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Co-occurrence of fumonisins with aflatoxins in home stored maize for human consumption in rural villages of Tanzania

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5 1 **Co-occurrence of fumonisins with aflatoxins in home stored maize for**
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7 2 **human consumption in rural villages of Tanzania**
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24 17

25 18 **Keywords:**

26 19 Aflatoxins, Co-occurrence, Fumonisins, Home stored maize, Tanzania

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1

2 **Abstract**

3 This study determined maize-user practices that influence the presence of fumonisin
4 and aflatoxin contamination of maize in food consumed in the rural areas of Tanzania.
5 Samples of the 2005 maize harvest in Tanzania were collected from 120 households
6 and examined for fumonisins and aflatoxins. Information on whether the maize was
7 sorted to remove defective (visibly damaged or mouldy) maize prior to storage and
8 whether the damaged and mouldy maize or the non-dehulled maize was used as food
9 was also collected. Also, the percentage of defective kernels in the samples was
10 determined. Ninety percent of the households sorted out defective maize, 53%
11 consumed the defective maize and 30% consumed non-dehulled maize. In 52% of the
12 samples, fumonisins were determined at levels up to 11,048 µg/kg (median, 363 µg/kg)
13 and in 15% exceeded 1000 µg/kg; the maximum limit (ML) for fumonisins in maize for
14 human consumption in other countries. Aflatoxins were detected in 18% of the samples
15 at levels up to 158 µg/kg (median, 24 µg/kg). 12% of the samples exceeded the
16 Tanzanian limit for total aflatoxins (10 µg/kg). Aflatoxins co-occurred with fumonisins
17 in 12% of the samples. The percentage defective kernels (mean, 22%) correlated
18 positively ($r=0.39$) with the fumonisin levels. Tanzanians are at risk of exposure to
19 fumonisins and aflatoxins in maize. There is a need for further research on fumonisin
20 and aflatoxin exposure in Tanzania to develop appropriate control strategies.

1 Introduction

2 Maize is a staple food for people living in sub-Saharan Africa, particularly those living
3 in the rural areas. The cereal is frequently contaminated with fumonisins (Shephard et
4 al. 1996, Miller 1995) and aflatoxins (Miller 1995). Visibly damaged and mouldy
5 maize contains more fumonisins and aflatoxins compared to visibly good quality maize
6 (Fandohan et al. 2005, Kpodo et al. 2000, Sydenham et al. 1990). The fumonisins of
7 agricultural and health importance are fumonisin B₁ (FB₁), fumonisin B₂ (FB₂) and
8 fumonisin B₃ (FB₃) (Miller 1995, Doko and Visconti 1994). Studies in animals
9 demonstrate that fumonisin B₁ (FB₁) causes liver cancer in male BD IX rats and female
10 B6C3F1 mice and kidney cancer in male Fischer 344 rats (International Agency for
11 Research in Cancer (IARC), 2002). In humans, FB₁ has been epidemiologically
12 associated with high rates of oesophageal cancer reported in the former Transkei region
13 of South Africa (Rheeder et al. 1992) and in China (Wang et al. 2000;, Chu and Li
14 1994). The International Agency for Research on Cancer (IARC) declared FB₁ as group
15 2B carcinogen; possibly carcinogenic to man. The Joint FAO/WHO Expert Committee
16 on Food Additives (JECFA) assigned a provisional maximum tolerable daily intake
17 (PMTDI) of 2 µg/kg bw/d for FB₁, FB₂ and FB₃, alone or in combination (WHO 2002).
18
19 Aflatoxins of public health importance that occur naturally in cereals exist in four
20 forms, namely aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂) with AFB₁
21 being the most potent form of the aflatoxins (International Agency for Research on
22 Cancer [IARC] 1993). These toxins are acutely toxic, immunosuppressive, mutagenic,
23 teratogenic, and carcinogenic.

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8 2 Fumonisin and aflatoxins have a widespread occurrence in maize worldwide. In Africa,
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10 3 fumonisin in maize have been extensively studied in South Africa (Shephard et al.
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12 4 2007, Shephard et al. 1996, Sydenham et al. 1990). Reports are also available on the
13
14 5 presence of fumonisin in other African countries including Kenya (Kadera et al. 1999),
15
16 6 Benin (Fandohan et al. 2006), Ghana (Kpodo et al. 2000) and Zimbabwe (Gamanya
17
18 7 and Sibanda 2001). Doko et al. (1996) detected fumonisin in 92.5% of maize samples
19
20 8 from Eastern and Southern Africa (including 9 samples from Tanzania) suggesting a
21
22 9 widespread occurrence of the toxins in that region. Also widespread occurrence of
23
24 10 aflatoxins in maize has been reported in African countries (Shephard, 2003; Williams
25
26 11 et al. 2004). For instance, mean total aflatoxins in 45% and 38.8% of samples of maize
27
28 12 collected from farmers in Nigeria and Benin were 200 and 105 $\mu\text{g}/\text{kg}$, respectively
29
30 13 (Shephard, 2003). The author also reported that total aflatoxins in 15 of 16 samples of
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32 14 Kenkey (a fermented maize product in Ghana) ranged from 6.15-196.1 $\mu\text{g}/\text{kg}$. Samples
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34 15 of maize collected from households in Kenya affected by outbreaks of aflatoxicosis in
35
36 16 2004 contained aflatoxins at levels up to 20,000 $\mu\text{g}/\text{kg}$ (Azziz-Baumgartner et al., 2005).
37
38 17 Also a survey of aflatoxins in food supplies in Tanzania showed high contamination of
39
40 18 these toxins in maize. In this survey, 11.2% of 472 samples of maize contained total
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42 19 aflatoxins at levels up to 69.3 $\mu\text{g}/\text{kg}$ (United Republic of Tanzania (URT) 1989). Thus,
43
44 20 dietary exposure to fumonisin and aflatoxins among Tanzanians consuming maize is
45
46 21 likely. It is estimated that the annual per capita consumption of maize in Tanzania is
47
48 22 112.5 kg (which is equivalent to 308g per day) and the national maize utilization is
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50 23 estimated to be three million tons per year (Food Security Department (FSD) 1996).
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52 24 The Tanzania Food and Nutrition Centre (TFNC), a governmental institution promoting

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5 1 intake of nutritious food advocate daily per capita consumption of 771 g for non-
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7 2 dehulled maize flour or rice and 790 g for dehulled maize flour for adequate energy
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9 3 intake for an adult individual living in communities relying on these cereal products for
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11 4 food (Tanzania Food and Nutrition Centre [TFNC] 1997).
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16 6 Despite maize being a staple food for the majority of Tanzanians, no further studies
17
18 7 have been conducted to understand the magnitude of the fumonisin and aflatoxin
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20 8 problem in Tanzanian maize. Data on occurrence and level of fumonisins and
21
22 9 aflatoxins in Tanzanian maize are needed for use in fumonisin and aflatoxin exposure
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24 10 assessments that will provide information on the extent of fumonisin and aflatoxin
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26 11 intake among Tanzanians and facilitate decision making for appropriate preventive
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28 12 actions.
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35 14 This paper reports fumonisin and aflatoxin contamination of maize and maize user
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37 15 practices that influence their presence in food consumed in the rural areas of the main
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39 16 maize producing regions of Tabora, Kilimanjaro, Ruvuma and Iringa in Tanzania.
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45 46 47 18 **Materials and Methods**

48 49 50 19 *Study areas and selection criteria*

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53 20 The study was conducted in the villages of Nyabula (Iringa region), Litapwasi
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55 21 (Ruvuma region), Kikelelwa (Kilimanjaro region) and Kigwa (Tabora region) in
56
57 22 Tanzania (Figure 1). [Insert Figure 1 about here] The regions were chosen based on
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59 23 their high maize production capacity compared to other regions of the country (FSD
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5 1 1996). For each region, the Regional Administrative Director suggested the leading
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7 2 district in terms of maize production and the District Administrative officer suggested a
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9 3 village with households relying on maize as staple food and located in an area that was
10
11 4 accessible by road was selected for the survey. In each village, 30 households who (in
12
13 5 accordance with the views of the village executive officers) could have some stock of
14
15 6 maize were selected for the survey. Therefore, a total of 120 households; 30 selected
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17 7 from each of the villages were visited for collection of samples of stored maize and
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19 8 data on maize sorting and user practices.
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24 9 *Climatological data of the study areas*

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27 10 Data on agro-ecological location for each region were obtained from the Ministry of
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29 11 Agriculture, Food Security and Cooperatives, Dar es Salaam, Tanzania. Data on mean
30
31 12 temperature and relative humidity recorded over 35 years (1971 – 2005) for Ruvuma
32
33 13 and Iringa were obtained from the Tanzania Meteorological Agency (TMA), Dar es
34
35 14 Salaam Tanzania. These climatological data are summarized in table 1. [Insert Table I
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37 15 about here]
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42 16 *Sampling of maize*

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45 17 In January 2006, five to six months after harvest, sampling of maize for human
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47 18 consumption was done in all the selected households. In the households, maize was
48
49 19 found stored in shelled or unshelled form. For stores where maize was found stored in
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51 20 unshelled form, the the stock was divided into four equal parts. Nine cobs were then
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53 21 taken from each of the 4 parts; three from different random points on the upper, centre
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55 22 and bottom layers of the stock. All the cobs were shelled after which the kernels were
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57 23 mixed to obtain a homogeneous sample. In case of households where maize was found
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1 stored in shelled form (commonly in 2 to 6 bags, each of about 100 kg), a probe (grain
2 trier) was used to draw incremental samples from various points of each bag. The
3 incremental samples were thoroughly mixed to compose an aggregate sample. In both
4 cases, at least one kilogram of the well mixed sample was packaged in khaki paper
5 bags, sealed and then transported to the Tanzania Food and Drugs Authority laboratory
6 in Dar es Salaam for analysis.

7

8 *Collection of information on maize sorting and user practices*

9 During sampling and by use of a simple questionnaire, the head of each household was
10 requested to give information on whether the maize sampled had been sorted prior to
11 storage. He was also requested to give information on whether the defective maize was
12 discarded or used as animal feed, raw material for local brews or as human food. In
13 addition he was requested to give information on type of staple food for the household
14 and whether they consumed non-dehulled maize meal (Dona) or dehulled maize meal
15 (Sembe). Dona is a widely used Swahili word for stiff porridge made from non-
16 dehulled maize meal, whereas Sembe is a Swahili word for stiff porridge made from
17 dehulled maize meal.

18 *Determination of fumonisins*

19 Fumonisin B₁ and B₂ in the maize were determined by an HPLC method based on
20 Sydenham et al. (1992) and the slight modifications made by Samapundo et al. (2006).

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22 *Extraction and clean-up.*

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1 Fumonisin were extracted overnight from 15 g of a finely ground portion of the maize
2 with 40 ml of methanol: water (3:1, v:v) in 100 ml glass bottles fitted on a horizontal
3 laboratory shaker. The slurry was filtered through Whatman No 1 filter paper and the
4 bottle rinsed with 10 ml of the mix of methanol and water. The pH of the filtrate was
5 measured and values were always within the normal pH range for maize based material
6 (between 5.8 and 6.5).

7
8 A 10 ml aliquot of the filtered extract was applied to a strong anion exchange (SAX)
9 cartridge (Varian, Bond-Elut LRC, 500 mg, Varian Belgium NV/SA, Belgium) fitted
10 to a solid phase extraction manifold (Alltech, 24- Port SPE Vacuum Manifold System,
11 ALLTECH Associates Inc, Lokeren, Belgium). Before applying the extract, the SAX
12 cartridge was conditioned with 5 ml of methanol, followed by 5 ml of a methanol-water
13 mix (3: 1, v:v). After application of the extract, the SAX cartridge was then washed
14 with 8 ml of the methanol-water mix (3:1, v: v), followed by 3 ml of methanol. The
15 fumonisins were eluted from the cartridge with 10 ml of 1% (v: v) glacial acetic acid in
16 methanol. The eluate was collected and evaporated to dryness at 60°C under a gentle
17 stream of nitrogen using a Nitrogen Evaporator (PIERCE model 18780, Reacti-Vap)
18 coupled with a dry bath (PIERCE Reacti-Therm), both supplied by Rockford, Illinois,
19 USA.

20 *HPLC analysis.*

21 The dry fumonisins were dissolved in 200 µl of methanol. The solution was thoroughly
22 mixed with 200 µl of a derivatizing reagent prepared by dissolving 40 mg of ortho-
23 phthaldehyde in a mixture of 1 ml of methanol, 5 ml of 0.1 M sodium tetraborate and
24 50 µl of β-mercaptoethanol. Twenty µl of the mixture was injected within 4 min into

1 the HPLC for analysis using a reversed-phase HPLC fluorescence detection system. A
2 Waters HPLC system consisting of a Waters 600 pump and controller was used. The
3 system was connected to a Shimadzu SIL-10ADvp auto injector. Chromatographic
4 separations were performed on a Discovery C8 column (100 X 4.6 mm, 5 μ m;
5 SUPELCO, Supelco Park, Bellefonte, PA, USA). Methanol – 0.1 M sodium
6 dihydrogen phosphate (75:25, v:v) mixture adjusted to pH 3.35 with orthophosphoric
7 acid was used as mobile phase. The flow rate of the mobile phase was 1 ml/min.
8 Fluorescence of the fumonisin OPA derivatives was detected at wavelengths of 335nm
9 (excitation) and 400nm (emission) using a Shimadzu RF-10AXL fluorescence detector
10 and recorded with a Shimadzu C-R3A Chromatopac integrator.

11
12 The limit of detection of the analytical method, defined as the mean value of the blank
13 readings plus three standard deviations, was 53 μ g/kg for fumonisin B₁ and 47 μ g/kg for
14 fumonisin B₂. To evaluate suitability of the method blank samples of maize were
15 spiked with FB₁ and FB₂ each at 100, 200, 300, 400 and 500 μ g/kg. Average recovery
16 values were 106% [five samples, relative standard deviation (RSD) of 16.6%] and 92%
17 (five samples; RSD of 15.3%) for FB₁ and FB₂ respectively. The results were corrected
18 for recovery.

19 *Determination of aflatoxins*

20 Aflatoxin B₁, B₂, G₁ and G₂ were determined in the maize in accordance with the
21 method described by Stroka et al. (2000) and slight modification made by Samapundo
22 et al. (2007).

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5 1 *Extraction and clean-up procedure.* Briefly, aflatoxins were extracted from 20 g of a
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7 2 finely ground portion of the maize with 40 ml of an acetonitrile-water solution (3:1, V:
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9 3 V) in a 100 ml glass bottle fitted on a horizontal laboratory shaker. The suspension was
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11 4 filtered through two layers of Whatman No 1 filter paper and the bottle rinsed with 10
12
13 5 ml of the acetonitrile-water solution. Four ml of the extract were adjusted to pH 7 by
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15 6 0.1 M NaOH solutions and then diluted by at least 36 ml of phosphate buffer saline
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17 7 solution (pH 7.4, NaCl 0.15 M). The diluted extract was then passed through AflaStar
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19 8 immunoaffinity column (IAC) (Romer Lab, Coring System Diagnostix GmbH,
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21 9 Gernsheim, Germany) fitted to a solid phase extraction manifold (24- Port SPE
22
23 10 Vacuum Manifold System, ALLTECH Associates Inc, Lokeren, Belgium) and allowed
24
25 11 to flow through without application of vacuum. Twenty five ml of water was then used
26
27 12 to rinse the container that held the extracts before passage through the column.
28
29 13 Aflatoxins were eluted by use of 3 ml of methanol applied as 0.5 ml aliquots which
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31 14 were held in the bed for about 3 minutes. A slight vacuum was applied to remove any
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33 15 liquid remaining in the bed.

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40 16 *HPLC analysis.*

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42 17 Twenty μ l of the eluate was injected to the HPLC for analysis using a reversed-phase
43
44 18 HPLC fluorescence detection system. No derivatisation of the aflatoxins was performed
45
46 19 (Samapundo et al., 2007). A Waters HPLC system consisting of a Waters 600 pump
47
48 20 and controller was used. The system was connected to a Shimadzu SIL-10ADvp auto
49
50 21 injector, a Shimadzu RF-10AXL fluorescence detector and Shimadzu C-R3A
51
52 22 chromatopac integrator. Chromatographic separations were performed on a Bondapak
53
54 23 ODS column (250 x 4.6mm, 5 μ m pore size). A methanol-water mixture (1:1, v:v) was
55
56 24 used as mobile phase. Flow rate of the mobile phase was set at 0.7ml/min. Fluorescence
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1 of the aflatoxins was recorded at wavelengths of 360nm (excitation) and 440nm
2 (emission).

3
4 The limit of detection of the analytical method (also defined as the mean value of the
5 blank readings plus three standard deviations) was 0.6µg/kg for each of AFB₁ and
6 AFG₁ and 0.07µg/kg for each of AFB₂ and AFG₂. To assess the repeatability of the
7 method, AFB₁ and AFG₁ were spiked into blank maize samples, each at 0.76, 3.81 and
8 6.85µg/kg and recovered, on average, by 89% (three samples; RSD of 15.7%) and
9 107% (three samples; RSD of 19.8%), respectively. The blank maize samples were
10 also spiked with AFB₂ and AFG₂, each at 0.56, 1.31 and 1.675µg/kg which, on average,
11 were recovered by 87%. The RSD value for three maize samples spiked with the AFB₂
12 was 14.3% and AFG₂, 22.9%. The results were corrected for recovery.

13 14 *Determination of percentage defective kernels*

15 The samples of maize were subjected to examination of percentage defective (broken,
16 wrinkled, visibly mouldy, rotten or discoloured) kernels. Two hundred kernels were
17 randomly taken from each sample and the defective kernels counted out and expressed
18 as percentage of the 200 kernels.

19 20 *Statistical analysis of data*

21 The statistical package used was Stata version 9 (Stata 9.0; Statacorp, Texas, USA).
22 Correlation analysis was used to determine relationship between percentage defective
23 kernels and fumonisin or aflatoxin levels in the samples. Means were compared by
24 analysis of variance (ANOVA).

1

2 **Results and discussion**

3 *Sorting and use of damaged and mouldy maize*

4 Table II shows the number of households who sorted maize before storage and the
5 number of these households who discarded the defective maize and who used it as food,
6 raw material for local brews or animal feed. Out of the 120 households, 110 sorted
7 maize prior to storage separating insect damaged and mouldy maize from the main
8 harvest.

9
10 [Insert Table II about here]

11
12 However, 50 out of the 110 households still admitted to consuming the damaged or
13 mouldy maize. The number of households (24 out of 30) who admitted that they
14 consume damaged or mouldy maize as food in Tabora was higher compared to the
15 number in Kilimanjaro (12 out of 30), Iringa (8 out of 30) or Ruvuma (6 out of 30).

16 The finding that people in Tanzania consumed sorted damaged or mouldy maize is in
17 contrast to a recent report that in the former Transkei region of South Africa, almost all
18 (161) households who participated in a study of exposure to fumonisins in maize used
19 the sorted mouldy maize as animal feed or raw material for beer (Shephard et al., 2007).

20 Consumption of mouldy maize either as direct food or via local brews is indicative of
21 the magnitude of the problem of food insecurity in the rural places (Sydenham et al.
22 1990). In this study relatively fewer households, 30 used the damaged or mouldy maize
23 as animal feed and 19 as raw material for beer. Only 21 of the 110 households said they

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5 1 discard the damaged or mouldy maize. According to the participants to our study,
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7 2 defective maize was separated from good quality maize and used within the first two
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9 3 months after harvest in order to avoid further deterioration which is normally
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11 4 experienced in unsorted maize stored for prolonged time.
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16 6 The observation that people consumed damaged and mouldy maize is of great
17
18 7 importance as mouldy maize contains more fumonisins and aflatoxins compared to
19
20 8 visibly good quality maize. Studying the fate of fumonisins and aflatoxins during
21
22 9 processing of maize to food products in Benin, Fandohan et al. (2005) found that
23
24 10 sorting and winnowing reduced mean aflatoxin level in maize from 6.57 µg/kg to 2.67
25
26 11 µg/kg and mean fumonisin level from 4800 µg/kg to 1500 µg/kg. According to the
27
28 12 researchers the fumonisins were significantly recovered in the discarded mouldy and
29
30 13 damaged kernels. High levels of fumonisins in mouldy maize have been reported by
31
32 14 other researchers in Ghana (52,670 µg/kg) (Kpodo et al. 1996) and South Africa (49
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34 15 900 µg/kg) (Sydenham et al. 1990) Thus, in order to prevent use of mouldy maize as
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36 16 food, farmers should be educated and made to realise the presence of high levels of
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38 17 mycotoxins in the mouldy fraction as well as health effects of the toxins.
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45 *Consumption of Dona*

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48 19 The participants to this study said that they consume *Dona* or *Sembe* as their staple
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50 20 food. From the 120 households, 36 respondents said they consume *Dona*. These
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52 21 respondents represent 90% of households in Kilimanjaro and 27% in Tabora.
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54 22 Households in Iringa and Ruvuma consumed *Sembe*. People consuming *Dona* are at a
55
56 23 higher risk of exposure to mycotoxins than those consuming *Sembe* because *Sembe* is
57
58 24 made from dehulled maize. Dehulling reduces the mycotoxin content in maize by
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1 removing the more contaminated pericarp and germ (Fandohan et al. 2005, Miller
2 1995). Fandohan et al.(2005) reported that in preparation of maize food products in
3 Benin, about 34% of aflatoxin in the maize was removed with the discarded hulls and
4 embryo and that the mean fumonisin content decreased from 2890 $\mu\text{g}/\text{kg}$ (in the raw
5 maize) to 1350 $\mu\text{g}/\text{kg}$ (in the de-hulled maize). It is worth noting that, communities
6 consuming *Dona* maize meal as staple food may use it for preparation of
7 complementary foods for their children (Shephard et al. 1996). Children consuming
8 *Dona* based foods might be at an even higher risk of exposure to mycotoxin given that
9 their food needs per kilogram body weight per day are higher than in adults.

10 *Fumonisin contamination*

11 Fifty two percent of the 120 samples were contaminated with FB_1 at levels up to 6125
12 $\mu\text{g}/\text{kg}$ (median, 206 $\mu\text{g}/\text{kg}$). FB_2 were determined in 36% of the samples at levels up to
13 4923 $\mu\text{g}/\text{kg}$ (median, 239 $\mu\text{g}/\text{kg}$). Total fumonisins (FB_1+FB_2) in the contaminated
14 samples were recorded for 52% of the samples, at levels ranging from 61– 11,048
15 $\mu\text{g}/\text{kg}$ (median, 363 $\mu\text{g}/\text{kg}$). Levels determined by this study compare very well with
16 levels determined by other studies which analysed home-grown maize in rural areas of
17 Africa. Gamanya and Sibanda (2001) reported FB_1 levels ranging from 4000-8000
18 $\mu\text{g}/\text{kg}$ in samples of maize from villages in Zimbabwe. Sydenham et al. (1990) and
19 recently Shephard et al (2007) reported similar fumonisin levels in good quality home
20 grown-maize from the high oesophageal area of the former Transkei region of South
21 Africa (maximum levels of 7900 $\mu\text{g}/\text{kg}$ for FB_1 , 3770 $\mu\text{g}/\text{kg}$ for FB_2 and 10,140 $\mu\text{g}/\text{kg}$
22 for total fumonisins). A similar maximum level of 12 000 $\mu\text{g}/\text{kg}$ was reported by
23 Fandohan et al. (2006) for the 1999-2000 harvest of maize in Benin. However, the
24 highest level of 6125 $\mu\text{g}/\text{kg}$ for FB_1 and 11,048 $\mu\text{g}/\text{kg}$ for total fumonisin determined

1 by this study are higher than the respective contaminations of 165 $\mu\text{g}/\text{kg}$ and 225 $\mu\text{g}/\text{kg}$
2 reported previously by Doko et al. (1996) for samples of maize from Tanzania. The
3 same authors reported total fumonisin levels of 370 $\mu\text{g}/\text{kg}$ for maize from Botswana,
4 135 $\mu\text{g}/\text{kg}$ for maize from Malawi and 2735 $\mu\text{g}/\text{kg}$ for maize from Zimbabwe. A
5 relatively lower maximum level of 4222 $\mu\text{g}/\text{kg}$ for total fumonisins was also reported
6 by Kpodo et al. (2000) for maize in Ghana. As opposed to the current study, the other
7 studies analysed maize from market outlets which is known to contain low levels of
8 contamination compared to home stored maize (Shephard et al., 1996). Just like found
9 by other researchers (Kpodo et al. 2000, Doko et al. 1996), FB_1 was more prevalent and
10 present at higher levels than FB_2 . On average, FB_2 corresponded to 31% of the total
11 fumonisins recorded in this study.

12
13 The occurrence and level of mycotoxins in a country vary from one geographical
14 region to another (Fandohan et al. 2006, Hell et al. 2000). [Insert Table III about here]
15 Table III shows occurrence and distribution of the fumonisin contaminated samples at
16 different levels of contamination in the different regions. Fumonisin were widespread
17 (70%) in Tabora compared to Ruvuma (50), Kilimanjaro (44%) and Iringa (43).
18 During the maize production season of 2005, Tanzania experienced drought which
19 might have influenced contamination of maize with fumonisins. Drought stress
20 condition might have been severe in Tabora compared to the other regions resulting in
21 more favourable conditions for fungal attack and fumonisin formation in maize in that
22 region (Miller 2001). Tabora is generally warm and one of the two regions that
23 experience lower rainfall of 800 – 1000mm/year compared to the other regions which
24 receive 900 – 2000mm/year.

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7 2 Despite Kilimanjaro being less prone to drought, relatively higher levels of fumonisins
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9 3 were observed in samples from this region. Seven percent of samples from Kilimanjaro
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11 4 contained fumonisin levels above 4000 $\mu\text{g}/\text{kg}$, the MTL in whole maize products in the
12
13 5 US (Soriano and Dragacci 2004). Farmers in Kilimanjaro region harvested maize from
14
15 6 May to July, a period which coincided with the main rain period of March to June
16
17 7 (Table I). Under rainfall condition, mature maize experiences prolonged periods of high
18
19 8 water content that may favour mould growth and mycotoxin formation. Gamanya and
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21 9 Sibanda (2001) reported that FB_1 concentration in food in Zimbabwe decreased from
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23 10 regions with high rainfall and annual moderate temperature to low rainfall regions.
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31 12 Since Tanzania (just like most other African countries) does not have a MTL for
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33 13 fumonisins, contamination results obtained in this study were compared with the MTL
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35 14 of 1000 $\mu\text{g}/\text{kg}$ set for fumonisins in maize for human consumption in other countries
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37 15 including the European Union (van Egmond et al. 2007) and Switzerland (Soriano and
38
39 16 Dragacci 2004). It was observed that 15% of the 120 samples (at least 10% of samples
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41 17 from each of Kilimanjaro, Tabora, Iringa and Ruvuma) exceeded this limit (Table III).
42
43 18 Shephard et al. (1996) indicated that the MTL of fumonisins set in developed countries
44
45 19 were based on low maize consumption (as low as 8.8 g of maize/person/day). This
46
47 20 means that with the high per capita maize consumption of 308g of maize/person/day
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49 21 in Tanzania (FSD 1996) an appropriate MTL would be set far below the 1000 $\mu\text{g}/\text{kg}$.
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51 22 In this case, more than 15% of the samples would exceed the appropriate MTL if it
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53 23 existed in Tanzania.
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5 1 *Aflatoxin contamination.*

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7 2 The AFB₁ was determined in 12% of the samples at levels ranging from 5 – 90 µg/kg
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9 3 (median, 38µg/kg). AFG₁ was determined in 9% of the samples at levels ranging from
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11 4 4 – 89 µg/kg (median, 29 µg/kg), AFB₂ in 8% of the samples at a range from 1-20
12
13 5 µg/kg (median, 6 µg/kg) and AFG₂ in 10% of the samples at levels from 1 – 17 µg/kg
14
15 6 (median, 3 µg/kg). Total aflatoxins (AFB₁+AFB₂+AFG₁+AFG₂) were recorded for
16
17 7 18% of the samples at levels ranging from 1-158 µg/kg (median, 24 µg/kg). The study
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19 8 confirms a previous report by the United Republic of Tanzania [URT] (1989), that
20
21 9 Tanzanian maize is contaminated with unacceptable levels of aflatoxins.
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27
28 11 Table IV shows distribution of aflatoxins contaminated samples in the regions.
29
30 12 Occurrence (%) of aflatoxin contaminated maize was significantly higher in Tabora
31
32 13 compared to the other regions (P< 0.016). Thirty seven percent of samples from Tabora
33
34 14 were contaminated with AFB₁ or total fumonisins. Aflatoxins were determined in 20%
35
36 15 of the samples from Kilimajaro. Only a small fraction (7%) of samples from each of
37
38 16 Iringa and Ruvuma were contaminated with aflatoxins. [Insert Table IV about here]
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42 17 As explained for the case of high occurrence of fumonisin contamination, the warm and
43
44 18 dry climatic conditions prevailing in Tabora region could be attributed to these
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46 19 observations.
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52 21 Tanzania regulates maximum limits of AFB₁ and total aflatoxins in food at 5µg/kg and
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54 22 10µg/kg respectively (Tanzania Bureau of Standards [TBS] 2004). In general 11% and
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56 23 12% of the 120 samples were contaminated with AFB₁ and total aflatoxins at levels
57
58 24 above the Tanzania MTL of 5 µg/kg and 10 µg/kg, respectively. Samples from Tabora
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1 accounted for 77% and 64% of the samples exceeding the MTL of 5 µg/kg (AFB₁) and
2 10µg/kg (total aflatoxins) respectively. With the exception of one sample, all
3 AFB₁contaminated samples exceeded the limit of 5 µg/kg.

4 *Co-occurrence of aflatoxins with fumonisins*

5 Fumonisin and aflatoxins co-occurred in 12 out of the 120 samples; 7 from Tabora, 4
6 from Kilimanjaro and one from Ruvuma. Total fumonisin levels in the co-contaminated
7 samples ranged from 111 – 11,048 µg/kg (mean, 2157µg/kg) and aflatoxins from 1-
8 151 µg/kg, (mean, 44µg/kg). Fifty eight percent of the 12 samples were co-
9 contaminated with fumonisins and aflatoxins at levels above the respective MLs of
10 1000 µg/kg and 10 µg/kg. Co-occurrence of aflatoxins with fumonisins has also been
11 reported for maize in Ghana (Kpodo et al. 2000) and in Benin (Hell et al. 2000b).
12 Important to note as well is that presence of fumonisins and aflatoxins in Tanzanian
13 maize is indicative of the presence of *Fusarium* and *Aspergillus* moulds in the maize.
14 *Fusarium* and *Aspergillus* genera of moulds exist in various species that produce other
15 forms of mycotoxins including nivalenol (NIV), Zearalenone (ZEA), Deoxynivalenol
16 (DON) (Sydenham et al. 1990) and Ochratoxin A (Miller 1995, WHO 2002) in food.
17 Zearalenone occurred in two out of 9 samples of maize from Tanzanian which were
18 analysed by Doko et al. (1996) and in one of those samples the toxin co-occurred with
19 fumonisins. This observation is in accordance with the views of other scientists that
20 people consuming maize are at a high risk of exposure to multiple mycotoxins
21 (Shephard et al. 1996, Miller 1995, Sydenham et al. 1990, Kpodo et al. 2000). Thus
22 efforts need be taken to study *Fusarium* and *Aspergillus* species contaminating maize
23 in Tanzania with a view to understand their geographical distribution and potential to

1 produce toxins in food. The information will be useful in formulation of strategies that
2 can target more than one type of mycotoxins.

3 *Relationship between percent defective kernels and fumonisins or aflatoxins*

4 All the samples contained defective kernels irrespective of whether they were drawn
5 from maize that had been sorted before storage or not. Eighty eight percent of the
6 samples contained defective kernels at levels above 7%; the ML recommended by the
7 Codex Alimentarius Commission for maize or corn (Codex Alimentarius Commission
8 (CAC) 1995). All the samples (100%) from Kilimanjaro, 93% from Tabora, 87% from
9 Iringa and 70% from Ruvuma exceeded the ML of 7%. The means of percent defective
10 kernels were not significantly different among the regions ($P>0.3644$). [Insert Table V
11 about here]

12
13 Table V shows a general increase in fumonisin levels from low percentage defective
14 samples to high percentage defective samples. For all the samples containing less than
15 7% defective kernels, fumonisin levels were lower than MTL of 1000 μ g/kg. The
16 maximum fumonisin level increased from 685 μ g/kg (defective range $\leq 7\%$), through
17 5989 μ g/kg (defective range, $7 < 30$) to 11 048 μ g/kg (defective range, $\geq 30\%$). Though
18 weak, there was positive correlation between fumonisin contamination and extent of
19 defective kernels in the sample ($r= 0.39$). Similar observations were made by Charnly
20 (1995) who reported that incidence of defective kernels in maize may be used to
21 predict the likelihood of fumonisin contamination in a maize stock. Nonetheless,
22 despite the presence of more than 7% defective kernels in 100% of the samples from
23 Kilimanjaro, only 44% of samples from that region were contaminated with detectable

1 fumonisins. This observation suggests that more studies are needed to investigate
2 effectiveness of sorting as a means for reduction of fumonisins in maize.

3
4 Aflatoxins were not detected in any of the samples that contained less than 7%
5 defective kernels (Table 7). This finding is in agreement with reports by other
6 researchers who observed a positive relationship between aflatoxins and insect damage
7 in maize in Benin (Hell et al. 2000a, Setamou et al. 1998).

8 9 **Conclusions**

10 This study confirms that fumonisins and aflatoxins are widespread contaminants of
11 maize intended for human consumption in Tanzania. It shows that populations in the
12 rural areas of Tanzania are at a risk of exposure to unacceptably high levels of
13 fumonisins and aflatoxins. Based on their consumption of *Dona* and the high fumonisin
14 contamination determined in samples from the Kilimanjaro region, people in that
15 region are at a relatively higher risk of exposure to fumonisins. Children consuming
16 *Dona* based complementary foods might be at an even higher risk of exposure to these
17 toxins. The findings of this study should trigger further research that will generate data
18 on the fumonisin and aflatoxins exposure among Tanzanians (children inclusive). The
19 exposure data and information on the extent to which dehulling performed by
20 householders actually reduces mycotoxin contamination of maize are needed to provide
21 further scientific basis for strengthening mycotoxin control strategies in this and other
22 developing countries.

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5 1 Despite the existence of regulatory limits for AFB₁ and total aflatoxins in maize for
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7 2 human consumption in Tanzania, a substantial fraction (11%) of maize in rural places
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9 3 contained unacceptable levels of these toxins. The observation is in agreement with
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11 4 views of other authors (Shephard et al. 1996; Williams et al., 2004) that enforcement
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13 5 of MTLs for mycotoxins in foods is a measure that is not protective for people in rural
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15 6 settings. Rural populations consuming own grown crops would eat their produce
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17 7 unchecked for safety limits. Therefore appropriate technologies for minimizing
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19 8 fumonisins in maize during farming, transport, storage and preparation for use need to
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21 9 be adopted by farmers and households in the rural places. These could include adoption
22
23 10 of fungal resistant varieties and good agricultural and handling practices such as use of
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25 11 fertilizers in growing maize and sorting to remove mouldy maize prior to storage and
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27 12 use. In view of this, agricultural extension officers advising farmers on good
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29 13 agricultural practices should be provided with a mycotoxin control package of good
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31 14 agricultural practices that they should advocate to farmers in the rural areas for
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33 15 adoption. Other measures could include campaigns to sensitize the public on effects of
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35 16 mycotoxins in human health and their impact on the economy. As a complementary
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37 17 strategy mothers should be advised to exercise care in selection ingredients for
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39 18 complementary foods and when a possibility exist, maize should be replaced with other
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41 19 least contaminated cereals such as sorghum and millets (Munimbazi and Bullerman
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43 20 1996). Maize containing less than 7% defective kernels contained relatively low
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45 21 contamination of fumonisins and aflatoxins, suggesting that proper sorting of maize
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47 22 prior to consumption is an important measure for the reduction of fumonisin content in
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49 23 maize. However, more studies need be conducted to investigate the extent to which
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5 1 sorting as practised by householders themselves can be relied on as a measure for
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7 2 reduction of mycotoxin contamination in maize in Tanzania.
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11 4 The ongoing efforts to discover fungi resistant crops and decontamination techniques
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13 5 for fumonisins in food will probably provide a long lasting solution to the problem of
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15 6 fumonisin contamination of the staple food for the subsistence-farming systems in the
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17 7 developing countries.
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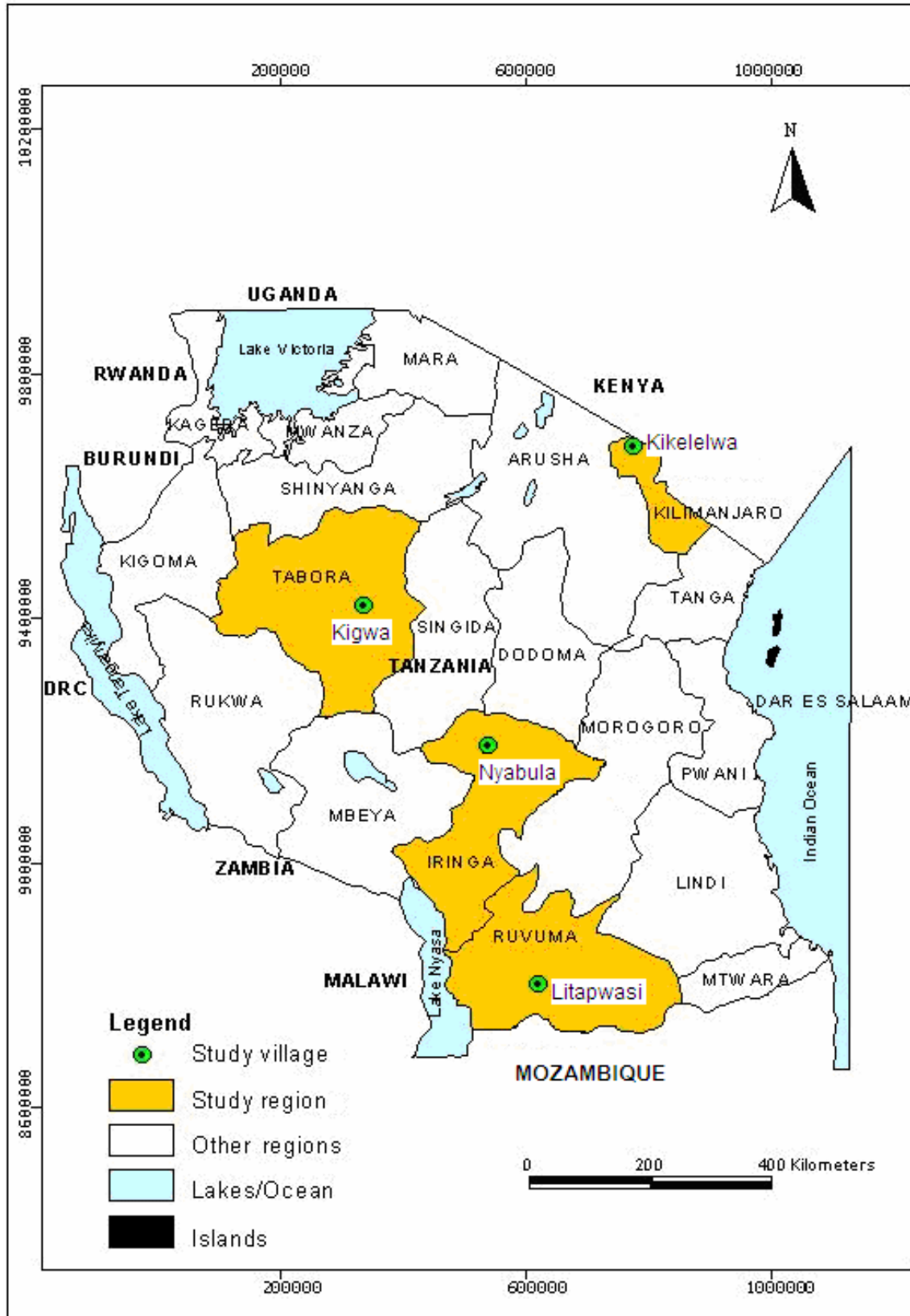
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1. Figure 1: Map of Tanzania showing study sites

1 **Table I: Climatological data for Tabora, Ruvuma , Kilimanjaro and Iringa regions of Tanzania**

Region	Agroecological zone	Sub- zone	Altitude (m)	Range of mean rainfall (mm/year)	Rainfall season	Mean of minimum to maximum temperatures (°C) from 1971-2005	Mean of minimum to maximum relative humidity (%) from 1971-2005
Tabora	Plateau	Western	800 – 1500	800 – 1000	November –April	nr	nr
Ruvuma	Plateau	Southern	800 – 1500	900 – 1300	November – April	16-27	51-77
Iringa	Southern Highlands	Southern	1200 – 1500	800 – 1400	December- April	15-27	47-64
Kilimanjaro	Northern Highlands	Northern	1000 – 2500	1000 – 2000	November – January and March – June	nr	nr

2 nr, no records

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14 5 **Table II. Regional differences in number ^a of households who sorted ^b maize prior to storage and those who used part of the**
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16 **damaged and mouldy maize as feed, local brew and food**
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Region	Sorted prior to storage (n)	Discarded and mouldy maize (n)	Used damaged and mouldy maize as		
			Feed (n)	Liquor (n)	Food (n)
Tabora	26	1	1	0	24
Kilimanjaro	30	0	16	2	12
Iringa	26	4	7	7	8
Ruvuma	28	16	6	0	6
Overall	110	21	30	9	50

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36 7 ^a, No out of 30 households in the region; ^b, households sorted out damaged and mouldy maize to avoid further deterioration of the
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38 8 whole harvest during storage.
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1 Table III. Median fumonisin levels and occurrence of detectable samples at different contamination ranges in Tanzania

Region	FB ₁			FB ₂			Total fumonisins			
	Median ($\mu\text{g}/\text{kg}$)	Occurrence (%) at different ($\mu\text{g}/\text{kg}$) ranging from LOD ^a - ≤ 1000	Occurrence (%) at different ranges >1000 ^b	Median($\mu\text{g}/\text{kg}$)	Occurrence (%) at different ($\mu\text{g}/\text{kg}$) ranging from LOD ^c - ≤ 1000	Occurrence (%) at different ranges >1000 ^b	Median ($\mu\text{g}/\text{kg}$)	Occurrence (%) at different ranges ($\mu\text{g}/\text{kg}$) ranging from LOD ^d - ≤ 1000	Occurrence (%) at different ranges ($\mu\text{g}/\text{kg}$) ranging from 1000 ^b ≥ 4000	Occurrence (%) at different ranges ($\mu\text{g}/\text{kg}$) ranging from >4000
Kilimanjaro	363	30	14	323	30	10	524	30	7	7
Tabora	257	60	10	131	47	3	363	53	17	–
Iringa	145	30	13	291	30	3	501	30	13	–
Ruvuma	144	37	13	485	14	6	155	37	13	–
Overall	206	40	12	239	30	6	363	35	15	2

2 ^a, The limit of detection for FB₁ (53 $\mu\text{g}/\text{kg}$); ^b, MTL set for fumonisins in maize for human consumption in other countries (van Egmond et
3 al., 2007; Soriano and Dragacci 2004); ^c, The limit of detection for FB₂; (47 $\mu\text{g}/\text{kg}$); ^d, The limit of detection for FB₁ (53 $\mu\text{g}/\text{kg}$) or FB₂
4 (47 $\mu\text{g}/\text{kg}$) or sum of these limits (100 $\mu\text{g}/\text{kg}$) as the case may be.

1 Table IV. Occurrence and levels of AFB₁ and total aflatoxins in maize in Tanzania

Region	AFB ₁			Total aflatoxins		
	Occurrence (%) at different ranges (µg/kg)		Range (µg/kg)	Occurrence (%) at different ranges (µg/kg)		Range (µg/kg)
	LOD ^a -≤5	> 5 ^b		LOD ^c -≤10	>10 ^b	
Tabora	3	34	5 – 90	7	30	5 – 158
Kilimanjaro	0	3	80 ^b	13	7	1 – 80
Ruvuma	0	3	15 ^b	3	3	7 – 26
Iringa	0	3	58 ^d	0	7	13 – 58
Overall	12	11	5 - 90	6	12	1 - 158

2 ^a, The limit of detection for aflatoxins AFB₁; (0.6µg /kg); ^b, MTL for AFB₁ or total aflatoxins in maize for human consumption in
3 Tanzania; ^c, The limit of detection for AFB₁ or AFG₁ (0.6µg /kg), AFB₂ or AFG₂ (0.07µg /kg) alone or in combination as the case may be;
4 ^d, one level present.

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3 **Table V. Comparison of occurrence and levels of fumonisins and aflatoxins in maize with different levels of defective kernels**4 **(n=120)**

Samples with defective kernels		Total fumonisins		Total aflatoxins	
Percent defective kernels	No of samples	No of samples	Range ($\mu\text{g}/\text{kg}$)	No of samples	Range ($\mu\text{g}/\text{kg}$)
$\leq 7^{\text{a}}$	15	5	143 - 685	-	-
$7 < 30$	88	43	61 - 5989	14	1 - 158
≥ 30	17	12	65 - 11 048	8	1 - 151

5 ^a, ML of defective kernels in maize

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