Screening of Aflatoxin-Producing Fungi in Maize and Groundnuts from Three Regions in Tanzania

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Abstract

This study screened aflatoxin-producing fungi in maize and groundnuts from Dodoma, Singida and Iringa regions in Tanzania, and assessed the level of peoples' awareness on aflatoxins health effects. One hundred and twenty samples (20 each crop) were collected and inoculated on Sabouraud dextrose agar and one hundred and eleven samples were observed to have fungal growths that were identified to belong to four genera, namely *Aspergillus*, *Rhizopus*, *Mucor* and *Dermatophyte* with occurrence frequencies of 75, 2.5, 5 and 10%, respectively. Among the four screened genera of fungi, *Aspergillus* was observed to be the major aflatoxin-producing fungi. Five species of genus *Aspergillus*, namely *A. flavus*, *A. parasiticus*, *A. niger*, *A. fumigatus*, and *A. terreus* were isolated with frequencies of 39.1, 10, 7.5, 15.8 and 2.5%, respectively. Macromorphology and micromorphology of isolated *Aspergillus* spp were also observed using a light microscope. Furthermore, it was noted that the prevalence of fungi and aflatoxins contamination is more in groundnuts than in maize. In terms of awareness in individuals concerning aflatoxins, only 34% respondents in Dodoma, 29.9% in Singida and 24% in Iringa were aware of aflatoxins. Therefore, the creation of awareness and sensitization on aflatoxins health effects to the people is an important part of intervention ways to forestall and control aflatoxins in Tanzania.

Keywords: Aflatoxins, Maize, Groundnuts, *Aspergillus* spp, *A. flavus*, *A. parasiticus*, *A. niger*, *A. fumigatus* and *A. terreus*.

Introduction

Maize (*Zea mays*) and groundnuts (*Arachis hypogaea*) are the major crops grown for economic and nutritional values in many tropical and subtropical areas of the world, and are also amongst the most susceptible host crops to *Aspergillus* spp, fungal species responsible for aflatoxin contamination (Guo et al. 2009). There is also a strong consensus that aflatoxin contamination of staple food crops like maize and groundnuts possesses big threats to public health, trade and livelihoods in Tanzania (Nyagi et al. 2016, Kamala et al. 2018). For example, the outbreak of an acute aflatoxicosis reported in Dodoma and Manyara regions of Tanzania in 2016 resulted into adverse effects like vomiting, abdominal disorders, diarrhea, jaundice and even death due to insufficient knowledge on aflatoxin-producing fungi and aflatoxins contamination (Kamala et al. 2018). The studies on aflatoxins are extensively done elsewhere in the world

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(Fountain et al. 2015, Kumar et al. 2017, Sserumagaa et al. 2020), while very little researches have been done on aflatoxin-producing fungi in Tanzania (Nyagi et al. 2016, Mohammed et al. 2018, Sasamalo et al. 2018). Therefore, the present study aimed at screening and identifying aflatoxin-producing fungi in groundnuts and maize from selected areas of Tanzania that experience low annual rainfall and high temperature which favor the growth of aflatoxin-producing fungi. The study further assessed the level of awareness on aflatoxins and its health effects in the society.

Materials and Methods
Study area
The samples were collected from three different regions, namely Dodoma, Singida and Iringa regions of Tanzania where Dodoma (6°10'23"S 35°44'31"E) and Singida (04°49'S 34°45'E) form part of the semi-arid central zone of Tanzania due to low and erratic rainfall. Dodoma records average annual rainfall of 600 mm and temperature ranges from 15 to 36 °C (Wikipedia/Dodoma 2021) and Singida records average annual rainfall of 240 mm and temperature ranges from 14 to 32 °C (Wikipedia/Singida 2021, World Weather Online 2021). Iringa (7.77°S 35.69°E) records average annual temperature of 13-25 °C and the average rainfall is 740 mm (Wikipedia/Iringa 2021). The screening and identification of aflatoxin-producing fungi in given samples were conducted at Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

Sampling and sample size
One hundred and twenty (120) maize and groundnut samples (harvested six months before) were collected in May 2020 from the three regions and cultured for fungal growth. Forty samples (20 each crop) were collected from each region making 120 samples in total. The samples were collected randomly from the local markets and households by using sterilized spoons and ziploc bags. The samples were stored at room temperature (25-27 °C)

until further use. The sample size was calculated based on an estimated prevalence rate (Habib et al. 2015, Ibrahim et al. 2017). One hundred and eighty (180) people from selected regions responded to the questionnaire.

Growth and identification of fungi
Finely grinded samples were inoculated into a labeled sterile Sabouraud Dextrose Agar (CM41-Oxoid, UK) medium impregnated with chloramphenicol and incubated at room temperature (25-27 °C) for 3-7 days. Plates were examined grossly for characteristic growth of *Aspergillus* species such as obverse and reverse color according to the method described by James and Natalie (2001) and Bandh et al. (2012). Czapek Dox Agar (CM0097-Oxoid, UK) was used as secondary differential media for specific identification and growth of *Aspergillus* species. The media was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 minutes. The growths were stained using lactophenol on clean glass slide. The slides were observed under 10x and 40x magnifications of a light microscope and the fungi were identified by observing standard specifications given by Barnett and Hunter (1999) and the monograph by Raper and Fennell (1965) was used for identification of *Aspergillus* species (Bandh et al. 2012, Ibrahim et al. 2017).

Data analysis
Data generated through the questionnaire was analyzed using descriptive statistics. The following formula was used:

$$Fr\ (\%) = \frac{\text{Number of samples with a species or genus}}{\text{Total number of samples}} \times 100$$

Where; Fr is isolation frequency. The raw data from questionnaire was analyzed by the software program "Statistical Package for the Social Sciences" (SPSS) to get the level of awareness on aflatoxins and their health effects in society.

Results and Discussion

The isolation frequency of different fungal genera from maize and groundnuts, prevalence of *Aspergillus* spp isolated from maize and groundnuts, macromorphology and micromorphology of different *Aspergillus* spp are presented in Tables 1, 2, 3 and 4, respectively. Furthermore, respondents' awareness on aflatoxins by regions and respondents' awareness on aflatoxins' health effects are presented in Tables 5 and 6, respectively. Among the one hundred and twenty (120) maize and groundnuts samples, 111 samples were observed to have fungal growths belonging to four genera, *Aspergillus*, *Rhizopus*, *Mucor* and *Dermatophyte* whose isolation frequencies were 75, 2.5, 5 and 10%, respectively (Table 1). Among the four genera of fungi, *Aspergillus* was observed to be the major one known to produce aflatoxins. Five different species of *Aspergillus*, namely *A. flavus*, *A. parasiticus*, *A. niger*, *A. fumigatus*, and *A. terreus* were isolated with occurrence frequencies of 39.1, 10, 7.5, 15.8 and 2.5%, respectively (Table 2). Macromorphology and micromorphology of different *Aspergillus* spp isolated were also observed using light microscope (Table 4). Furthermore, it was noted that the prevalence of fungi and aflatoxins contamination is more in groundnuts than in maize (Table 3).

Table 1: Isolation frequency (Fr) of different fungal genera isolated from maize and groundnuts

Genus	*Aspergillus*	*Dermatophyte*	*Mucor*	*Rhizopus*
No. of Isolates	90	12	6	3
Fr (%)	75	10	5	2.5

Table 2: Prevalence of *Aspergillus* spp isolated from maize and groundnuts

Aspergillus isolated	No. of isolates	Isolation Frequency (%)	Prevalence (%) (n=90)
A. flavus	47	39.1	52.2
A. parasiticus	19	15.8	21.1
A. niger	9	7.5	10
A. fumigatus	12	10	13
A. terreus	3	2.5	3.3
Total	90	75	100

Table 3: Prevalence of *Aspergillus* spp isolated between maize and groundnuts

Aspergillus spp	*A. flavus*	*A. parasiticus*	*A. niger*	*A. fumigatus*	*A. terreus*
In Maize	18	8	3	3	-
In Groundnuts	29	11	6	9	3

Table 4: Macromorphology and micromorphology of different *Aspergillus* spp isolated from maize and groundnuts

Description	Macromorphology	Micromorphology
Yellow to green colonies *A. flavus*		
Dark greenish colony *A. fumigatus*		
Black colony *A. niger*		
Reverse white to brown *A. terreus*		
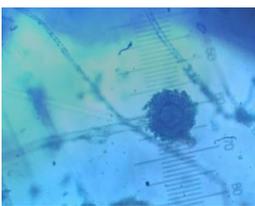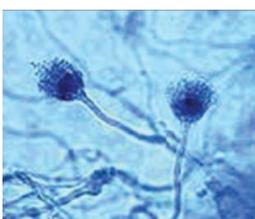
The predominant *Aspergillus* species observed during the study was *A. flavus* (39.1%). This is in agreement with the findings of Fapohunda et al. (2012), Davari et al. (2015), Fakruddin et al. (2015) and Ghaemmaghami et al. (2016, 2020) who reported *Aspergillus* section *flavi* to be the predominant species for producing aflatoxins in various feeds. This might have been that *A. flavus* can adapt to different geographical locations especially the sub-tropical and tropical regions of a country. Generally, high water activity and high humidity are favourable conditions for *Aspergillus* growth (Fernández-Cruz et al. 2010). Environmental conditions for the production of aflatoxins by *A.* section *flavi* are 33 °C and 0.99 a_w (Milani 2013). Thus, typical hot and humid atmosphere and substandard storage conditions are required to synthesize aflatoxins in agricultural products (Atanda et al. 2013). Some of the metabolites produced by these members of *Aspergillus* section *flavi* are well-known to possess encoding genes for aflatoxin production (Ibrahim et al. 2017). Also the prevalence of fungi and aflatoxins contamination was observed to be more in groundnuts than in maize, and this is in agreement with findings of Agbetiameh et al. (2018).

In terms of awareness of people regarding aflatoxins and its health effects, 34, 29.9and 24% of the respondents in Dodoma, Singida, and Iringa, respectively were aware of aflatoxins though with minimal knowledge (Tables 5 and 6). Participants who were aware of the fungi mentioned change of taste and unpleasant smell of the food as the effects of mould contamination which led to stomach-ache and diarrhea. But they were not aware of complicated health issues (Table 6).

Table 5: Respondents' awareness on aflatoxins by regions

Region	Dodoma	Singida	Iringa
Awareness on aflatoxins (%)	34%	29.9%	24%

Table 6: Respondents' awareness on aflatoxins' health effects

S.N.	Questions	Yes (%)	No (%)	Don't know (%)	Total %
1	Can aflatoxins lead to stomach aches and diarrhoea?	53.9	23.9	22.2	100
2	Can aflatoxins cause teratogenic defects /death?	15.6	53.3	31.1	100
3	Can aflatoxins reduce child growth?	18.3	44.4	37.3	100
4	Can aflatoxins cause liver cancer?	17.2	48.3	34.5	100
5	Can aflatoxins impair immunity?	12.8	54.4	32.8	100
6	Can aflatoxins cause kidney failure?	19.4	51.7	28.9	100
7	Can aflatoxins lead to death?	25.6	46.6	27.8	100

Conclusion

This study presents the findings on the isolation frequencies of different fungal genera and the prevalence of *Aspergillus* spp isolated from maize and groundnuts obtained in three regions of Tanzania. The macromorphology and micromorphology of different *Aspergillus* spp were also shown. The study also revealed that knowledge about aflatoxin producing fungi, aflatoxins and their complicated health effects upon consumption is little known in Tanzania. Therefore, raising awareness and sensitizing people about aflatoxins is an important part of interventions necessary to prevent and control aflatoxins in Tanzania. This could be done by preparing manuals and brochures about aflatoxin-producing fungi and their health effects in Kiswahili and local languages. These materials could be distributed and used for training, conducting seminars and in mass media like radio and television for wider dissemination of the information to the population.

Conflict of Interest
Authors declare no conflict of interest.

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