AATF Project 6 (PI006)
Aflatoxin Biological Control Project

6.1 Background

Aflatoxin is a genotoxic, immunosuppressive carcinogen that frequently contaminates maize and groundnuts. The toxicity of aflatoxins to humans and animals has led to regulatory controls. The International Institute of Tropical Agriculture (IITA) in collaboration with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and other partners have developed an indigenous biological control technology to mitigate aflatoxin contamination in Nigeria. In 2009, the partners obtained a provisional registration of the trade name AflaSafe™ from the apex Nigerian regulatory body, National Agency for Food and Drugs Administration and Control (NAFDAC) and conducted farmer-scale evaluation in Kaduna and Oyo states.

6.2 Summary of Achievements

Nearly 2 tonnes of AflaSafe™ was produced in IITA using a laboratory scale manufacturing protocol. In collaboration with Kaduna State Agriculture Development Project and UNDP Pampaida Millennium Villages Project, AflaSafe™ was tested by farmers in five zones in Kaduna and Oyo States. Maize and groundnut were treated at various crop growth stages and different dosage rates to determine the optimal time and dosage for treatment. For each treated plot, an untreated control field was identified. Prior to treatment, soil samples were taken to measure the native population of *Aspergillus*. Soil and grain were sampled at harvest to determine the extent of changes in the *Aspergillus* community structure in favour of the applied biocontrol strains. NAFDAC officials monitored the deployment of AflaSafe™ and harvest of three fields.

Aflatoxin contamination is highly variable in nature. The efficacy of AflaSafe™ was calculated from aflatoxin data of 51 treated maize fields whose companion control fields had aflatoxin contamination. Groundnut from all 8 farms and 13 control maize farms did not have a detectable level of aflatoxin. On an average, AflaSafe™ reduced aflatoxin concentration by 79% in the 51 treated maize fields. Maize grains from 74% AflaSafe™ treated fields had an aflatoxin concentration below the Nigerian aflatoxin regulation limit (4ng/g) and 100% of the samples complied with the US standard (20ng/g). In contrast, only 3% maize in the untreated fields satisfied the Nigerian regulation limits and 27% exceeded the US limits. Therefore, AflaSafe™ can significantly lower aflatoxin risk and improve market opportunities. Aflatoxin reduction was similar for 10kg/ha and 20kg/ha application rates. Therefore, 10kg/ha is recommended for treating fields with AflaSafe™. Plot size did not have any effect on the efficacy of AflaSafe™. The appropriate time of application of AflaSafe™ is about 15 to 22 days before flowering when the height of the crop is between 'waist' and 'chest' high. Further analyses (aflatoxin concentration after storage and the incidence of released strains in soil and grain) are in progress.

Contacts were made with three food and feed manufactures to establish farmer-industry linkages for marketing of maize and groundnut from AflaSafe™ treated farms.

6.3 Technical Objectives

6.3.1 Obtain approval from the National Agency for Food and Drugs Administration and Control (NAFDAC) for testing the atoxigenic strains for aflatoxin biocontrol in farmers' fields.

i. Progress

- Two NAFDAC officials from the Registration and Regulation department monitored the deployment of AflaSafe™ on one maize farm, and the harvest of three maize farms and one of groundnut. These officials asked that we submit the following data for these four treated fields (three for maize and one for groundnut) and accompanying controls
    - Aflatoxin concentration in grains
    - Frequency of occurrence of released strains in soil before AflaSafe™ application
    - Frequency of occurrence of released strains in the soil at harvest
    - Frequency of occurrence of released strains in grains at harvest
    - Frequency of occurrence of released strains in the soil at the planting time of next season's crop to determine the carry-over of the inoculum
- Necessary inspection fees were paid to enable NAFDAC officials to monitor the trials.

ii. Next steps

NAFDAC has provided provisional registration of AflaSafe™ for a period of two years. During this period, on-farm data needs to be gathered to demonstrate the efficacy of AflaSafe™ in reducing aflatoxin. NAFDAC will continue to monitor the trial activities during the testing process. Subsequently, a report of efficacy trials will be submitted to complete the documentation needed for full registration. The time consuming process of gathering the requested data (particularly on the occurrence of released strains) will be completed by July 2010. In addition, certification for the manufacturing facility at IITA Ibadan will need to be obtained from the Inspectorate Services of NAFDAC

6.3.2 Test the efficacy of bio-competitive strains of *Aspergillus flavus* in reducing aflatoxin concentration in maize and groundnut in farmers' fields in Nigeria

Efficacy of AflaSafe™ at harvest in the four zones in Kaduna State

Natural occurrence of aflatoxin concentration at harvest was highly variable (below detectable level to 35.8ng/g). Out of the 72 farms that were treated, aflatoxin concentration was below detectable level in 21 treated and their companion control fields. Therefore, 51 treated fields and their untreated counterparts where aflatoxin expression occurred were taken into consideration for further data analysis. Average aflatoxin concentration in the treated fields across all zones was 3.3ng/g (range 2.1 to 5.1ng/g), while the corresponding mean concentration was 15.4ng/g (range 9.8 to 27.2ng/g) in the control fields (Table 4). The average reduction of aflatoxin resulting from AflaSafe™ application was 79% (range 57% to 87%) across all the zones. Apart from Lere, aflatoxin concentrations in other zones were within the Nigeria regulatory limits (<4ng/g). Pampaida zone (87%) had the highest reduction in aflatoxin concentration and the least was in Lere zone (57%). Late application of AflaSafe™ is responsible for the low efficacy in Lere.

Table 4. Mean aflatoxin content of maize samples after application of AflaSafe™ across four zones in Kaduna State.

Zone	Number of fields	B-aflatoxin* concentration (ng/g)		Reduction**** (%)
		Treated**	Control***	
Maigana	22	2.1	9.8	78
Pampaida	10	3.6	27.2	87
Lere	9	5.1	11.8	57
Birnin-Gwari	10	2.3	12.6	82
Mean		3.3	15.4	79

*B-Aflatoxin = Sum of aflatoxins B_1 and B_2 in each sample.

**Treated = fields in which AflaSafe™ was applied.

***Control = Fields in which AflaSafe™ was not applied.

****Reduction (%) = (100-(mean of treated/mean of control) x 100). Fields with no aflatoxins in control plots were excluded from analysis.

More than 74% of the samples in the AflaSafe™ treated fields were within the Nigerian aflatoxin regulation limits (<4ng/g) and 100% of the samples complied with the US regulation (<20ng/g). In contrast, only 3% of the samples in the untreated fields satisfied the Nigerian regulation limits and 27.3% were above the US regulatory limits. Therefore, 71.5% more farmers (74.5% in treated minus 3% in untreated) improved their maize quality to acceptable Nigerian aflatoxin standards by using AflaSafe™ in the study sites (Table 5).

Table 5. Frequency of newly harvested maize grain samples having different levels of aflatoxin concentration in AflaSafe™ treated and untreated fields in Nigeria.

Aflatoxin	Treated fields**	Untreated fields***

(ng/g)*	Number	Proportion (%)	Number	Proportion (%)
<4	38	74.5	1	3.0
>4 - 10	10	19.6	12	36.4
>10 - 20	3	5.9	11	33.3
>20	0	0.0	9	27.3
Total	**51**		**33**	

*<4 = Nigeria regulation limit, <10 = World Food Program acceptable limit, <20 = US regulation limit, >20 = unacceptable level of aflatoxin.
**Treated = fields where AflaSafe™ was applied.
***Control = Fields where AflaSafe™ was not applied. Number of fields = Number of fields with aflatoxin values within the specified range. Proportion = (Number of fields/total) x 100. NB: Fields with no aflatoxins in control plots were excluded from calculations.

Effect of rate of application on the efficacy of AflaSafe™

The study was conducted to determine the optimum rate of application of AflaSafe™ required for reduction of aflatoxin. Aflatoxin reduction of 81% and 79% at 10 and 20kg/ha application rate, respectively were not significantly different from each other (Figure 9). Both application rates (10kg/ha and 20kg/ha) of AflaSafe™ reduced mean aflatoxin to acceptable Nigerian regulatory limits (<4ng/g) in the treated fields, while the aflatoxin concentration in the untreated control fields exceeded the standard used by the World Food Program (10ng/g) for purchasing maize.

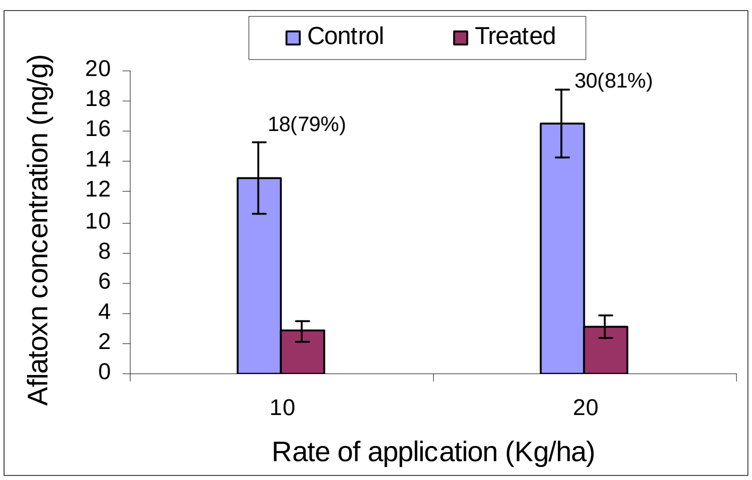

Figure 9. Effect of rate of AflaSafe™ application on the concentration of aflatoxin in maize in Kaduna state. The numeral on top of each bar is the number of samples analysed and the numeral in parenthesis is the percentage reduction of aflatoxin in treated fields compared to control fields. Control fields with no aflatoxins were not included in this analysis.

Effect of field size on the efficacy of AflaSafe™

This study was conducted to determine whether field size had any effect on the efficacy of AflaSafe™. Farmers who used AflaSafe™ had fields of different sizes. Mean aflatoxin concentrations in the treated fields were

<4.0ng/g (mean 2.4ng/g), while in the control fields the concentration were from 10 to 33ng/g (mean 17.1ng/g). Average aflatoxin reduction ranged from 75% to 95% in fields of various sizes, but the maximum reduction (95%) was for >1.5 to 3 ha field size. However, field size did not have significant effect on the efficacy of AflaSafe™ (Table 6).

Table 6. Effect of field size on the efficacy of AflaSafe™ in farmers' field in Kaduna state.

Field size (ha)	Number of fields	B-aflatoxin* concentration (ng/g)		Reduction* (%)
		Treated**	**Control*****	
0.25	3	3.5	33.0	89
> 0.25 to 0.5	17	2.8	14.8	81
>0.5 to 1.0	24	3.4	13.9	75
>1.0 to 1.5	3	1.7	10.0	83
>1.5 to 3.0	4	0.7	13.7	95
Mean		**2.4**	**17.1**	**85**

*B-Aflatoxin = Sum of aflatoxins B_1 and B_2 in each sample.
**Treated = fields in which AflaSafe™ was applied.
***Control = Fields in which AflaSafe™ was not applied.
***Reduction (%) = (100-(mean of treated/mean of control) x 100).
NB: Fields with no aflatoxins in control plots were excluded from calculations.

Effect of crop height on the efficacy of AflaSafe™

The purpose of this analysis is to determine the appropriate stage of maize height when AflaSafe™ application leads to optimum reduction in aflatoxin. The height of the crop (measured in relation to height of a person and used as a proxy for stage of the crop) at the time of AflaSafe™ application and the number of days between AflaSafe™ application and 50% flowering were recorded. On an average, the 'waist' high crop flowered after 19-22 days while the crop height 'above head' flowered 1-5 days after AflaSafe™ application. Aflatoxin was more variable in the control than in the treated fields. A high percentage aflatoxin reduction (94% to 100%) was achieved in fields where AflaSafe™ was applied when the crop was 'waist' to 'chest' high. Fields in which AflaSafe™ was applied when the crop was of 'above head' height (or 1-5 days before flowering) resulted in only 63% reduction in aflatoxin. Therefore, the efficacy of AflaSafe™ decreased with increase in crop height, i.e., late application of AflaSafe™ (Figure 10). These visual descriptive parameters are easier for a farmer to understand for deployment of AflaSafe™ than using the maize growth stage.

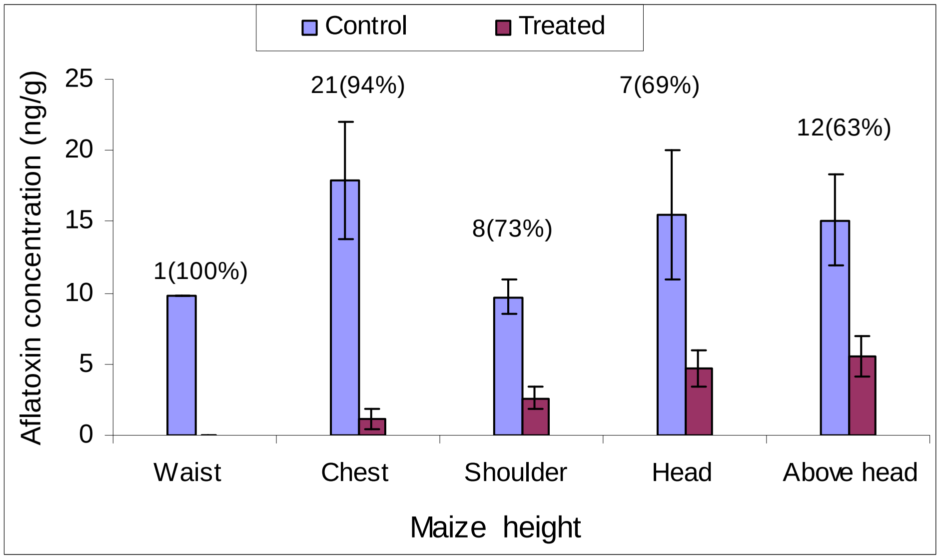

Figure 10. Effect of height of maize on the efficacy of AflaSafe™ in Kaduna State. The numeral on top of each bar is the number of samples analysed and the numeral in parenthesis is the percent reduction of aflatoxin for the descriptive height. Correspondence between crop height and time interval between AflaSafeTM application and flowering are as follows: waist = 19–22 days between AflaSafeTM application and 50% flowering; chest = 15–18 days between AflaSafe™ application and 50% flowering; shoulder = 10–14 days between AflaSafe™ application and 50% flowering; Head = 5–9 days between AflaSafe™ application and 50% flowering; and Above head = 1–4 days between AflaSafe™ application and 50% flowering. Control fields with no aflatoxins were not included in the calculations.

Conclusion

The ecologically sound biological control approach can add value to other simple technologies for aflatoxin management, since aflatoxin contamination would be slight even if fungi colonised the grains in spite of using these technologies. Adoption of this biocontrol technology in other parts of Nigeria will increase the supply of good quality maize at a reasonable price for value-added markets and enhance food safety, thereby improving the health of people. Improved market access will also increase economic returns to processors and producers, providing incentives for producers to adopt aflatoxin management technologies including biocontrol.

iii. Challenges faced

- There was much more demand from farmers to treat their fields with AflaSafe™ than the amount of inoculum the lab-scale manufacturing process could provide. A plan for commercial scale production of AflaSafe™ needs to be developed.
- Aflatoxin contamination is highly variable from field to field. As a result, several untreated fields had undetectable levels of aflatoxin. Such fields were not included in analysis. Therefore, field efficacy trials need to be carried out

in a large number of fields so that efficacy can be measured in a significant number of fields where aflatoxin contamination occurs.

- Late application of AflaSafe™ in some fields (particularly in Lere) resulted in its low efficacy. Farmers need to apply AflaSafe™ at the correct growth stage to obtain full benefits from AflaSafe™ application.

iii. Next steps

a. AflaSafe™ was supplied in 2009 free of cost to the farmers who participated in testing the biocontrol technology. Obviously, it is not possible to supply the technology free of cost in the coming years. It is necessary to determine farmers' willingness to pay and develop a business plan for commercialisation of the technology.

b. Although the current method of production and delivery of inoculum has worked well, improvements on the formulation of AflaSafe™ and inoculum delivery mechanisms need to be implemented to reduce the cost of the product.

c. Second year large scale efficacy test of AflaSafe™ will be conducted and full registration of the product with NAFDAC will be sought.

6.3.3. Product Deployment

Explore opportunities to link farmers using biocontrol technology with food and feed processors for use of aflatoxin-safe maize and groundnut.

i Progress

Farmer-industry linkages were discussed with Nestle Nigeria. Nestle offers a 13-15% premium for maize consignments that meet the company's aflatoxin standard (4ng/g). Nestle carries out an education programme to enable farmers to produce aflatoxin-safe maize. It was not possible to work with Nestle in 2009 for several reasons. The key reason was the need for Nestle to buy maize in bulk from a single source – something that large traders can offer, but not individual unorganised farmers. Nestle is willing to work with the project partners in 2010 by including AflaSafe™ as a component in their recommended aflatoxin management practices. Further discussions will take place with Nestle during February-March to develop collaborative work plans for 2010.

ii. Challenges faced

Although the private sector has been supportive and realises the need for aflatoxin management for sourcing good quality raw material, their willingness to pay a premium for the aflatoxin-safe maize and -groundnut is still not clear (except for Nestle). There is a need to build mutual trust between the farmers' organisations and the private sector so that each considers the other as an equal and dependable partner, and share the mutual goal of producing and marketing safe food. There is an urgent need to identify a private sector manufacturing facility to produce and market AflaSafe™.

iii. Next steps

The business plan is ready for circulation and comments from stakeholders in order to factor in all the suggestions of key stakeholders. There is a need for farmers to be organised so that as a group they can offer maize in bulk to large scale food processors and meet the high demand of niche markets.

6.3.3 Testing of biological control in maize field in Kenya

For the past seven years, significant aflatoxin outbreack in Eastern and Central regions of Kenya caused deaths and most recently the Government has put a ban on the marketing of over 2.3 millions bags of contaminated maize The four most affected districts in Kenya are Makueni, Kitui, Machakos and Thika. Food samples from some of the affected households contained 20 to 8,000ppb aflatoxin. The strains of *Aspergillus flavus* in the affected Kenyan districts where aflatoxicosis occurred are some of the most potent producers of aflatoxin ever found and unique in Africa. In a survey, 55% of market maize samples collected in Makueni, Kitui, Machakos and Thika districts had aflatoxin levels > 20ppb, 35% of the samples had aflatoxin levels > 100ppb and 7% of the samples exceeded 1,000ppb.

In preliminary research activities, several native atoxigenic strains from the aflatoxin affected districts in Kenya have been identified during the last three years. These strains are able to reduce aflatoxin contamination by up to 90% in laboratory tests.

AATF in partnership with IITA and The Kenya Agricultural Research Institute (KARI) has initiated the process for the evaluation of the biocontrol technology in Kenya in collaboration with the Kenya Plant Health Inspectorate Services (KEPHIS) which will entail the registration of AflaSafe™ and the local atoxigenic stains contained in the product.