Systematic investigation of aflatoxins and associated fungi in soils: An example of Kenyan maize fields

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Introduction

- Soil is a complex matrix in terms of chemical and physical diversity driven by land use, geography and climate (Boivin *et al.*, 2004)
- Distribution of mycotoxins in soil is heterogenous (Meyer *et al.*, 2022), therefore suitable monitoring and sampling strategies are needed (Kenngott *et al.*, 2022), analogue to sampling strategies in food and feed mycotoxin monitoring (Miraglia *et al.*, 2005)
- Aflatoxigenic fungi as well as AFTxs has been evidenced in soils from maize fields in aflatoxin hot-spots regions (Accinelli *et al.*, 2008)
- There is a need for reliable strategies for aflatoxin monitoring in soils, considering the above mentioned factors
- In this study, soil samples were collaboratively analysed at chemical level by the UKL (soil physicochemical properties & mycotoxin concentrations in soil), at field level by the Kenyan Partner KALRO (CFU values and soil fertility) and at molecular level by MRI-KA (ITS sequencing and mycotoxin biosynthesis potential)

The aim of this work is to present an interdisciplinary approach for the systhematic investigation of mycotoxigenic fungi and mycotoxins in hot-spot regions, in example of maize fields in Makueni, Kenya

Afla Z

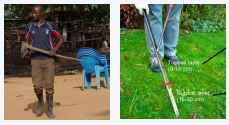
Project stages

Recruiting small holder farmers
Capacity building and information campaigns

Defining experimental area Makueni, sites and treatments (4):
Conventional-, conservation-tillage, *Trichoderma* and push-pull

Sampling strategy for soil and maize
4 treatments × 4 field replicates × 5 pseudo replicates × 2 positions × 2 Depths = Total 320

Sampling in Kenya: Soil and Maize
CFU values at field level (KALRO)



Soil chemical and molecular analysis in Germany
MRI-UKL

Project results

AFTxs in soil samples/UKL

- 320 soil samples were analyzed via LC-FLD/LC-MS (Albert *et al.*, 2021)
- AFTx were non detected in soil samples
- Time between sampling and shipping > 3 months
- AFTXs degradation in soil → UV/microbial degradation

CFU values *Aspergillus spp.* soil/KALRO

- Abundance of *Aspergillus* in soils increasing in that order: conventional < conservation-tillage < *Trichoderma* < push-pull.
- CFU values were approximately 2x higher (p<0.01) in the upper (0-15 cm) than in the lower soil layer (15-30 cm)
- The depth effect was more evident in push-pull.
- CFU levels were in general higher in soils than in maize cobs.

Molecular analysis/MRI-KA

- In total 16 composite samples were analyzed corresponding to 4 replicates/treatment
- Following species were determined *A. parasiticus, A. flavus/A. parasiticus* (3/16), *A. minisclerotigenes* (4/16), *Penicillium spp.* (8/16), *A. niger/A. carbonarium* (12/16)
- All investigated species *A. flavus/A. parasiticus* were able to synthetize at least 1 Aflatoxin (max. conc.=37.6 µg/mL AFB1)
- All investigated species *A. minisclerotigenes* sythetized AFB1 (max. conc.=116.6 µg/mL)

Discussion and outlook

- The absence of AFTXs in soil samples is attributed to degradation (Angle and Wagner, 1980; Pankaj *et al.*, 2018), since samples were stored for longer than 3 months and no evidence of AFTX accumulation has been reported for soils. Method as well as sampling strategy was tested and validated previously (Albert *et al.*, 2021 & Kenngott *et al.*, 2022)
- CFU values in soils allow for differences in the treatments (Angle *et al.*, 1982) → land cover used for push-pull may support fungal growth, in particular at the topsoil
- Aflatoxigenic species were detected in the investigated samples, however preliminary data does not allow for correlations with treatments
- AFTX biosynthesis was confirmed for the investigated *A. flavus/A. parasiticus* and *A. minisclerotigenes* species isolated from the soil. This indicates the potential of mycotoxigenic species to biosynthesize mycotoxins *in situ* (Accinelli *et al.*, 2008)
- The conception and design of this study allowed us to assess differences in fungal biomass according to the investigated treatments and it demonstrated that aflatoxigenic species do occur in the soil and they have the potential to produce mycotoxins
- Further studies are necessary to investigate:
  - Environmental realistic concentrations in the soil, including factors that may contribute to residual concentration (e.g. storage/transport of samples and possible degradation)
  - Factors triggering population abundance in soils as well as mycotoxin biosynthesis

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