capacity or impaired intestinal barrier function which may increase the risk of enteric infection and impaired nutrient absorption (Smith *et al.*, 2012) therefore mediating impaired growth.

Limiting of AFs or FBs contamination in food crops and subsequent dietary exposure by application of simple intervention measures in a subsistence farming community has been also reported in Guinea (Turner *et al.*, 2005) and South Africa (Van der Westhuizen *et al.*, 2010) from a non-randomised controlled studies. There are many other potential post-harvest intervention procedures to mitigate AFs and FBs contamination including chemical deactivation such as nixtamalization, sorting by mechanical means with near-infrared spectroscopy (Pearson *et al.*, 2004) and application of modified atmospheres (Samapundo *et al.*, 2007). However, adoption of these strategies in rural areas of developing countries like Tanzania is challenging given the lack of necessary facilities, low technology and low capital. Other strategies to reduce exposure include

enforcement of a maximum limit of AFs and FBs in maize. However, enforcement of a maximum limit would not be effective for people in the rural areas who consume what they harvest without official check of safety.

The strength of this study was the involvement of the health and agriculture extension officers during the delivery of the intervention. The positive findings and feedback from health and agriculture extension officers and farmers is encouraging, providing evidence for the feasibility of scaling up the intervention. A limitation of this study is lack of baseline data on various outcomes. The fact that AFs and FBs estimated intake and WAZ were measured only at post-intervention means that it is not possible to unambiguously relate the observed group difference in the mentioned variables to the effect of the intervention. However, the randomisation and consistency between the contamination and intake variables increase our confidence on the internal validity of the findings. Another limitation of this study is the unbalanced dropout. As observed in Figure 1, reasons were not related to the intervention. Those lost to follow-up were similar in baseline characteristics to their peers. This study used maize as the only source of AFs and FBs intake. It is possible that other crops used for complementary food would also contribute to AFs and FBs intake. However, the contribution of roots, tubers and other cereals that are susceptible to mycotoxin contamination to the diet was quite small. Another limitation is lack of sickness information, such as acute respiratory infection and diarrhoea, that might have confounded the study outcomes. An additional limitation could be the consequence due to the usage of insecticides, which if not properly handled could result into increased exposure to insecticide residues. During the review process, we noted that the study was possibly underpowered as 30 clusters per arm were required to obtain the hypotheses effect. We carried out an additional power analysis and obtained a power of 95% for the proportion of children exceeding PMTDI for total FBs intake.

## 5. Conclusions

Post-harvest intervention measures are effective in mitigating AFs and FBs contamination of maize thereby reducing risk of dietary exposure to the toxins in Tanzanians infants at risk. The developed mitigation package may be promoted for widespread implementation as a public health measure for people in the rural areas who consume what they harvest without official check of safety. Moreover, the relatively simple intervention package may be generalised in other communities where high prevalence of growth retardation, environmental conditions and poor agricultural practices favour production of AFs and FBs in food crops.

Despite several limitations, the results of this randomised controlled trial are promising, and highlight that it may

be time for agricultural interventions to also consider mycotoxin contamination of crops to achieve improved nutrition and health outcomes. In addition, the post-harvest intervention measures are aligned with local customs and policies in Tanzania. As such the intervention package applied in the present study may be integrated into the existing community health and agriculture programs in Tanzania. These simple intervention measures, at the farmers' level, are useful in developing countries, where resources are limited and sophisticated technologies are lacking.

## Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/WMJ2017.2234>.

**Table S1.** Aflatoxin and fumonisin contamination in maize for intervention and control groups at baseline and end of intervention.

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