Bundesanstalt für Landwirtschaft und Ernährung

AflaZ

ptble
Projektträger Bundesanstalt für Landwirtschaft und Ernährung

MRI Max Rubner-Institut

JKI Julius Kühn-Institut

EAFF Eastern African Farmers Federation

KALRO Kenya Agricultural & Livestock Research Organization

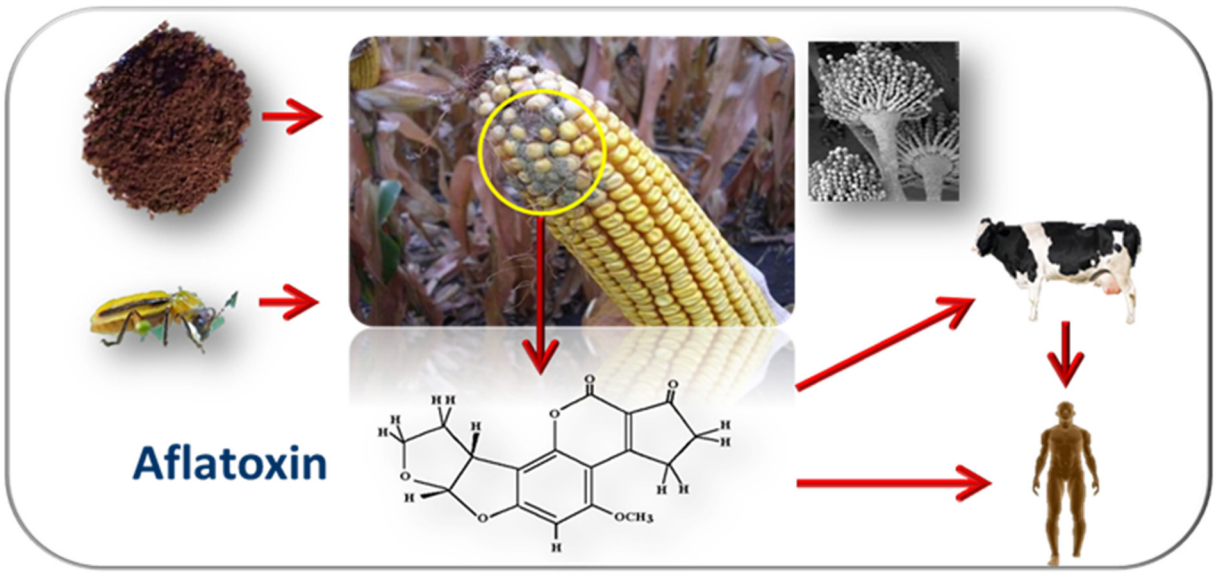
FRIEDRICH-LOEFFLER-INSTITUT FLI Bundesforschungsinstitut für Tiergesundheit Federal Research Institute for Animal Health

UNIVERSITÄT KOBLENZ · LANDAU

Project-Update

Project title:	**Development and implementation of sustainable strategies to improve food-safety and retain nutritional values by reducing fungal infestation and aflatoxin contamination in the food-chain in Kenya as model region for Sub-Saharan Africa (AflaZ)**
Geographical focus:	Kenya
Call reference:	Reference: 323-06.01-03-2816PROC11 Funding code: 2816PROC11
Cooperating partners:	Max Rubner-Institut; Julius Kühn-Institut; Friedrich-Loeffler-Institut; Universität Koblenz-Landau; Kenya Agricultural & Livestock Research Organisation; East African Farmers Federation
Duration:	1.10.2018 - 31.12.2023
Budget:	**1.674.559,79 €**

Diagram of the core topics that AflaZ focuses on:



Aim of the project:

Due to the consumption of foods heavily contaminated with aflatoxins (especially the staple foods maize and milk), the population of Kenya and other African countries is regularly exposed to toxin levels far above the recommended limits. Children and sick people are particularly at risk from the health effects associated with mycotoxin ingestion.
The BLE-funded project, **AflaZ**, focuses on improving food safety and the quality standard of milk, maize and products thereof. Kenya was chosen as a model region because it is a high-risk area for aflatoxin contamination due to fungal infestation in the food sector. The AflaZ project develops effective and sustainable methods to analyse and monitor fungal infestations and aflatoxin contamination both in the field and in storage in order to reduce them in the long term. In addition, the AflaZ research programme includes extensive capacity building strategies. These include cooperation with local institutions, farmers, students and other stakeholders and thus enable sustainable knowledge transfer (dissemination), which ensures cultural acceptance of the recommendations and sustainable integration of the new methods by the local population.

The following core topics are in focus of the AflaZ project:

• Isolation and identification of aflatoxin-producing fungi from maize and associated soil samples from Kenya, as well as their microbiological and molecular biological characterization for the development of effective and sustainable monitoring and prevention strategies.

• Analysis of the transfer of aflatoxin from the feed to cow milk, so-called carry over, as well as analysis of a possible reduction/degradation in the aflatoxin content in the subsequent processing of the milk into cheese and yoghurt. Identification of an aflatoxin-specific biomarker in the blood of dairy cows and development of a suitable analysis method.

• Analysis of aflatoxin derivatives formed by the fungus or plant through metabolization that may contribute significantly to "masked" aflatoxin contamination; comparison and development of a standardised methodology for the application of fast-screen tests for aflatoxin, and establishment of an APP for mobile aflatoxin analyses in the field.

• Determination of soil aflatoxin content and physico-chemical parameters as vitality factors for soil organisms and for maize plants, as well as field insects as pests on the maize plant and vectors of the spread of spores of aflatoxin-producing fungi.

• Training and capacity building of Kenyan PhD students within the AflaZ project as multipliers for dissemination of AflaZ research results to farmers, as well as their education and training in the new methods. Strengthening of communication and cooperation between Kenyan partner institutions and other local research institutions. Dissemination of the project results via the eGRANARY platform and the PAEPARD blog spot, as well as recommendations to governmental and non-governmental organizations in Kenya. A TV documentary about the AflaZ project and topic-specific radio programs is developed and is repeatedly broadcasted to reach broader sections of the population.

Results of the fifth year of the project (2022):

After the restrictions caused by the Corona pandemic in 2020 and 2021, the situation eased somewhat in 2022 and work within the AflaZ project was able to take place almost without restrictions again in 2022. In Kenya, regional droughts and local floods caused difficulties in maize cultivation and crop failures in 2022, which made the work for certain work packages difficult in some cases, so that some trials will have to be made up in 2023. However, the project objectives have not changed in the course of project implementation.

At the **Max Rubner Institut** (MRI) in Karlsruhe, Mrs. Alexandra Schamann, who worked on **WP1** and parts of **WP2** as a doctoral student, successfully completed her doctoral thesis in December 2022 with "Magna Cum Laude". As part of her work, the diversity of aflatoxin formation was investigated on a genomic and chemical-analytical level in different Kenyan isolates of *Aspergillus flavus* and *A. minisclerotigenes*, as well as *A. flavus* and *A. parasiticus* isolates from the MRI strain collection in Karlsruhe. Since *A. minisclerotigenes* plays an important role in the regular and often fatal aflatoxicosis outbreaks in Kenya, it was sequenced in addition to *A. flavus* and *A. parasiticus* fungal strains using MiSeq sequencers (short reads) and PacBio technology (long reads). Comparative genome analyses of different isolates of *A. flavus* and *A. minisclerotigenes* were then used to elucidate variations in the architecture of the aflatoxin gene clusters and to identify differences in the prevalence of aflatoxin formation. In a comprehensive monitoring of the aflatoxin biosynthesis of *Aspergillus flavus*, the precursors of aflatoxins and thus the aflatoxin biosynthesis pathway were also investigated. Furthermore, the assertiveness between aflatoxin-producing and non-aflatoxin-producing *Aspergillus flavus* strains was monitored/analysed using a droplet digital PCR system (ddPCR). In addition, the conditions under which aflatoxin-producing Aspergilli actively produce aflatoxin and the conditions under which aflatoxin production or growth of these fungi is reduced were investigated.

The competition trials with the MRI isolate of the mycoparasitic fungus, *Trichoderma afroharzianum*, and *Aspergillus flavus* in **WP3** were continued and expanded. The effectiveness of the MRI isolate *T. afroharzianum* MRI349 as a bio-control species for the containment of *A. flavus* and the associated reduction of the aflatoxins produced could be confirmed again. The plant pathogenic fungus *Fusarium verticilloides* isolated from Kenyan soil and maize samples was also used in competition trials. Here, too, *T. afroharzianum* MRI349 proved to be an effective control organism. In 2022, 12 additional *Trichoderma* strains and the biocontrol strain *Trichoderma harzianum* (product name: Trianum-P) used in Kenya were included in the competition trials. Individual interesting metabolites isolated from the fungal samples are currently being analysed by mass spectrometry with the support of the AflaZ partner MRI-Kiel. The significance of these metabolites in the process of competition is currently being investigated in challenge tests at MRI-KA.

In **WP3.2**, the **Julius Kühn-Institut** (JKI) has improved the antifungal assay for high-throughput extracts established in 2021 for *A. flavus* and used it for the identification of extraction conditions, antifungal activity and compounds of some African plants (*Ocimum gratissimum, Lippia adoensis, Xylopia aethiopica, Melia volkensii*) (See publication Karimi et al. 2022). In addition, other plant material (*Plectranthus barbatus, Psidium guajava, Zanthoxylem chalybeum*) from Kenya was tested for antifungal activity. As part of the work under WP3.2, a formulation of essential oils with starch was produced that is effective against *A. flavus* on agar and leaves. Initial tests with whole plants under semi-free-range conditions on the durability and efficacy of the antifungal compounds and extracts under field conditions proved difficult.

In 2022, the Kenyan doctoral student Beatrice Tenge was able to carry out a two-month research stay at the **MRI Detmold**, during which she continued to work on the methods for determining aflatoxins (**WP4**).

At the **University of Koblenz-Landau**, the experiments on aflatoxins and soil quality (sampling and analysis of mycotoxins in soils, determination of the role of soil properties in the degradation of aflatoxins, effects of aflatoxin B_1 on the soil microbiome) were completed in 2022 as part of **WP5**, which also concluded the laboratory activities of the soil analysis work package. The data were statistically analysed and a total of three publications were written on this. A demo version of an open source web app to support data collection in Kenya for the AflaZ project was developed, which is to be finalised and ready for use in 2023.

Kenyan PhD student Ginson Riungu (**WP6**) further investigated the diversity of insects, the severity of damage caused by the insects and their ability to transmit *Aspergillus* spores in Kenya, which revealed a high overall diversity of insects that varied from region to region. Fields with intercropping had a higher number of species and a higher number per taxa, and an increase in the number of predators and parasitoids was observed. It was found that maize grown alone as a monoculture showed the highest damage by fall armyworms. The rainfall in the two rainy periods in Kenya in 2022 was not sufficient at all locations for the maize plants to grow adequately for the trials. In Kilifi, the drought was particularly severe, resulting in crop failures. Some of the WP6 trials will therefore be repeated in March 2023.

At the **MRI in Kiel**, the freeze-drying and analysis of the basic fodder from the transfer trial was carried out in 2022 as part of **WP7**. The samples were then successfully analysed for mycotoxin content. The analyses of the yoghurts produced from the trial milk and the "Edam type" cheese have been carried out and the data evaluated. In the milk samples, the corresponding mycotoxin aflatoxin M_1 could only be detected in the aflatoxin-exposed group. No *Aspergillus* toxins were detectable in any of the other milk samples. The hypothesis that aflatoxin M_1 may be degraded from the milk during yoghurt production must be rejected, as the mycotoxin is fully recovered in the yoghurt. The mycotoxin cyclopiazonic acid is also transferred from milk, if the milk is contaminated by CPA, into Edam-type cheese to a considerable extent. With these experiments, the practical work of WP7 is completed. The preparation of the publication of the main results will extend into the year 2023.

At the **Friedrich-Loeffler-Institut** (FLI), the practical work on **WP7** was completed in the course of 2022. In order to investigate toxicologically negative effects of the toxin-producing *A. flavus* strains as well as the *A. flavus* biocontrol strains on animal health, extensive feeding trials were carried out with subsequent analyses of the animals' blood and urine. A panel of clinical-chemical and haematological parameters was determined in the blood, which, in addition to hepatotoxic effects, also allowed possible effects on energy, fat and protein metabolism, as well as on the differentiated red and white blood count to be recorded. It was found that neither liver-associated enzymes, nor metabolites of the energy metabolism, nor of the protein and lipid metabolism were influenced by the feed. Calcium and phosphorus as well as the electrolytes chloride and sodium were also unaffected by feeding. The effects of the biocontrol strains on the health parameters of the animals were tested on the one hand by blood tests and on the other hand by a direct histopathological assessment of the liver. This is considered to be the main target organ of aflatoxins and is described as the central site of action. In all samples, only minor changes occurred, mainly lymphohistiocytic portal infiltrates and focal hepatocellular vacuolisation, which, however, are not of pathological significance. Furthermore, various aflatoxin biomarkers that can be used for the assessment of exposure were established within WP7. For this purpose, suitable enzymatic and chromatographic methods were developed and the applicability of the biomarkers was investigated with the help of the feeding experiment.

The **Kenya Agricultural & Livestock Research Organisation (KALRO)**, **WP8**, managed the AflaZ trial fields in the three counties of Kisumu, Makueni and Kilifi, which are considered hotspots for aflatoxin contamination of maize, in 2022. Grain samples from these regions and from trial fields with different treatments (conservative tillage, *Trichoderma* application, push pull and conventional tillage) were used for detection and quantification of aflatoxins (determination of total aflatoxin content) by ELISA method.

The **Nagoya Protocol** contract documents (Prior Informed Content (PIC) and Mutually Agreed Terms (MAT)) were submitted to the district representatives of the Ministry of Agriculture in Makueni, Kisumu and Kilifi in August 2022 and finally signed by the district representatives, KALRO, and the Kenya Wildlife Services (KWS) in the following months.

The practical work of the **East African Farmers Federation (EAFF), WP9** was completed by the end of 2021. The knowledge dissemination activities in 2022 were focused on continuing to educate on the functioning, use and benefits of the various agricultural technologies and applications for aflatoxin control through the channels used by EAFF such as the e-GRANARY platform. In December 2022, the EAFF-led AflaZ TV documentary on aflatoxin issues in Kenya, AflaZ project activities and results on prevention strategies was completed. All project partners provided their own information including film footage to ensure comprehensive coverage of the entire project. The AflaZ documentary was also linked to the AflaZ page on the Max Rubner-Institut homepage.

In October 2022, an **AflaZ partner meeting** was held in Nairobi/Kenya by the coordination (**WPX**) of the AflaZ project with the support of the Kenyan partners KALRO and EAFF. After extensive preparation, the meeting was successfully organised from 10.10.2022 to 14.10.2022 with the participation of almost all German and Kenyan project partners. Several meetings with intensive exchange between the participants in Nairobi and Kisumu and visits to AflaZ trial fields and AflaZ partner institutions took place.

Key statements and policy advice:

1) The AflaZ project focuses on the problem of maize infestation by aflatoxin-producing fungi such as *A. flavus* and *A. parasiticus*. In the course of the investigations, as well as in current studies, it has become apparent that *A. minisclerotigenes* also plays a significant role in the aflatoxin contamination of maize in Kenya. This species is probably also the cause of the mostly fatal aflatoxicosis outbreaks in Kenya. In addition, investigations of both maize and soil samples from Kenyan trial fields also identified various fungi of the species *Fusarium*. Therefore, it was decided to include selected *Fusarium* isolates in e.g. the competition studies with *Trichoderma* Biocontrol fungi, as there are often highly plant-pathogenic strains of *Fusarium* that infect maize, cereals and other crops such as fruit and vegetables, form mycotoxins there and are often precursors for less pathogenic species such as *Penicillium* or *Aspergillus*.
2) It is possible to effectively inhibit mycotoxin-producing fungi with a combination of synergistic acting inhibitory influences in storage (e.g. with light of a certain wavelength and low grain moisture content) and partly already in the field by using natural antagonistic fungi. In this context, the use of biocontrol strains (*A. flavus*) already used commercially, has to be assessed critically for various reasons. The alternative use of a commercial product with *Trichoderma harzianum*, which was investigated in AflaZ, shows good, but partly ambivalent results regarding the infestation with *A. flavu*s in the field. The use of an MRI isolate of *T. afroharzianum* as a biocontrol strain seems promising.
3) Antifungal plant ingredients from local Kenyan associated plants, as well as the amino acid arginine, which could be easily sprayed on, showed promise as another approach to inhibit aflatoxin-producing fungi.
4) Infestation and effectiveness of prevention methods (e.g. assertiveness of biocontrol species against toxin-producing species) can be tested and monitored in the laboratory using molecular ddPCR technology.
5) Rapid aflatoxin detection methods continue to be used and implemented in field trials in Kenya. A survey of farmers and demonstration of the rapid tests showed that a test with smartphone app evaluation is preferred. However, it also became apparent that due to the high costs of the required test strips, an app development is not sufficiently feasible at this time point. Discussions with individual company representatives regarding cooperation to reduce costs have not been promising so far, so that further considerations should be carried out in addition to the project, e.g. organisation and implementation of maize tests for aflatoxins via EAFF. In the course of this circumstances, the development of the APP was transferred to another partner within the AflaZ project with additional funding (UKL). The app is in the final development phase with support from MRI-KA and KALRO.
6) A method for the determination of aflatoxin concentration in soil/plant was developed and successfully validated. The method was further optimized for the analysis of plant matrices (food) to be used in the study of the soil/plant system. The transmission rate of aflatoxin in cow milk is investigated in a feeding study, as well as a possible aflatoxin reduction in the downstream dairy products yogurt and cheese. This reduction does not take place. In addition, a specific biomarker was developed to measure aflatoxin contamination in the dairy cow.
7) Maize fields are cultivated using conventional methods as well as the methods developed in the AflaZ, the infestation with aflatoxin is compared and the farmers are trained in the new methods as well as specialist knowledge about the health problems of aflatoxin intoxication. In this way, a sustainable aflatoxin reduction is possible and achievable. Through the training of Kenyan doctoral students, partly also additionally of Erasmus doctoral students from Kenya, partly also in tandem with German doctoral students, within the AflaZ project, but also of farmers, the multiplication of the findings from AflaZ on site is guaranteed. The AflaZ consortium was able to talk directly to farmers and farmer representatives during visits to AflaZ fields in October 2022 and engage in an intensive exchange about the project results, prevention strategies and health relevance of mycotoxins in maize.