Research Proposal: AflaGoggles for Aflatoxin Detection

Description

AflaGoggles for Screening Aflatoxin Contamination in Maize

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Geographical Locations

Nigeria, USA

Project Duration

10/01/2013 - 09/30/2015

Executive Summary

The present proposal responds to the Peanut and Mycotoxin Innovation Lab (PMIL) request for proposals on feasible mycotoxin mitigation and monitoring systems in developing countries. The proposed project would provide innovative diagnostic and monitoring tools for mycotoxin management. The success of the proposed project would help to improve human nutrition and health, assist in fighting rural poverty, and would eventually benefit economic development in these countries.

Aflatoxin contamination in maize is a major food safety issue worldwide. The problem is of special importance in African countries because maize is used as a staple food. A primary limitation to controlling ingestion of contaminated food in these countries is the lack of affordable and feasible methods for farmers on small village farms to screen for aflatoxin contamination. Due to the high cost associated with any existing aflatoxin detection methods, it is of urgent need to develop portable, rapid, and non-invasive technology for aflatoxin detection in maize for these farmers.

The Mississippi State University and USDA have been working on a collaborative research project to develop rapid and non-invasive methods for aflatoxin detection. The research found that under UV excitation, there is a fluorescence shift toward longer wavelengths in the blue-green spectral region for maize kernels with high aflatoxin content. Based on the above results, this project proposes to develop a greatly simplified device called AflaGoggles for aflatoxin screening in maize. The device will use special narrow bandwidth optical filters. The spectral properties of the optical filters will be defined by evaluating knowledge from previous spectral based aflatoxin detection research. AflaGoggles would be similar to a pair of sun glasses without any built-in electronics. Looking through AflaGoggles, the human eye will be the detector for aflatoxin contamination.

The proposed project will be implemented over two years. The first year will be a feasibility and proof of concept study. The second year will focus on building the prototype AflaGoggles and initial trials of the device. A total of six objectives have been established for the proposed project. The objectives will be fulfilled along the timeline of project implementation. Meanwhile, the proposed project would also work on strengthening human and institutional capacity. The project plans include hiring a post- doctoral research scientist to carry out a significant part of the project tasks. The proposed project will also

employ a staff/student in the focus country to help with project implementation and field trials. Proper training will be provided for the post-doctoral researcher/staff/student. Their work would eventually benefit both, the US-based and the focus country institutions. For future perspectives, upon successful implementation of the proposed project, the research team plans to expand the work into a scaled-up project which would lead to the commercialization of the proposed AflaGoggles. Future development would involve industrial partners and consideration for other crop products such as peanuts and pistachios.

Project Description

Goal

The goal of the project is to develop portable, fluorescence spectral-based technology for rapid and non-invasive aflatoxin detection in maize. A detection device, AflaGoggle, will be developed in the project.

Relevance and Justification

One of the principal food crops in Africa and other developing areas of the world is maize, and aflatoxin contamination of maize is regarded as a highly important food safety problem in these areas and worldwide (Robens, 2008). The first step in controlling aflatoxin contamination is to accurately detect the toxin. However, current aflatoxin detection methods are quite expensive for use by African farmers. For example, the current USDA approved methods for aflatoxin screening are costly chemical- based analytical methods. These methods include high performance liquid chromatography (HPLC) or thin layer chromatography (TLC). The steps involved in the inspection process are initial sampling, mixing of samples, sub-sampling, sample grinding, sub-sampling of ground materials, and finally chemical analysis (USDA, 2002). It is obvious that the screening process is expensive and time consuming.

The major constraint in the target countries is that there is no affordable and feasible method available to farmers in small village farms, traders or consumers to screen for aflatoxin contamination. Often, there is no aflatoxin assessment performed at all. Therefore, the development of low cost alternative approaches including portable, rapid, and non-invasive technology for aflatoxin detection in maize is urgently needed.

It has been a continuing effort within the research community to find a way to rapidly and non- destructively detect aflatoxin contamination in maize. One of such attempts is the black light test (Gloria et al., 1998). The black light test involves the examination of bright greenish yellow fluorescence (BGYF) on whole-kernel maize or a stream of coarsely ground maize meal under 365nm ultraviolet (UV) light. This method is a rapid and low-cost approach. However, the black light test is not a reliable test for aflatoxin contaminated maize (Wicklow, 1999). Generally, fluorescence is emitted from the intermixed aflatoxin and the BGYF compound, regardless of whether it is from toxin or non-toxin-producing *A. flavus* strains. This results in high rates of false detection because the black light-based visual inspection cannot differentiate the fluorescence signals. The black light only reveals the broad fluorescence response from the samples. However, it cannot identify the source of fluorescence emission. For this reason, the black light test is only used as a presumptive test rather than for quantitative or even qualitative analysis.

One of the more recent developments in this area is the use of spectral technology, including the use of fiber optic spectrometers (Pearson, et al., 2001) and imaging spectrometers (or hyperspectral imagers). Yao, et al. (2010) found that there exists a fluorescence shift toward longer wavelengths in the blue-green spectral region for maize kernels with high aflatoxin content. A series of subsequent studies and publications, building on this discovery, have demonstrated an average detection accuracy of 70-90% for aflatoxin contaminated maize kernels even when atoxigenic *A. flavus* was used in sample preparations (Yao, et al., 2013). Based on this information, we believe it is possible to develop portable, fluorescence spectral-based technology for rapid and non-invasive aflatoxin detection in maize.

The above studies pointed out the possibility of using two narrow wavelength bands for the detection of ultra-violet (UV) light-excited hot (contaminated) maize kernels. The detection algorithm is based on fluorescence emission from the two narrow wavelength bands. The assumption for this project is that the narrow wavelength spectral information could be utilized in a greatly simplified device.

The main output from the project would be a simple spectral device similar to a pair of sun glasses (we will call them AflaGoggles). There are no electronics attached to AflaGoggles. Instead, special narrow wavelength band pass filters will be integrated within the lens of the Goggles. AflaGoggles will work with a UV-LED flash light which provides fluorescence excitation of contaminated maize kernels. The human eye will be the final detector for such contamination. Research will be implemented to determine filter specifications and to configure the AflaGoggles.

The main outcome of the project would provide the end users (farmers, traders, and consumers) with a low-cost, simple device for aflatoxin contamination screening in maize. It may be used in the field, storage, market, as well as by consumers. There is no advanced training required for using AflaGoggles. The end users would have an affordable and feasible method for detecting aflatoxin contamination in maize.

Research Plan

Objective(s)

1. Survey existing methods for low-cost, rapid, and non-invasive screening of aflatoxin contamination in maize
2. Survey current applications using narrow bandwidth filtering
3. Identify two potential narrow bandwidth spectral bands for aflatoxin screening
4. Develop feasible method to use the identified narrow bandwidth spectral bands and provide initial design of the AflaGoggles
5. Prototype construction of the AflaGoggles and the development of screening procedures with the prototype
6. Implement trials to test AflaGoggles for feasibility in real world situations and also for continuous improvement of the screening procedures

Role of Each Scientist/Partner

Dr. Haibo Yao

Associate Research Professor, Mississippi State University (MSU). The main role of MSU is to provide expertise in hyperspectral imaging and its application in aflatoxin detection. MSU will carry out feasibility study and the construction of prototype devices. MSU will also lead the effort in field test studies and dissemination of intellectual property.

Dr. Robert Brown

USDA/ARS- Southern Regional Research Center. Dr. Robert Brown is a research plant pathologist at ARS. USDA will provide expertise in mycotoxigenic fungi, plant pathology, as well as laboratory space and fungal inocula. USDA will also be involved in prototype design, experiment planning, aflatoxin analysis of the test samples, and dissemination of intellectual property.

Dr. Abebe Menkir

International Institute of Tropical Agriculture, Nigeria. Dr. Menkir is the in-country collaborator. He will provide expertise in maize agronomy, as well as participate in prototype design and field testing. Dr. Menkir will be a key resource for technology transfer and commercialization.

Annual Work Plan, Milestones and Timeline

Year 1, October - November, 2013

Survey of existing screening methods.

Milestone

is to survey existing methods for low-cost, rapid, and non-invasive screening of aflatoxin contamination in maize. One focus area will be the black light screening method. The black light method has high levels of false positives. It sees broad spectrum of fluorescence emission from the contaminated samples. General requirements and guidelines will be created for low-cost, rapid, and non-invasive screening of aflatoxin contamination in maize.

Year 1, December 2013 - January, 2014

Survey of current filtering methods.

Milestone

is to survey any current methods and applications using narrow bandwidth optical filtering. This work will provide a guideline for design and implementation of the proposed AflaGoggles. The scope of the survey is not limited to sensing and detection of food contaminants. The focus will be on the interaction of narrow bandwidth optical filters and the human eyes. Reviews will be generated for methods using narrow bandwidth filtering.

Year 1, February- June, 2014

Identify two narrow bandwidth spectral bands.

Milestone

is to identify two potential narrow bandwidth spectral bands for aflatoxin screening. The objective will be fulfilled with results from our current rapid aflatoxin detection research and incorporated with new requirements generated from Objectives 1 and 2. Small batches of trial experiments will be implemented with the identified optical filters. In the meantime, test plots will be established to create artificially infected maize ears with *Aspergillus flavus* in the field. The infected maize ears and kernels will be used in the later development of the AflaGoggles. The identified two bands will be used in subsequent implementation of the project.

Year 1 and 2, July- December, 2014

Formulate the screening method and initial design.

Milestone

is to develop feasible methods to use the identified narrow bandwidth spectral bands and provide initial design of the AflaGoggles. In this phase of the project, experts in optical design will be consulted to develop methods that will utilize the two narrow bandwidth bands from Objective 4. The methods developed will be tested with contaminated maize samples and compared with measurement results from chemical lab analyses. The deliverable of this part of the project will be the initial design of the AflaGoggles.

Year 2, January- June, 2015

Prototype construction and screening procedure development.

Milestone

is to construct the prototype AflaGoggles and to develop screening procedures with the prototype. In this phase of the project, detail design of the AflaGoggles will be finalized and reviewed. Prototype AflaGoggles will be subsequently constructed. Detailed aflatoxin screening procedures along with the prototype will be developed in alignment with the final design, review, and prototype construction. These procedures will also be reviewed. Test plots will be established to create artificially infected maize ears in the field using *Aspergillus flavus* inocula.

Year 2, July- September, 2015

Initial field test.

Milestone

is to carry out initial field trials of the AflaGoggles to test their feasibility with the maize samples obtained from the test plots. The results will be statistically compared with sample aflatoxin contents measured with approved chemical analysis methods. Meanwhile, in order to test the feasibility of the AflaGoggles in a real world situation, prototypes will also be sent to the focus country (Nigeria) for field testing. To do this, a staff/student will be hired by the project funds to maintain a test plot. The staff/student will be trained in the US before the actual implementation of field tests. This approach will also strengthen the human and institutional capacity in the focus country. These test results will provide opportunities for continuous improvement of the prototypes and the screening procedures. The deliverable of this part to the project work will be the project report, prototype AflaGoggles and related screening methods. A follow-up proposal for large scale commercial applications will also be prepared.

Scale-up and Marketing Plan

If the project is successful, we will develop a follow-up project to commercialize the AflaGoggles. In the beginning of the commercialization effort, it will be necessary to refine the prototype AflaGoggles based on the results and feedback from the initial field trials and project implementation. Through this process we can improve the performance of AflaGoggles and make sure it suits the needs of actual users from the small farms of the focus country.

The new project will identify appropriate subcontractors or OEM suppliers for the initial production of AflaGoggles. The goal is to produce good quality, cost effective AflaGoggles. The new project will ensure the production of a small batch of AflaGoggles to be used and tested in the focus country. The project team will follow up with the production and the end users. Data will be collected to evaluate the efficacy and acceptance of AflaGoggles. This process will eventually lead to large scale production and application of AflaGoggles.

Large scale application is the ultimate goal for the development of the AflaGoggles. The scope of this is beyond a research project. We plan to solicit help from industry, non-profit organizations, venture capital investment, as well as government agencies to ramp up the production and create distribution networks. This effort is not limited in the focus country, but could be implemented around the globe. Countries with severe aflatoxin contamination such as sub-Saharan Africa and South East Asia will be the first to benefit by the large scale application of AflaGoggles. Finally, the project team will also extend the technology to similar applications including, but not limited to, aflatoxin screening in peanuts, pistachios, and other tree nuts.

Gender Research Strategy

To ensure that both women and men (smallholder farmers and/or processors) can benefit from the outputs of the research from this program, gender will be considered at all stages of this proposed research project. Gender equality and empowerment of women and girls, however, cannot be considered without considering the socio-cultural context in which males and females live. Therefore, we will certainly approach this topic as students desirous of learning about and becoming familiar with the socio-cultural factors impacting gender in Nigeria.

The technology itself, AflaGoggles, facilitates implementation as an aflatoxin control tool by either men or women. Use of AflaGoggles holds the promise of increasing men's and women's health and well- being, while improving safety and increasing economic productivity. Also, there is no advanced training required to use AflaGoggles, negating lack of education as a potential hindrance to advancing gender equality.

Inclusion of women in this research project will be promoted throughout every facet of the project. This includes staff hiring considerations in Nigeria and selection of farmers and processors who participate in the project. In order to implement this we will partner with any Nigerian organizations, public or private, that can enhance our efforts to improve gender equality and female empowerment.

Lastly, using USAID indicators, we will evaluate our performance at closing key gender gaps and empowering women and girls.

Outcomes and Impacts

The main output from the project would be a simple spectral device called AflaGoggles. These come outfitted with special narrow wavelength band pass filters will be integrated within the lens. AflaGoggles will work with a UV-LED flash light which provides fluorescence excitation of contaminated maize kernels. The human eye will be the final detector for such contamination.

The main outcome of the project would provide the end users (farmers, traders, and consumers) with a low-cost, simple device for aflatoxin contamination screening in maize. It may be used in the field, storage, market, as well as by consumers. There is no advanced training required for using AflaGoggles. Therefore, end users would have an affordable and feasible method for detecting aflatoxin contamination in maize. This would serve to enhance food security, human health and trade.

USAID Mandate Responsiveness

- **MDGs -** Poverty/Hunger: Improved Health: Raised Rural Incomes: Sustainable Development
- **Foreign Assistance Framework -** Governance: Human Capacity: Economic Structure: Persistent Dire Poverty: Global Issues (HIV and Infectious Diseases, climate change, biodiversity)
- **IEHA -** Science and Tech Applications: Increased demand for peanuts (maize): Market Access: Increased Trade
- **USAID Focal Areas -** Greater incomes: Greater value and market demand: Public Health: Food Security: Sustainable Value Chain: Improved Human Capacity

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