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Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania

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ABSTRACT

Knowledge on the presence of mycotoxins in Africa is fragmentary, although it can be assumed that both concentrations and prevalence in food commodities is high. The present study focuses on the presence of *Fusarium* species and their associated mycotoxins in maize from two geographically distant agro ecological systems in Tanzania. In a two-year survey, both *Fusarium* species and concomitant mycotoxins were surveyed in the Northern highlands (Hanang district) and the Eastern lowlands (Kilosa district). Parallel with this, a questionnaire on agricultural practices in both agro-ecosystems was included in this study. This allowed us to put the presence of the toxigenic *Fusarium* species and their mycotoxins within a relevant agricultural framework.

Fusarium verticillioides, *Fusarium graminearum* and *Fusarium poae* were the predominant species in both locations although the population in the Eastern lowlands was slightly more complex comprising also *Fusarium culmorum*, *Fusarium avenaceum* and *Fusarium sporotrichioides*. The predominant presence of *F. verticillioides* resulted in a high prevalence of fumonisins in both regions. The importance of *F. graminearum* in the population was reflected by the presence of deoxynivalenol in the mycotoxin analysis. Although the agricultural practices differed significantly amongst both locations, only few significant correlations were detected between mycotoxin presence and crop rotation, storage conditions, and insect control measures.

1. Introduction

Maize is the most important cereal grown and consumed in Tanzania, providing 60% of dietary calories and more than 50% utilizable proteins to the population. The crop is cultivated in all 21 regions of mainland Tanzania, predominantly by smallholder farmers in the rural areas, on about two million hectares or 45% of the cultivated area. The consumption of maize is estimated to be 112 kg annually per capita equivalent to 308 g per day per capita (Katinila, Verkuijl, Mwangi, Anandajayasekeram, & Moshi, 1998; Mboya, Tongoona, Derera, Mudhara, & Langyintuo, 2011).

Unfortunately, maize production in Africa is known to be highly vulnerable to contamination with toxigenic fungi and their secondary metabolites, called mycotoxins. Mycotoxins attract worldwide attention because of their impact on human health, animal productivity and economic losses (Bhat, Rai, & Karim, 2010; Wagacha & Muthomi, 2008). Mycotoxin formation occurs during crop growth in the field and during storage. Field toxigenic fungi predominantly enclose *Fusarium* spp. (also *Aspergillus* could occur on mature, dry kernels) whereas storage fungi comprise mainly *Penicillium* and *Aspergillus* spp (Bhat et al., 2010; Logrieco, Bottalico,

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Mule, Moretti, & Perrone, 2003). The most important toxins from an agricultural and human health point of view comprise fumonisins (FB_1, FB_2), type-A trichothecenes (including T-2 toxin and HT-2 toxin), type-B trichothecenes (including deoxynivalenol (DON)), aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), ochratoxins (OTA) and zearalenone (ZEA) (Pitt, Taniwaki, & Cole, 2013; Shephard, 2004; Wagacha & Muthomi, 2008). In the field, predisposing conditions leading to fungal growth are high temperature and humidity, poor soil fertility, drought and insect damage, monsoons and unseasonal rains during harvest. Poor harvesting, drying and storage practices, improper transportation, marketing and processing contributes to fungal growth of mainly storage fungi. The former conditions and practices prevail in Africa and on top of that, diets consist mainly of maize, which entails high daily exposure to mycotoxins (Bhat et al., 2010; Bhat & Vasanthi, 2003; Thompson & Henke, 2000; Wagacha & Muthomi, 2008). Vomiting, diarrhea or other gastro-intestinal problems and immunosuppression are general symptoms of mycotoxicosis in humans (Bhat et al., 2010). In addition, mycotoxins are known to be potentially carcinogenic, mutagenic, teratogenic and neurotoxic (Bryden, 2007; Frisvad, Smedsgaard, Larsen, & Samson, 2004; Gelderblom et al., 2001; Rheeder et al., 1992; Riley et al., 2001; Wagacha & Muthomi, 2008). Children that are chronically exposed to mycotoxins show signs of impaired growth (Gong et al., 2002; Gong et al., 2004; Kimanya, De Meulenaer, Roberfroid, Lachat, & Kolsteren, 2010). In addition, Marasas, et al. (2004) suggest that fumonisin consumption is a risk factor for development of neural tube defects in unborn children and related birth defects such as craniofacial abnormalities. Beside these direct health risks, mycotoxin contamination of the food chain has also an enormous economic impact. Losses from rejected shipments and lower prices for inferior quality can be devastating for developing countries export markets. Direct costs to farmers include reduced income as a result of crop losses, lower prices for inferior quality, increased livestock mortality and reductions in livestock productivity, fertility and immunity. The cost of reduced labor force due to illness and costs from hospitalization or other health care services are problems that are often overlooked (Bhat & Vasanthi, 2003; Bryden, 2007).

Mycotoxins are considered as unavoidable contaminants of food, therefore, the goal is to minimize contamination of maize and maize products by application of good agricultural practices (GAP) during production and-, harvest and good storage practices (GSP) during storage. These include growing resistant varieties, crop rotation, fertilization, insect management, irrigation, proper drying and removal of damaged kernels. A promising long-term strategy is breeding for resistance (Wagacha & Muthomi, 2008). But so far, high levels of genetic resistance have been difficult to achieve (Clements & White, 2004; Munkvold, 2003a). The knowledge that mycotoxins have serious effects on humans, animals and countries' economies has also led to the establishment of regulations on mycotoxins levels in food and feed. Worldwide, approximately 100 countries had developed specific limits by the end of 2003, representing approximately 87% of world inhabitants (FAO, 2004). Still, the majority of African countries have no specific mycotoxins regulations. Even for the few countries with established regulations, enforcement is limited due to reliance on subsistence farming and home produced food (FAO, 2004; Shephard, 2008).

In this context, this paper is presenting results of an inventory of local agricultural practices and their linkage with the presence of mycotoxigenic *Fusarium* species and their associated mycotoxins (FB1, FB2, DON, ZEA, T-2 toxin and HT-2 toxin), in two maize producing agro-ecological zones (AEZ) of Tanzania. The results of this study are useful for guiding the establishment of workable agricultural based strategies to prevent mycotoxins contamination of maize and minimize related human exposures in Tanzania.

2. Materials and methods

2.1. Research design

The study was conducted in two AEZ of Tanzania; Eastern lowlands (Morogoro region, Kilosa district) and Northern highlands (Manyara region, Hanang district). Both zones are main maize growing areas.

Kilosa is one of the districts in Morogoro region, which lies between 6°S and 8°S and 36°30′E and 38°E, consisting of mostly flat lowland. The area experiences an average of eight months of bimodal rainfall distribution whereby in good years short rain starts from October to January, followed by long rains in mid-February through May. Mean annual rainfall ranges around 600 mm in lowlands and average temperature is about 25 °C. In Kilosa, more than 80% of the population depends on agriculture and the district offers a variety of agro-ecological conditions for farming. Thus a variety of food crops is grown, including maize, rice, millet, cassava, beans, bananas and cowpeas. The surplus produces of these food crops are also used as cash crops. The crops are predominantly grown by small-scale farming (average farmland is less than one hectare). In addition, farming is characterized by limited use of inputs such as improved seeds, fertilizers and/or manure, and the majority of the farmers use hand hoes for cultivation (Benjaminsen, Maganga, & Abdallah, 2009; Morogoro Region Socio-Economic Profile, 1997).

Hanang is one of the districts in Manyara region, which is located within 3°S and 6°S and 33°E and 38°E. Its elevation is between 1000 m and 2000 m above sea level. Climate in highlands is more temperate with an average temperature of 20 °C. The zone usually experiences two rainfall seasons during the year, with short scanty rains during the months of October to December and long rains from February to May. The average rainfall in highland zone varies from 700 mm to 900 mm. In Hanang, growing maize in association with beans or pigeon peas is the most common cropping system. Pigeon peas are considered a commercial crop as less than 10% of production is consumed at home. The farming is usually semi-mechanized as a majority of the farmers use animal force for ploughing, planting and transportation of harvests (Investment and Socio-Economic Profile Manyara Region, 2013; Nkonya et al., 1998).

2.2. Field sampling and sample size

A two stage sampling was conducted during 2011/12 and 2012/13 cropping seasons (Zeller, Schwarze, & van Rheenen, 2002). The 2011/12 sampling involved 40 villages scattered around both districts to represent different agro-ecological conditions. Five maize growing households were randomly chosen from each village and approximately one kg of maize was collected from each household. Samples were collected from different points in the batch until approximately one kg was obtained. Per village, the five samples were composited to maintain one sample of one kg. The composite samples were sent to the laboratory, air dried to maintain field status, frozen for 24 h to kill insects and kept at 4 °C until required for analysis. The second sampling in 2012/13 was done analogously, only then ten villages were selected scattered over each district and four households per village participated.

2.3. Isolation and identification of Fusarium species by real time PCR

From each sample, three randomly picked grains were surface-sterilized for 30 s in 1% NaOCl, washed for 30 s with 70% EtOH, washed with distilled sterile water, dried for five minutes, placed on PDA plates (potato dextrose agar, Oxoid Belgium, 39 g PDA/l) and