



Tropentag 2015, Berlin, Germany
September 16-18, 2015

Conference on International Research on Food Security, Natural Resource
Management and Rural Development
organised by the Humboldt-Universität zu Berlin and the Leibniz Centre for Agricultural
Landscape Research (ZALF)

Developing an aflatoxin biocontrol product against aflatoxin-producing *Aspergillus* spp. in Zambia

Juliet Akello^a, Joseph Atehnkeng^b, Mweshi Mukanga^c, Henry Njapau^d, Thomas Dubois^e, Peter J. Cotty^f, Ranajit Bandyopadhyay^b

^a International Institute of Tropical Agriculture (IITA-Zambia), Lusaka, Zambia

^b IITA-Ibadan, Oyo Road, Ibadan, Nigeria

^c Zambia Agriculture Research Institute, Mount Makulu Central Research Station, Zambia.

^d National Institute for Scientific and Industrial Research (NISIR), Lusaka, Zambia

^e The World Vegetable Center (AVRDC) Eastern and Southern Africa, Duluti, Arusha, Tanzania

^f USDA-ARS, School of Plant Sciences, University of Arizona, Tucson, USA.

Introduction

Food quality and safety issues resulting from widespread aflatoxin contamination is an obstacle to improved health status and livelihoods of smallholder maize and groundnut farmers, and hampers their linkages to markets in Zambia. Produced by toxigenic isolates of *Aspergillus* section Flavi, aflatoxins are highly toxic carcinogenic substances associated with stunting, immune system suppression, liver rot and even death of humans (Azziz-Baumgartner *et al.* 2005; Tchana *et al.* 2010; Khlangwiset *et al.* 2011). Similarly, domestic animals reared on aflatoxin contaminated feeds often exhibit higher mortality rates and compromised quality of meat and/or milk products depending on the toxin level (Robens and Richard 1992). Economically, the presence of aflatoxins in produce hampers economic growth by barring regional and international trade due to strict regulations that are in place. Zambia for instance used to be a net exporter of groundnuts to Europe and South Africa but is currently among the lowest exporters of this crop because aflatoxins have been detected in grain and processed commodities at levels that exceed acceptable international limits for export (IITA/ ICRISAT, unpublished; Sitko *et al.* 2011).

To minimize aflatoxin contamination in highly susceptible crops, the International Institute of Tropical Agriculture (IITA) and its partners have developed an environmentally friendly and

cost-effective biocontrol product known as aflasafe for use in Western and Eastern African countries where aflatoxin problem is also common. However, no such product exists in Zambia. Furthermore, knowledge on *Aspergillus* spp. community structure is limited in Zambia and there is a need to understand the interaction between aflasafe and *Aspergillus* in the soil to interpret product performance. The main aim of this study was to establish the relationship between aflasafe and the soil community profile of *Aspergillus* spp., and their potential effects on aflatoxin levels in maize and groundnut.

Material and Methods

To develop the aflasafe biocontrol product for Zambia, grain samples (maize and groundnut) and soil samples were collected from homesteads or fields cropped to either maize or groundnuts, respectively. The samples were obtained from the districts of Chibombo, Kabwe, Kapiri-Mposho, Mkushi, Mumbwa, and Serenje in Central Province, and from Chadiza, Chipata, Katete, Lundazi, Mambwe, Nyimba, Petauke, and Vubwe in Eastern Province. *Aspergillus* spp. were isolated from the soil and milled grain samples using semi-selective media (i.e. modified rose Bengal medium for *Aspergillus* section Flavi) (Cotty 1994; Probst *et al.* 2007). The isolated *Aspergillus* strains were characterized to species level using morphological and microscopic techniques.

The toxicity of all *A. flavus* L-strains were assessed using qualitative and quantitative thin layer chromatography. The genes of all *A. flavus* L-strains that did not produce aflatoxin were sequenced using SSR technique. The strains that were found to lack the genes for aflatoxin production were co-inoculated with aflatoxin producing strains to determine their ability to outcompete the toxin-producing *Aspergillus* spp. Eight *A. flavus* L-strains were selected and used to formulate two Zambian aflasafe products (aflasafe ZM01 and aflasafe ZM02).

The efficacy of the two candidate aflasafe biocontrol products in reducing aflatoxins were tested on-farm by applying in maize and groundnut fields, 2-3 weeks before flowering at a rate of 10 kg/ha. Each treated field was paired with a control field. Soil was sampled from all selected fields

(both treated and untreated fields) prior to aflasafe application and 3 months after crop harvest, while crops were collected at harvest. Microbiological assessments were performed for soil and grain, and aflatoxin assessment for grain, from both treated and control fields.

Results and Discussion

A total of 4853 *Aspergillus* spp were isolated from the soil and grain samples. Morphological and microscopic characterization showed *A. flavus* (74%) as the most predominant species followed by *A. parasiticus* (23.7%), *A. tamarii* (2%) and lastly *A. niger* (0.3%). Toxicity assessment indicated that the majority of *Aspergillus* spp were aflatoxin producers (toxigenic) with less than 10% manifesting atoxigenicity status (Fig.1). Consequently, heavy aflatoxin levels (>20 ppb) were detected in untreated grains, especially groundnuts. Treating maize and groundnut fields with the two formulated aflasafe biocontrol products comprising of non-toxin-producing *A. flavus* L-strains significantly reduced aflatoxin levels in maize and groundnut grains by 74-84% and 86-99%, respectively compared to the control fields.

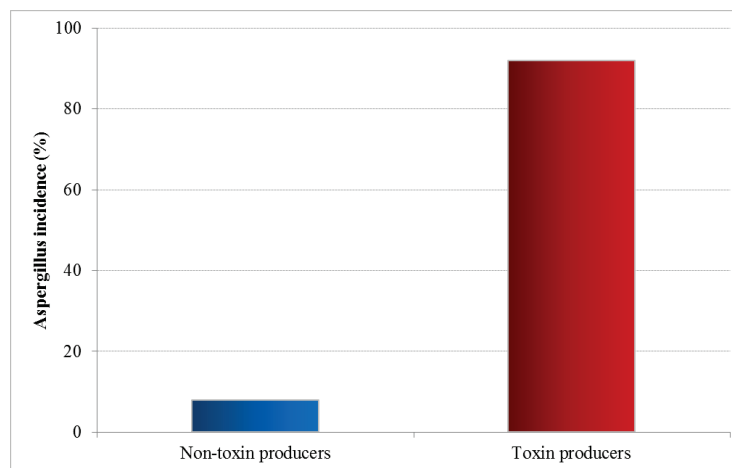


Figure 1: Incidence of aflatoxin producing strains in soil and grain samples collected after the 2011/2012 and 2012/2013 cropping seasons from Central and Eastern Provinces of Zambia

Similarly, field testing of country specific aflasafe products in Nigeria, Senegal, Burkina Faso and Kenya consistently demonstrated that the tested biocontrol products could reduce aflatoxin contamination of maize and groundnut by 80% to 90%. Moreover, the beneficial effects of the

aflasafe products in these countries were demonstrated to have carried over efficacy in reducing contamination from one season to the next (IITA, unpublished data).

Conclusions and Outlook

This study indicates that biocontrol holds great promise for fighting the aflatoxin burden on maize and groundnut in Zambia. Adoption of the biocontrol products aflasafe ZM01 and aflasafe ZM02 to address aflatoxin could improve grain nutritional quality and safety for better health and greater income of Zambian smallholder farmers. However, there is need to commercialize and upscale aflasafe deployment in the country if its health and trade benefits are to be reaped by the small-holder farmers and the nation at large.

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