# THE CONTRIBUTION OF LAND CERTIFICATION TO FARM MANAGEMENT PRACTICES: The CASE OF DANDI DISTRICT, OROMIA NATIONAL REGIONAL STATE, ETHIOPIA

## Mekonnen Abebe Diriba<sup>1</sup>

#### Abstract

This study had investigated farmers' perceived land tenure security after they had received certificate of land holding right. In addition, it had assessed the contribution of rural land certification scheme to rural land management practices, and the major challenges faced in Dandi District. Primary data for the study were collected through household questionnaires, focus group discussions and interviews of key informants. Farmers' perceived land tenure security had improved after being issued land holding certificates. As a result, better and sustainable land management and protection had also been achieved. However, lack of clear plot demarcation, uncontrolled selection of tree species for planting, the decline of indigenous tree species and financial constraints were some of the challenges observed.

Key words: Land certification, land management practice, land tenure

<sup>&</sup>lt;sup>1</sup>St. Mary's University; P.O.Box 1211, Addis Ababa, Ethiopia <<u>siyanemeko@yahoo.com</u>>

# Introduction

Land, as one of the natural resource bases, plays a substantial role in the national economy of a country. Particularly in Africa where the majority of the populations derive their livelihood from agriculture, land resource is the major source of household employment and income. However, increases in human and livestock population, dependence on traditional and unsustainable farm management practices, and climate change induced problems, have become a threat to the sustainability of agriculture in sub-Saharan Africa. Increasing population density and the increasing degradation of the natural resource base have become the leading causes to declining per-capita food production. Unlike earlier times of less intense population pressure, it has now become more difficult to support the growing population through traditional land use and land management practices, with little or no technical inputs (Omitiet al., 2000).

Ethiopia has been affected by land degradation (Betru*et al.*, 2005) and has adversely influenced the performance of the agricultural sector (Woldeamlak, 2003). Land degradation remains a major challenge and the issue of land management is a major environmental concern in Ethiopia where more than 80% of the population is rural and dependent on agriculture. Thus, improvements in land productivity become vital to enhance and sustain the welfare of this largely agrarian population (World Bank, 1989). Improvements in agricultural productivity require a more efficient and sustainable use of rural resources and it calls for the adoption of improved land management practices.

Research findings in the area of land management show that the adoption of land management practices are influenced by a host of factors. According to Desta (2012), land management decisions are influenced by different factors, such as, level of infrastructure development, quality of agricultural extension services, provision of conservation technical assistance, and type of land tenure policy. Kabubo-Mariara (2007) identified property rights as one of the key institutions that determine the adoption of different land management practices. Moreover, providing land certificate is believed to affect the application of technologies for the management of agricultural and natural resources, and particularly the adoption of land management practices. Lack of tenure security, among other factors was identified as contributing to the aggravation of land degradation as it discourages farmers from investing on their land and from taking adequate care to prevent soil erosion (Berhane and Fayera, 2005).

Though, there are people who argue that land titling has nothing to do in enhancing land investments, international institutions such as the United Nations for Human Settlement (UNCHS, 1999) and some researchers (Deininger, 2003; Gebremedhin *et al.*, 2003), revealed that there is close relationship between land tenure and property rights and secured property rights to encourage farmers to invest in land management practices.

Examples from several countries adequately support that land ownership rights and investment in land improvements are related. In Thailand, land ownership security was found to significantly explain incidences of land improvements (Feder and Onchan, 1987) while in India, improvements in land markets were found to be associated with increase in conservation investments on farm land (Pender and Kerr, 1998). There was a similar experience from Tigray, northern Ethiopia, where land tenure security was significantly and positively associated with long-term durable soil conservation investments such as stone terraces (Gebremedhin and Swinton, 2003).

The current Ethiopian government has been taking measures to implement land titling aimed at improving farmers' perceived land tenure security. Though the titling process provides certificates of holding and do not bestow ownership rights, this is considered as an incentive that will encourage farmers to sustainably manage the land they own. Nevertheless, the long-term impact of the measures taken by the government to improve agricultural production through land titling, including the current land policy of the country in relation to tenure security, needs to be periodically assessed. This study, therefore, was initiated to explore the contribution of rural land certification scheme in improving farmers' incentives to invest in land resource management practices.

# Methodology

#### Description of the study area

Dandi district is one of the eight districts in west Shewa zone of Oromia regional state of Ethiopia, located about 80 kilometers west of Addis Ababa. The main city of the district is known as Ginchi. Information obtained from the district Environmental Protection Land Administration and Use Office (EPLAUO) reveals that Dandi District is structured into 48 rural kebeles with the total household population of 172,842. The average family size for the district is 4-5 persons per household. The District has a total area of 174,617 ha, out of which, arable land is 72,664 ha, grazing land 1,980ha, forest land 96,685ha, unused land 1242 ha and others 2046 ha. The altitude of the area ranges between 1200-3288 meters above sea level and the average temperature is 16.55°C with an annual average precipitation of 700-

2300 mm. The major livelihood of the population in the area is mixed farming, which includes cultivation of various crops and animal husbandry. However, crop cultivation is the major source of income for most farmers. The major crops grown in the area are cereals, pulses and root crops. Enset has been commonly grown around homestead as subsidiary source of food. Grazing land is communally owned.

## **Research Design**

A survey method of research was adopted to obtain descriptive information involving both quantitative and qualitative data.

## Sampling Method

Purposive sampling technique was used to select one District and the desired number of *Kebeles*. Accordingly, Dandi District was selected from West Shewa zone of Oromia regional state. Out of the total 48 *kebeles* (the smallest administrative area) in the District, five of them were purposely selected as the provision of land holding certification has already been implemented. The *kebeles* selected for data collection were Boda Bosoqa, Honche Bite, Mareno Gonjeb, Dandi Sulu and Dandi Mumicha (Table 1).

Kebele	<b>Total Population</b>	Sample size
Boda Bosoqa	585	37
Honche Bite	387	25
Mareno Gonjeb	446	29
Dandi Sulu	460	29
Dandi Mumicha	476	30
	Boda Bosoqa Honche Bite Mareno Gonjeb Dandi Sulu	Boda Bosoqa585Honche Bite387Mareno Gonjeb446Dandi Sulu460

Source: Own survey

The total households of the five selected *kebeles* for the survey were 2356. Among these, only 150 sample households were selected based on the formula set out by Grosh and Munoz (1996).

The formula used was:  $e = (\sqrt{1-n/N})(\sqrt{p} * 1 - P/n)$  where E =error, n =Sample size (150), N=population (2356) and P=0.5 (50%)

The number of households selected from each *kebele* varies, depending upon their respective household size. Simple random sampling technique was adopted in order to select the desired subjects from a population of each *kebele*. Out of the total 2354 households residing in all these kebeles, 1944 (82.58%) are male-headed and 410 (17.42%) were female-headed, and all have registered and received land holding certificates. During the study period, in the district as a whole, a total of 28,261 households, 22,145 (78.36%) male-headed and 6,116 (21.64%) female-headed households, have been registered and got entitlement book of land holding certificate.

Data were collected on the level of land improvement activities, attitude of land right security, problems related to land certification, land holding registration status, issuance of certificate of holding and other basic information related to the study. The required training was given to 3 enumerators and one supervisor collecting the data so as to enable them to gather the necessary information as desired.

#### **Data Collection**

## Structured Questionnaire

Regarding the household survey, structured questionnaires, both open and close-ended questions, were formulated to solicit information on land management practices before and after land certification, farmers' perception of land tenure security before and after land certification and other related issues. The questionnaires were pilot-tested to check for ambiguities and redundancies and adjustments were made where necessary. Three farmers from each *kebele*, with a total of 15 farmers from 5 *kebeles*, were selected for the pilot test.. Since farmers in the study area speak the dialect *Afan Oromo*, the questionnaires that were initially prepared in English, were translated into the local language.

#### **Informal Survey**

#### Key informants interview

Key informants interview was administered to triangulate or crosscheck the data gathered through the other tools. For reason of manageability, 30 farmers, 6 from each *kebele*, were randomly selected for the interview. In addition, key-informants were also drawn from all development agents (DAs) working in the sample *kebeles*, and from District land Administration offices. Discussion with administrative bodies of 5 *kebeles* (2 from each *kebele*) was also held.

#### **Focus Group Discussion**

Focus group discussion was held with concerned officials from Environmental Protection and Land Administration and Use Authority (EPLAUO). Check-list of questions was prepared mainly focused on contribution of land certification, improvements made on land management practices as a result of land holding right, farmers' perception of land right security before and after land certification and other related issues. These facilitated in obtaining detailed qualitative information for triangulating data from household survey.

#### **Method of Data Analysis**

In analyzing quantitative data, descriptive statistical methods, frequency table, percentage, graphs and inferential statistics, chi-square tests of association and paired t-test were applied. Statistical Package for Social Sciences (SPSS, version 20.0) for windows was used. Descriptive statistics were used in analyzing the nature of land management practices before land certification and changes of farm management practices employed by farmers after the provision of land holding certificate, households' perception of land right security before and after land certification and the role of institutions in supporting farmers' efforts and challenges that farmers face in implementing various conservation practices. The qualitative data obtained from the focus group discussions and from key informants interviews were analyzed thematically. Chi-square ( $\gamma^2$ ) test of association was also used to test if there is association between land certification and farmers' investments in land management practices. Moreover, an independent t-test was also employed to compare means of numbers of trees planted by farmers before and after land certification.

## **Results and Discussion**

## Socio-demographic and Economic Characteristics of Respondents

The socio-demographic and economic characteristics of the respondents, land resource and related issues, improvements in land management practices as a result of land certification, and other related issues were evaluated. The data analysis was done with a 95% confidence interval.

The study's population is predominantly composed of male-headed households. Out of the total sample, 84% were male-headed households. Access to land was predominantly biased towards male-headed households showing gender imbalance in land accessibility in the area.

About three-fourth of the sample respondents were economically active. Around 78% of the farmers fall in the range of 18 - 60 years of age. The mean (51.2 years) and median (50.5 years) age of the respondents revealed that the landholders were generally elderly farmers in the study area. The age distribution of the respondents in the study was found to be normally distributed, with a positive skewness. The standard deviation for the age of the respondents was 13.1 years, showing the existence of variability in ages among the respondents (Table 2).

	Frequency			Per	cent
Age category	Male	Female	Total	Male	Female
18 - 30	7	0	7	4.7	0.0
31 – 35	12	0	12	8.0	0.0
36 - 40	15	5	20	10.0	3.33
41 - 45	12	2	14	8.0	1.33
46 - 50	17	5	22	11.33	3.33
51 – 55	18	4	22	12	2.67
56 - 60	17	3	20	11.33	2.0
61 – 65	11	2	13	7.33	1.33
66 - 70	10	1	11	6.67	0.67
70+	8	1	9	5.33	0.67
Total			150		

Table 2. Age Distribution of the Respondents

Source: Own Survey

Almost all farmers (98.7%) in the study area owned parcels of land which were registered and certified with primary book of entitlement. Very small proportion (1.3%) of the farmers own parcels of land that were not registered. The record of the Environmental Protection, Land Administration and Use Office (EPLAUO) of the District had shown that 98% of the households had parcels of land that were registered and the result from the household survey is a closest estimate of the official record. Some parcels were not registered and the households did not receive land ownership certificate because some of them were not willing due to lack of awareness and others had some border conflicts with their neighbors and their cases were being examined in the court of law during the period of land registration. During an interview session held with the district EPLAUO experts, it has been observed that registration and provision of land certification had taken place for those who were not benefitted previously. However, the majority (95.3%) of the respondents had indicated that their parcel of land was not demarcated. The district land administration office experts had acknowledged the failure to demarcate because of budget constraints. Unmarked boundary usually was a source of disputes over land (ECA, 2004). Absence of clear demarcation of boundaries could be a source of tenure insecurity. Place (2009) contended that certainty of retaining rights from actual or risk of dispute over rights has created feeling of tenure insecurity. The size of the land specified on the primary book of land certificate was based upon estimates in a traditional way by utilizing the local unit of measurement called Kert/Fechassa (Affan Oromo) which is roughly a quarter of one hectare. In terms of actual measurement, however, four Kerts may not be equivalent to one hectare. Similarly, the boundaries were demarcated by using physical and natural land marks, such as rivers, hills, rocks, trees and roads. Such demarcation is not dependable as one can move stones, and feeder roads may be changed over the years. Such practices of demarcation may have negative effects on perceived land tenure security of peasants and may aggravate land related disputes. Hence, the need for clear demarcation of boundaries, perhaps with the help of cadastral maps is crucial. This is also stipulated in the Land Proclamation of the Oromia Regional State (Proclamation No.130/2007, Art.15).

## Farmers' Perception of Land Tenure Security

Attempt has been made to analyze the perceptions of farmers in terms of land tenure security. The anticipation of future land redistribution scheme and fear of land expropriation by the government were the major factors for land tenure insecurity. With regard to land expropriation, the majority of the respondents had indicated that their fears have somehow been reduced after they obtained the land ownership certificates (Table 3).

Table 3: Responses of farmers	to issues related to perceived feelings of
land tenure security	

Question	Response	Frequency	%
Have you had fears that your parcels	Yes	100	66.7
of land could be expropriated by the government before land certification?	No	50	33.3
Do you expect that there will be	No	138	92.0
future land re-distribution after holding your land certificate?	Not sure	12	8.0
Has your overall feeling of tenure	Yes	125	83.3
security increased after land certification?	No	11	7.3
	Not sure	14	9.4

#### Source: Own survey

Prior to issuance of land certificates, the majority (66.7%) of the respondents had fears that their land could be expropriated anytime by the government. But, after receiving the certificates, the majority (92%) had responded that they do not expect any future land redistribution will occur. Overall, 83.3 % of respondents feel more secured after land certification, though some had expressed skepticism in light of the political climate (Table 3).

According to views expressed in focus group discussion and informants' interview, redistribution of land had focused at farmers owning plots of land

Mekonnen Abebe

larger than the *kebele* average. After receiving land holding certificates, however, many respondents had stated that they have less fear of land expropriation by the government. Similarly, the proportion of respondents with the belief that future land re-distribution may happen has decreased after land certification. However, many were aware that all land is state property and expropriation for special development venture could happen at any time with proper compensation.

Land security is believed to be maintained and further promoted by the additional benefit obtained from the secured land and certificate of holding. When farmers were able to lease out their land and use their certificates of holding as collateral to access credit service, they realize that they are exercising their property right and this further strengthen their security and tend to promote land conservation practices . However, the benefit obtained from leased land and credit access using the certificate as collateral was very limited even though it is declared as such in the Oromia Regional land proclamation (Proclamation No.130/2007, Art.5 sub art. 6).

# The Contribution of Rural Land Certification to farm Management Practices

Security of land tenure is a critical variable in determining the incentives to undertake various Land Management Practices (LMP). Hurni (2000) described the concept of LMP as the application of productivity enhancing farm practices such as terracing, fallowing, planting trees, using organic manure, construction of soil and water conservation structures, fencing on farm land. It is implicated that the issuance of land certification increases individual or community investment in land in the sense that secured land ownership considerably increases farmers' incentives to supply

labor to initiatives aiming at improving the fertility of individual or communal land (Deininger *et al.*, 2006). One of the key objectives of this research was to investigate the contribution of land certification towards improvement of land management practices.

In this study, 90.7% of the respondents had indicated that they were more involved in undertaking various land improvement practices on their parcels of land after they received land certificates (Table 4).

Thus, the provision of legally recognized land holding certificates can be considered as one of the motivating factors for engagement in better land management practices. Earlier studies have shown a positive impact on agricultural productivity as it eliminates anxiety and uncertainty of farmers for possible expropriation of their lands (Shimelles *et al.*, 2009; Abera*et al.*, 2012).

Nevertheless, there was variation in the types of land management practices. Most were engaged in tree planting, terracing on farmland, application of compost and organic manure, and fencing their farmlands. Among land management practices, Abera et al (2012) had shown that terracing, planting trees, application of compost, application of farm yard manure and construction of water harvesting structure have increased after land certification. Assefa (2010) had also observed similar results, in that, the majority of the households who received land certificates were involved in one or more of land improvement activities.

It seems that there is association between engagements in land management practices and obtaining land holding rights, i.e. land certification (Table 4).

Land Management	Response	Before	After	χ2
Practice		Certification	Certification	
Tree planting	Yes	74.5	92.7	
The planting	No	25.5	7.3	1.01
Soil bund Construction	Yes	20.8	91.3	
Son build Construction	No	79.2	8.7	7.17**
Stone bund Construction	Yes	27.3	84.8	
Stone bund Construction	No	68.7	15.2	16.60**
Compost & organic	Yes	75.7	93.4	
manure	No	24.3	6.6	9.01**
Equaina formland	Yes	63.1	95.1	
Fencing farmland	No	36.9	4.9	21.86**

Table 4: Percentage of farmers engaged in land management practices(LMP) before and after land certification

\*\*Significant at 5% probability level. Degree of freedom for each chi-square ( $\chi 2$ ) tests of association is 1. Source: Own Survey

Construction of soil and stone bund, application of compost and organic manure and fencing farmlands were all significantly associated (p<0.05) with land certification. However, according to key informants and focus group discussants, terrace construction was undertaken through mass mobilization in the form of soil conservation campaign. Significant increase of both soil and stone terrace construction was made possible through an effort exerted by individual framers on their respective homestead while on open sloppy areas through public work campaign.

With respect to tree planting, farmers use any tree species available irrespective of land certification, implying that farmers practice tree planting regardless of land certification to fulfill their demand for construction and firewood, as well as for generating additional income for their livelihood (Table 4). Around 74.5% of the farmers in the study area had managed to plant trees even though there was fear of land redistribution, i.e., before land certification. Farmers were planting fast growing trees, such as, *Eucalyptus spp.* and *Grevillea robusta* to maximize their income prior to land redistribution as they were unsecured of their holdings. The other reason was to protect their farm land from heavy erosion.

Overall, the findings of this study had shown farmers' willingness and motivation to invest in land management practices over time due to land certification. This finding is in consistent with the findings of Ogolla & Mugabe (1996), Besley*et al.* (1997), Deininger (2003), Shimeles *et al.* (2009) and Assefa (2010) who found that certification encourages landholders to invest in land. However, the result of this study is not in line with the findings of Tesfu (2011) in Amhara region and Place (2009) in Somalia and Kenya, who argued that tenure security has no association with the decision of farmers to invest in land management practices.

# Comparison of the Magnitude of Land Management Practices before and after Land Certification

#### Tree planting before and after land certification

A total of ten different types of tree species were identified and recorded in farmers' fields under study. The number of indigenous trees, *Olea africana* (Weyira), *Sesbania* and *Acacia* (Girar) planted by farmers after land certification have declined, while the other seven tree species had increased, though the magnitude of trees planted after land certification was significant only for *Eucalyptus* (Bahir zaf) and *Grevillia* (Table 5). Here, it is important to note that plantation of indigenous tree species with long duration to

maturity are not preferred for plantation by farmers. Farmers preferred to plant fast growing tree species, such as, *Eucalyptus* and *Grevillia robusta*, which may be associated with fear of tenure security. Farmers always anticipate expropriation from the plot if the land is needed for public use, and as such they go for fast growing tree species that can generate income in few years.

Type of tree species	Mean No of Before certification	trees planted After certification	Mean Difference	Std. Error Mean	t-test
Eucalyptus globulus	181.85	331.89	150.033	53.144	2.823**
Juniperous procera	28.33	36.87	8.540	6.186	1.381
Dombey atorrida	1.55	8.06	6.52	6.552	0.995
Hagenia abysinica	1.48	4.45	2.973	2.003	1.484
Grevillea robusta	0.05	2.59	2.547	0.47	5.409**
Olea Africana	0.15	0.03	-0.120	0.1	-1.178
Arundinaria alpine	1.47	4.17	2.7	2.5	1.067
Sesbania sesban	0.09	0.00	-0.087	0.09	-1.067
Acacia bussei	0.46	0.15	-0.307	0.19	-1.591
Fodder trees	0.00	0.11	0.330	0.24	13669

 Table 5: Mean number of tree species planted before and after land

 certification

\*p<0.05; Source: Own Survey

# Other types of land management practices before and after land certification

On average, farmers constructed 0.45 hectare more stone-bund terracing after land certification. Farmers in the study area had also applied compost and organic manure, on average on 0.25 hectares of additional land compared to the pre-land certification period. Fencing around farm land has also improved after land certification, and on average, farmers fenced 0.19 hectares of more lands compared to the pre-land certification period (Table 6). The significant increase in the magnitude of the three types of land management practices could be partly associated to the secured holding of land certificates and to the legal protection of tenure security that motivated farmers to undertake various land improvement activities. Soil bund terrace construction, on the other hand, has shown insignificant increase in terms of magnitude, indicating that due attention was not given though soil bund terracing seems to be easier to construct and economical in terms of labor and time than stone-bund terracing.

When households were asked why they practiced more conservation practices after land certification, they responded by stating that besides receiving legal landholding document at hand, they were given several relevant trainings on the benefits of adopting land management practices and the contribution of such practices in improving their farm productivity. EPLAUO experts had also confirmed offering recurrent trainings which helped them to bring about change in land management undertakings in the study area. In general, though farmers' motivation and their effort of undertaking various conservation practices seemed to be promising as a start, it had shown a positive effect on long term investment. It is difficult to conclude that the overall performance of land management activities practiced is as adequate as desired both in terms of magnitude and proportion. In this regard, diversifying as well as increasing the quantity of tree planting and construction of other terracing structures is crucial for land management undertaking to be robust enough.

Table 6: Mean values of land man	nagement practices (in ha) before and
after land certification.	

Type of Land Management Practice	Before Certification	After Certification	Mean Difference	Standard Error of mean	t-value
Soil bund construction	0.010	0.111	0.11	0.01	8.605
Stone bund construction	0.00	0.045	0.45	0.008	5.245**
Compost & manure	0.09	0.34	0.25	0.02	12.085**
Fencing the farmland	0.14	0.33	0.19	0.02	9.26**

\*\*p<0.05Source: Own Survey

#### **Challenges faced in undertaking Land Management Practices**

The study further identified the challenges that farmers were facing in the adoption of land management practices. Among the respondents, 43% had stated that they had faced challenges in engaging in different types of land management practices. Shortage of fund was mentioned as the most serious problem. This is related to cash income required to buy the necessary inputs and to pay wages to daily labourers. Unavailability of financial institutions for credit generation within the study area was a hindrance.

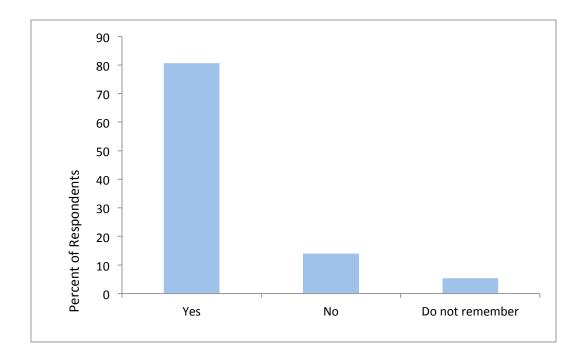
In a group discussion with farmers and district land administration experts it was pointed out that most farm plots were not clearly demarcated and sketched due to lack of budgets. The absence of clear demarcation of boundaries seem to be a source of disputes over land and had sometimes

54

created a feeling of tenure insecurity, which in turn decreases farmers' motivation for long term investment decision. Hence, demarcation and sketching of farmers plot with cadastral mapping system is crucial as promulgated in the Oromia rural land proclamation (Proclamation No.130/2007, Art.15 sub article 2.).

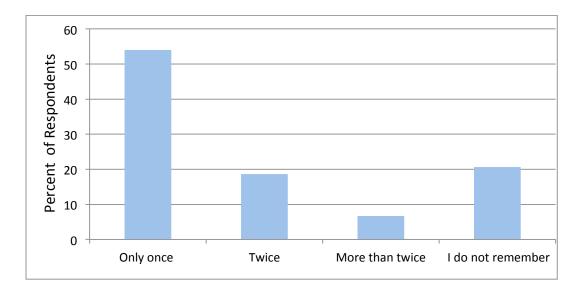
#### Land Redistribution

When farmers were asked on the occurrence of land redistribution in the study area, the majority (80.7%) had expressed that land redistribution had happened before certificate provisions (Fig. 2).



# Figure 2: Land Redistribution before the Provision of Certificate of Land Holdings

Plot of land of 54% of farmers was redistributed only once by the local government before the provision of land certificate. But, 18.67% of the respondents had stated that land apportioning and redistributing took place twice before land certificate was provided to each farmer (Fig. 3).



# Figure 3: Farmers' response on the frequency of land redistribution within the study area before certification

According to focus group discussions and key informants interviews, land redistribution did not have a holistic approach that covered all *kebeles* and was not as frequent as in the Northern part of Ethiopia. What they call redistribution, as affirmed by EPLAUO, was allocating unused land to the landless and transferring land to others when someone without family passes away. According to key informants, if some farmers had holdings more than the *Kebele* average (2.5 hectares), the remaining plot of land was redistributed to people having no holdings, and this was practiced only once before land certification. The finding of this research is in line with Assefa

(2010) in which he found simillar result that the level of farmers' confidence has increased through time as a result of certification.

#### Institutional interventions to enhance land Management Practices

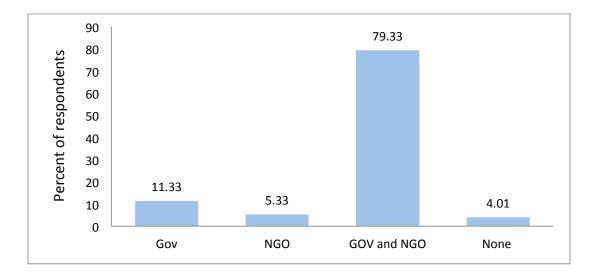
It is an undeniable fact that various governmental and non-governmental (both local and international) institutions provide various types of supports to boost the land management efforts of farmers in different parts of Ethiopia. Such efforts had a positive outcome in the collaborative efforts of enhancing farmers' capability to manage and invest on their land holdings (Table 6).

# Table 6: Institutional support and interventions towards landmanagement practices

No	Variable	Responses	Frequency	Percentage
	The Institutions which helps	Government	17	11.3
	the respondent in providing all	NGOs	8	5.3
1	the necessary support to	Both Gov't & NGOs	119	79.3
	enhance land management Practices	No support from any	6	4.0
		Technical Advice	7	4.7
		Material support.	5	3.3
2	The Kind of support obtained	Only tree seedlings	4	2.7
	from the mentioned institution	All three types of support	127	84.7
		Others if mentioned	7	4.7
	Evaluation of the respondents	Good enough	80	53.3
3	on the interventions of	No enough at all	48	32.0
	supporting institutions	No response	22	14.7

Source: own survey

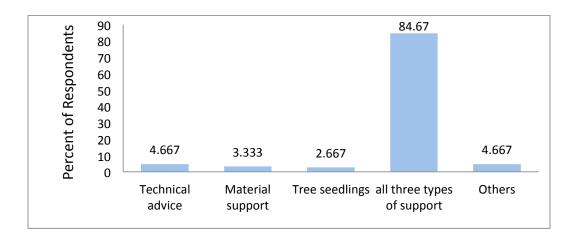
The level of support was more pronounced when the government agencies were working in conjunction with NGOs rather than individually alone, as indicated by 79.33% of respondents (Fig. 4).



#### Figure 4: Level of support provided to farmers in the study area

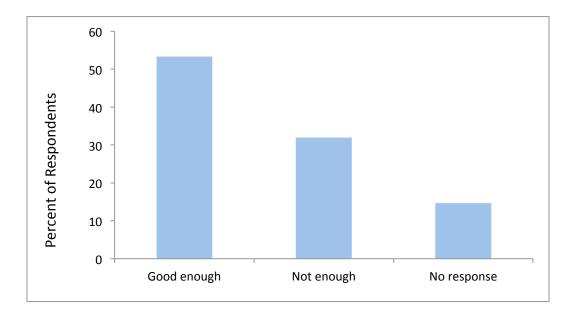
According to respondents, the kind of support obtained from the aforementioned institutions include, technical advices (4.7%), material support (3.3%), provision of tree seedlings (2.7%), and all three types of support (84.7%). So, the amalgamation of various forms of support computed was 94.4%, as some failed to respond (Fig. 5).

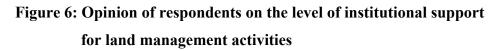
58



#### Figure 5: Types of support provided to farmers for land management

When farmers were asked on the adequacy of the interventions, 53.3% of the respondents stated that the intervention was rated as "good enough", while 32.0% considered that it was "not enough at all" and the remaining 14.7% were indifferent (Fig.6).





Thus, the various forms of support seemed to contribute immensely to the farmers' efforts and motivations for enhanced change on their land management practices. This finding is in line with Tesfu's (2011) study in that support rendered by both governmental and non-governmental institutions in the form of material provision and technical back-up had contributed at farm household level to undertake a variety of short and long-term conservation practices.

#### Conclusion

Land tenure insecurity is one of the bottlenecks in natural resource management in general and land management practices in particular. Land registration and certification program has been implemented in Oromia National Regional State since 2003 with the objective of registering all parcels of farm households and granting them legal certificates of land holding in order to ensure land tenure security. In this regard, farmers received primary book of certificate as a legal document which ensures usufruct land holding right of households.

Tenure insecurity restricts rights in land and reduces farmers' incentives to invest in land management practices. The program of land redistribution that took place in the study area had created a sense of insecurity among the farming community, and as a result, the land was deprived of attention and was exposed to soil erosion and mismanagement of farmland. With the issuance of certificates the comparative level of motivation to invest in land has shown considerable increase and farmers used significant amount of energy to improve their farmland holdings. Land tenure security and property rights eliminate anxiety and uncertainty of farmers and it has encouraged farmers to make long term investment decision on their holdings.

Support obtained from government and non-government institutions had contributed positively to enhance household's decisions to undertake some sort of conservation practices as technical support is crucial to adopt the required land conservation technologies.

Farming households face various challenges to undertake different farmland management practices which limit the progress of such undertakings. Though the intervention of institutions was found to be evaluated as good enough and contributed to enhance land management efforts, the overall progress of such activities were not robust enough in terms of mix of planted trees, in which a drastic decline of some indigenous trees has been observed. If the challenge of financial constraint faced by some farmers is solved, the issuance of certificate of land holding right promotes land management practices.

#### References

- Assefa Belay (2010). The Effects of Rural Land Certification in Securing Land Rights: A Case of Amhara Region, Ethiopia. MA Thesis, International Institute for Geo-information Science and Earth Observation, The Netherlands.
- Betru Nedessa, Jawad Ali and Ingrid Nyborg (2005).Exploring Ecological and Socio-Economic Issues for the Improvement of Area Enclosure Management: A Case Study from Ethiopia. A working Document of the Dryland Coordination Group (DCG). DCG Report No 38, May 2005.Dryland coordination Group c/o MiljeΦhuset G9 Grensen 9b, N-0159, Oslo, Norway.
- Birhanu Adenew and Fayera Abdi (2005). Land Registration in Amhara Region, Ethiopia Research Report 3, Natural Resources Group, International Institute for Environment and Development, 3 Endsleigh street, London WC1H 0DD, United Kingdom.
- Bisley, T. (1995). Property Rights and Investment Incentives: Theory and Evidence from Ghana. Journal of Political Economics, 103: 903-937.
- Deininger, K. (2003). Land Policies for Growth and Poverty Reduction. World Bank, Washington, DC; Oxford University Press, Oxford, UK.Deininger, K., Zevenbergenb, J., and Ali, D.A. 2006. Assessing the Certification Process of Ethiopia's rural Lands. Conference paper, Colleque international "Les frontiers de la question fonciére - at the frontière of land issues."Moutpellier, France.
- Desta Damene and Ignatius Mberengwa (2012). Determinants of farmers' land management practices: the case of Tole District, South West Shewa zone, Oromia National Regional State, Ethiopia. Journal of
- Feder, G. and R. Noronha (1987). Land rights, systems and agricultural development in Sub- Saharan Africa. World Bank researcher observer 2:143-169.
- Gebremedhin B. and Swinton S.M. (2003). Investment in soil conservation in northern Ethiopia: The role of land tenure security and public programs. Agricultural Economics, 29: 69-84.
- Hurni, H. (2000). Assessing sustainable land management (SLM). Agriculture, Ecosystems and Environment 81, 83–92.

- Kabubo-Mariara J., and Linderhof V. (2003). Tenure Security, Sustainable Land management and Poverty: Case Studies from Kenya. Agricultural Economics Research Institute (LEI), The Hague.
- Magalata Affan Oromo, "Oromia Rural Land Use and Administration Proclamation," Proclamation No. 130/2007.
- Ogolla B. D. with Mugabe, J. (1996). Land Tenure Systems, In Land We Trust; Initiative Publishers, Nairobi Kenya.
- Omit, J., Parton, Ehui, S., and Sinden, J. (2000). Some Policy implications of the resurface of rural factor markets following agrarian de-collectivization in Ethiopia. Human Ecology, 28:585-603.
- Pender, J and Kerr, J. (1998).Determinants of farmers' indigenous soil and water conservation investments in semi- arid India. Agricultural Economics, 19:113-125.
- Pender J., Ehui, S., and Place, F. (2006).Strategies for Sustainable Land Management in the East African Highlands. International Food Policy Research Institute. Washington D.C.
- Shimelles Tenaw, Zahidu, K.M, and Parvian, T. (2009). Effects of land tenure and property rights on agricultural productivity in Ethiopia, Namibia, and Bangladesh. Helsinki: University of Helsinki.
- Singh, K. (1994) Managing common pool resources. Principles and Case Studies. Oxford .
- Tesfu Kahsay (2003). The Effect of Land Tenure Systems on Soil Conservation Practices in Northern Ethiopia - A Case Study of Habru District in Amhara National Regional State (ANRS), Ethiopia. Research and Perspectives on Development Practice, Kimmage DSC series. Kimmage Development Studies Centre, Kimmage Manor, Whitehall Road, Dublin 12, Ireland.
- Woldeamlak, B. (2003). Land Degradation and Farmers' Acceptance and Adoption of Conservation Technologies in the Degil Watershed, Northwestern Highlands of Ethiopia, Social Science Research Report Series no.29, OSSREA, Addis Ababa.
- World Bank. (1989). Sub- Saharan Africa: from crises to Sustainable growth: a long-term perspective study. The World Bank: Washington D.C.

# MYCOTOXIN AND MYCOTOXICOSES: A REVIEW OF EXPOSURE, POTENTIAL HEALTH CONSEQUENCES AND INTERVENTIONS

## Mengistu Hulluka<sup>1</sup>

#### Abstract

Many species of fungi produce mycotoxins, a poisonous metabolite affecting the human and animal health. The most notable of which is Aflatoxin B. Mycotoxins are a big problem in agriculture infesting large number of crops and their toxic metabolite then end up in food and animal feed. Most of the toxins are actually toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic – cancer causing. Intervention strategies to reduce human exposure to mycotoxins are discussed.

<sup>1</sup> St. Mary's University, Addis Ababa, Ethiopia

#### Introduction

Mycotoxin is a toxic secondary metabolite produced by organisms of the fungi kingdom, commonly known as molds. The term mycotoxin is derived from a Greek word '*mykes*', meaning fungus, and '*toxicon*' meaning poison (https://www.thefreedictionary.com/mycotoxin).

Over 50,000 different species of mold are identified so far, but only about 200 present serious health risks to humans and animals. The toxin–producing fungi are referred to as toxic molds (Richard, J.L., 2007).

Many species of fungi produce different types of mycotoxins. One mold species may produce many different mycotoxins, and the same mycotoxin may be produced by several species. An example of mycotoxin poisons– *Claviceps purpurea* poisoning – from consuming ergot infested crops. Mycotoxins are found in all sorts of foods and beverages: grains, raisins, beer, wine, dark chocolate, and peanut butter (to name a few) can all contain measurable levels of mycotoxins (Bennet, J.W., 2003).

Mycotoxins are a big problem in agriculture. Over 25% of the world's agricultural production is contaminated by mycotoxins. The mycotoxins can then end up in food. If crops like wheat are stored poorly after harvest, toxic molds can grow and contaminate the wheat with mycotoxins. In the last ten years, mycotoxins have accounted for 30 - 60% of food and feed rejections at European Union borders (Kumar,M. et al., 2018).

General interest in mycotoxins rose in 1960 when feed-related mycotoxins called 'turkey X disease' which was later proved to be caused by aflatoxins, appeared in farm animals in England. Subsequently, it was found that aflatoxins are heptocarcinogens in animals and humans and this stimulated

research on mycotoxins (http://www.blacktoxicmolds.com/mycotoxinsmold.php).

# Mycotoxin types

Below are a list of some of the Mycotoxins and the associated fungi reported to have occurred throughout the world.

FUNGI	MYCOTOXIN
Aspergillus carbonarious	Ochratoxin A
Aspergillus flavus	Aflatoxin B
	Cyclopiazonic Acid
Aspergillus niger	Ochratoxin A
Aspergillus nomius	Alfatoxin B
Aspergillus parasiticus	Alfatoxin B
Aspergillus ochraceus	Ochratoxin A
	Penicillic Acid
Aspergillus oryzae	Cyclopiazonic Acid
Aspergillus terreus	Citrinin
Aspergillus versicolor	Sterigmatocystin
	5-Methoxysterigmatocystin
Chaetomium globosum	Chaetoglobosin C
	Chaetoglobosin A
	Chaetoglobosin B
Chaetomium thielavoideum	Sterigmatocystin
Chaetomium udagawae	Sterigmatocystin
Emericella nidulans	Sterigmatocystin
Memnoniel laechinata	Griseofulvin
	Dechlorogriseofulvin
	Epi-dechorogriseofulvin
	Trichodermin
	Trichodermol
Penicillium aurantiocandidum	Penicillic Acid
Penicillium aurantiogriseum	Penicillic Acid
Penicillium brasilianum	Penicillic Acid

FUNGI	MYCOTOXIN
Penicillium brevicompactum	Mycophenolic Acid
Penicillium camemberti	Cyclopiazonic Acid
Penicillium carneum	Mycophenolic Acid
	Roquefortine C
Penicillium citrinum	Citrinin
Penicillium chrysogenum	Roquefortine C
Penicillium commune	Cyclopiazonic Acid
Penicillium crustosum	Roquefortine C
Penicillium discolor	Chaetoglobosin C
	Chaetoglobosin A
	Chaetoglobosin B
Penicillium expansum	Citrinin
	Roquefortine C
Penicillium freii	Penicillic Acid
Penicillium griseofulvum	Cyclopiazonic Acid
	Griseofulvin
	Roquefortine C
Penicillium hirsutum	Roquefortine C
Penicillium hordei	Roquefortine C
Penicillium melanoconidium	Roquefortine C
	Penicillic Acid
Penicillium nordicum	Ochratoxin A
Penicillium palitans	Cyclopiazonic Acid
Penicillium paneum	Roquefortine C
Penicillium poloncium	Penicillic Acid
Penicillium roqueforti	Mycophenolic Acid
	Roquefortine C
Penicillium verrucosum	Citrinin
	Ochratoxin A
Penicillium viridicatum	Penicillic Acid
Stachybotrys chartarum	Satratoxin H
	6B-Hydroxydolabella-3E,8E,12-trien-14-one
	Epoxydolabellane A
	Isororidin E
	Isosatratoxin F

FUNGI	MYCOTOXIN
	MER-5003 Molecular Weight 470
	MER-5003 Molecular Weight 412
	Roridin E
	Roridin L-2
	Satratoxin G
	Trichodermin
	Trichodermol
Trichoderma longibrachiatum	Trichodermin
	Trichodermol
Trichoderma harzianum	Trichodermin
	Trichodermol
Trichoderma viride	Trichodermin
	Trichodermol

Source: National Treatment Centers for Environmental Disease (ntced.com) <u>http://www.ntced.org/diagnosing</u>-mold-exposure/mycotoxins-and-funguschart/

# Effect of Mycotoxin in Humans and Animals

When contaminated food is processed, mycotoxins enter the general human food supply. Animals fed contaminated food can pass mycotoxin transformation products into eggs, milk products, and meat. For example, contaminated poultry feed is suspected in the findings of high percentages of samples of aflatoxin-contaminated chicken meat and eggs in Pakistan. In Kenya, 125 people died in 2004 after they ate food contaminated with aflatoxin mycotoxins. Children are particularly affected by aflatoxin exposure, which is associated with stunted growth, delayed development, liver damage, and liver cancer (William, J.H., 2004).

Some toxic molds (such as *Stachybotrys chartarum*) grow in homes. These toxic molds carry mycotoxins on their spores. If these molds are disturbed,

they can also release large amounts of mycotoxins into the air on spores. The main way people are exposed to mycotoxins in their homes is by breathing the mycotoxin in. When there is toxic mold in buildings, there is a much greater amount of mycotoxins in the air. When people breathe-in mycotoxins, the mycotoxins can damage the lungs and create breathing problems.

Farm animals have died from eating feed contaminated with mycotoxins. There have also been cases of pets dying after eating pet food which contained mycotoxins. Health effects can depend on the length of time and the amount of mycotoxins a person was exposed to. The main types of long-term symptoms caused by mycotoxins are breathing problems, mental problems, and damage to internal organs. Mycotoxins can also suppress the immune system. This will then leave a person more vulnerable to getting other diseases too (http://www.blacktoxicmolds.com/mycotoxins-mold.php).

#### **Initial Mycotoxin Symptoms in Human**

After exposure to the mycotoxins, early symptom begins within five minutes. Full effects take 60 minutes. If skin exposure occurs, the skin die and slough (fall off). The symptoms can also depend on how sudden or gradual the mycotoxin exposure was. Being exposed to a large amount of mycotoxins suddenly might cause symptoms like vomiting and pain. The followings are top mold-associated symptoms in humans: a) fatigue and weakness, b) headache and light sensitivity, c) poor memory, difficult word finding, d) difficult concentration, e) morning stiffness, joint pain, f) unusual skin sensations, tingling and numbness, g) shortness of breath, h) appetite swings, body temperature regulation, i) increased urinary frequency or

increased thirst, J) red eyes, blurred vision, sweats, mood swings, sharp pains, k) abnormal pain, diarrhea, bloating, l) tearing, disorientation, metabolic taste in mouth, m) static shocks, n) vertigo – feeling light headed (Carnahan, J., 2015).

#### Important Types of Mycotoxins and Their Effect on Humans

#### Aflatoxins

Aflatoxins are poisonous and cancer causing chemicals that are produced by certain molds growing in soil, decaying vegetation, hay, and grains. Aflatoxins are regularly found in improperly stored staple commodities, such as, cassava, chilli, peppers, corn, cotton seed, millet, peanuts, rice, sesame seeds, sorghum, sunflower seeds, tree nuts, wheat, and a variety of species under conditions of high humidity and temperature and present a risk to human health that is insufficiently recognized.

The two major Aspergillus species that produce aflatoxins are *A. flavus*, which produces only B aflatoxins, and *A. parasiticus*, which produces both B and G aflatoxins. Aflatoxins M1 and M2 are oxidative metabolic products of aflatoxins B1 and B2 produced by animals following ingestion, and so appear in milk (both animal and human), urine and faeces (Williams, J.H. et al, 2004).

Aflatoxicol is a reductive metabolite of aflatoxin B1. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver. The evaluation of epidemiological and laboratory results carried out in 1987 by the International Agency for Research on Cancer (IARC) found that there is sufficient evidence in humans for the carcinogenicity of

naturally occurring mixtures of aflatoxins, which are, therefore, classified as Group 1 carcinogens, except for aflatoxin M1, which is possibly carcinogenic to humans (Robbins, C.A. et al, 2000).

Several outbreaks of aflatoxicosis have occurred in tropical countries, mostly among adults in rural populations with a poor level of nutrition for whom maize is the staple food. The clinical picture presented by cases indicated acute toxic liver injury, which was confirmed by morphological changes in liver autopsy specimens that were indicative of toxic hepatitis. Mortality rates in the acute phase were  $10\pm60$  % (Peraica, M. et al., 1999).

#### Ochratoxins

Ochratoxins are also secondary metabolites of Aspergillus and Penicillium strains, found on cereals, coffee and bread, as well as on all kinds of food commodities of animal origin in many countries. The most frequent is ochratoxin A, which is also the most toxic.

It has been shown to be nephrotoxic, immunosuppressant (Peraica, M., et al, 1999). The symptoms developed after 24 hours of transitory epigastric tension, respiratory distress. The presence of the mycotoxin in wheat from the granary was proved qualitatively by thin-layer chromatography. This mycotoxin has been proposed as the causative agent of endemic nephropathy, although the evidence for this is not substantial. This fatal renal disease occurs among rural populations in Croatia, Bosnia and Herzegovina, Yugoslavia, Bulgaria, and Romania, where it has been estimated that about 20,000 people are either suffering from or are suspected to have the disease. There is no acute phase of the illness; the first signs and symptoms of the disease are not specific and include fatigue, headache, loss of body weight and pale skin. In the advanced stage of the disease, the size

and weight of kidneys are remarkably reduced, with diffuse cortical fibrosis, usually without signs of inflammation.

Ochratoxin A is found more frequently and in higher concentrations in the blood of inhabitants from endemic regions than control regions. Many samples of locally produced food and feed collected in the endemic area contained ochratoxin A. It should be emphasized that the grain analyzed had been kept for many months in the inadequate food stores of individual families. In Tunisia, ochratoxin - A has been detected in high concentrations in the blood and food of patients with kidney impairment of unknown etiology. It has also been found in several countries, both in food and feed and in humans. So far, no cases of endemic nephropathy have been recorded in these countries. In endemic regions of Croatia, Bulgaria and Yugoslavia, the incidence of otherwise rare urothelial tumours of the pelvis and ureter is 50, 90 and100 times greater, respectively, than in non-endemic regions. It has been suggested that ochratoxin-A may be the causal agent for both endemic nephropathy and urothelial tumours. IARC classified ochratoxin-A as a compound possibly carcinogenic to humans (Peraica, M. et al, 1999).

#### Trichothecenes

Trichothecenes are mycotoxins produced mostly by members of the genus *Fusarium*, although other genera (e.g. *Trichoderma*, *Trichothecium*, *Myrothecium* and *Stachybotrys*) are also known to produce these compounds. To date, 148 trichothecenes have been isolated, but only a few have been found to contaminate food and feed. The most frequent contaminants are deoxynivalenol (DON), also known as vomitoxin, nivalenol (NIV), diacetoxyscirpenol (DAS), while T-2 toxin is rarer.

JAD 7 (1) 2017

Common manifestations of trichothecene toxicity are depression of immune responses and nausea, sometimes vomiting. The first recognized trichothecene mycotoxicosis was alimentary toxic aleukia in the USSR in 1932; the mortality rate was 60%. In regions where the disease occurred,  $5\pm$  40% of grain samples cultured showed the presence of *Fusarium sporotrichoides*, while in those regions where the disease was absent this fungus was found in only  $2\pm8\%$  of samples. The severity of mycotoxicosis was related to the duration of consumption of toxic grain. Such severe trichothecene mycotoxicoses, the consequence of continuous ingestion of toxins, have not been recorded since this outbreak. In several cases, trichothecene mycotoxicosis was caused by a single ingestion of bread containing toxic flour or rice.

In experimental animals, trichothecenes are 40 times more toxic when inhaled than when given orally. Trichothecenes were found in air samples collected during the drying and milling process on farms, in the ventilation systems of private houses and office buildings, and on the walls of houses with high humidity. There are some reports showing trichothecene involvement in the development of ``sick building syndrome''. The symptoms of airborne toxicosis disappeared when the buildings and ventilation systems were thoroughly cleaned. There are some reports that indicate that trichothecenes may have been used as chemical warfare agents in South-East Asia (Peraica, M. et al, 1999).

#### Fumonisins

Fumonisins are mycotoxins produced throughout the world by *Fusarium moniliforme* and related species when they grow in maize. Fumonisins B1 and B2 are of toxicological significance, while the others (B3, B4, A1 and

A2) occur in very low concentrations and are less toxic. In India, a single outbreak of acute food-borne disease possibly caused by fumonisin B1 has been reported. In the 27 villages involved, the individuals affected were from the poorest social strata, who had consumed maize and sorghum harvested and left in the fields during unseasonable rains.

The main features of the disease were transient abdominal pain, borborygmus and diarrhoea, which began half an hour to one hour following consumption of unleavened bread prepared from moldy sorghum or moldy maize. Patients recovered fully when the exposure ceased and there were no fatalities.

Fumonisin B1 was found in much higher concentrations in the maize and sorghum from the affected households than from controls. Fumonisin B1 was found more frequently and in much higher concentrations in maize in regions of Transkei, China and north-east Italy with a higher incidence of oesophageal cancer than other regions. It was postulated that the high incidence of oesophageal cancer was related to the presence of this mycotoxin in maize, which is a staple food in these regions. The incidence and concentration of aflatoxin B1, deoxynivalenol and fumonisins B1, B2 and B3 were recently determined in maize samples from an area of China (Haimen) with a high incidence of primary liver cancer and from an area with a low incidence. Aflatoxin B1 was found in low concentrations in almost all maize samples from both these areas, but the incidence and concentration of deoxynivalenol and fumonisins were much higher in the samples from the area where the incidence of primary liver cancer was high. An IARC working group classified the toxins from F. moniliforme as possibly carcinogenic to humans.

The impact of other mycotoxins on human health was reported in persons occupationally exposed to large amounts of different mycotoxin-producing fungi (farmers, workers in silos, etc.). In such cases, exposure to spores via the respiratory tract seems to be of considerable importance (Peraica, M. et al, 1999).

# Zearalenone

Zearalenone (previously known as F-2) is produced mainly by *Fusarium graminearum* and related species, principally in wheat and maize but also in sorghum, barley and compounded feeds.

Zearalenone and its derivatives produce estrogenic effects in various animal species (infertility, vulval oedema, vaginal prolapse and mammary hypertrophy in females and feminization of males, atrophy of testes and enlargement of mammary glands). In Puerto Rico, zearalenone was found in the blood of children with precocious sexual development exposed to contaminated food.

Zearalenone was also found together with other Fusarium mycotoxins. The proportion of late-term abortions (gestational age  $16\pm27$  weeks) was higher among farmers. The risk associated with grain farming was higher after the harvest, in seasons with a poor quality harvest and in pregnancies with multiple fetuses, which suggests that mycotoxins in grain induce labour at an early stage of pregnancy. Pulmonary mycotoxicosis has been reported in ten persons exposed to large quantities of fungal hyphae and spores during the cleaning of silos. The clinical picture developed several hours afterwards, with burning eyes, throat and chest, irritating cough and fever. There was no wheezing, cyanosis or other sign of bronchospasm. In five patients, chest X-rays revealed reticular and fine nodular features

# Mengistu Hulluka

compatible with interstitial pneumonitis. Histological study of a lung biopsy from one patient showed a multifocal acute process, with primary involvement of terminal bronchioles containing numbers of various spores. Cultures from lung biopsy material revealed at least five fungal species, including one *Fusarium* and one *Penicillium*. However, blood samples were not checked for the presence of mycotoxins. In contrast with the findings in patients with farmer's lung disease, these patients did not develop positive serological reactions to thermophilic actinomycetes or to extracts of fungi obtained from hay or silage. The patients were followed for periods of  $1\pm10$  years; they continued their work, avoiding massive re- exposure to fungal dust, and during the observation period there were no further incidents (Peraica, M. et al, 1999).

#### Ergot

Ergot is the common name of the sclerotia of fungal species within the genus *Claviceps*, which produce ergot alkaloids. The sclerotium is the dark-coloured, hard fungal mass that replaces the seed or kernel of a plant following infestation. Ergot alkaloids are also secondary metabolites of some strains of *Penicillium, Aspergillus* and *Rhizopus* spp. The ca.40 ergot alkaloids isolated from Claviceps sclerotia can be divided into three groups:

± derivatives of lysergic acid (e.g. ergotamine and ergocristine);

± derivatives of isolysergic acid (e.g. ergotaminine);

± derivatives of dimethyl ergoline (clavines,e.g.agroclavine).

The source of the ergot strongly influences the type of alkaloids present, as well as the clinical picture of ergotism. *Claviceps purpurea* produces ergotamine-ergocristine alkaloids, which cause the gangrenous form of

ergotism because of their vasoconstrictive activity. The initial symptoms are oedema of the legs, with severe pains. Paraesthesias are followed by gangrene at the tendons, with painless demarcation. The last recorded outbreak of gangrenous ergotism occurred in Ethiopia in  $1977\pm78$ ; 140 persons were affected and the mortality was high (34%) (King, B., 1979).

The other type of ergotism, a convulsive form related to intoxication with clavine alkaloids from *Claviceps fusiformis*, was last seen during 1975 in India when 78 persons were affected. It was characterized by gastrointestinal symptoms (nausea, vomiting and giddiness) followed by effects on the central nervous system (drowsiness, prolonged sleepiness, twitching, convulsions, blindness and paralysis). The onset of symptoms occurred 1±48 hours following exposure; there were no fatalities. Ergotism is extremely rare today, primarily because the normal grain cleaning and milling processes remove most of the ergot so that only very low levels of alkaloids remain in the resultant flours. In addition, the alkaloids that are the causative agents of ergotism are relatively labile and are usually destroyed during baking and cooking (Peraica, M., et al, 1999).

Intervention strategies to reduce human exposure to mycotoxins <a href="https://www.iarc.fr/en/publications/pdfs-online/wrk/wrk9/IARC\_WGR9">https://www.iarc.fr/en/publications/pdfs-online/wrk/wrk9/IARC\_WGR9</a>

Intervention strategy varies in resources required, complexity, and useful scale. For effective implementation, all it requires is social consent and political will. Some interventions are complicated and resource-intensive, and others are simple to implement on a community or even household scale.

The most important intervention types are discussed below:

- Regulation
- Dietary diversity
- Genetic resistance
- Biological control
- Primary prevention
- Post-harvest intervention
- Chemoprevention

# **Regulation**

Corporate, international, and governmental regulatory frameworks are necessary in the reduction of mycotoxin levels in food and feed. The available evidence shows that the development of a functioning food safety system begins in the corporate sector, both for domestic consumption and for export crops. As capacity and appropriate legal frameworks and enforcement structures are put in place, contamination levels in crops eventually decrease. However, the positive impact on subsistent farmers is usually limited, with the benefits generally going to larger farmers. Enforcement of risk-based food law is critical to public health and economic viability, and drives the development and sustained use of intervention technologies. For example, after the reporting of several deaths in children in Africa due to consumption of aflatoxin-contaminated meal, a decision was made in 1966 by the FAO /WHO/UNICEF Protein Advisory Group to set a limit of 30 ppb aflatoxin in protein supplements made from groundnuts.

Aflatoxin contamination levels can vary widely, from products that meet the strict maximum levels set by the European Commission [2 ug/kg for AFB<sub>1</sub>; 4ug/kg for total aflatoxins (sum of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG2)] for cereals and nuts for direct human consumption (European Commission for food safety, 2010). **7932** 

# Dietary diversity to mitigate mycotoxin exposure

Dietary diversity is a good way to improve nutrition and health. Aspects important for a healthy diet include the number of different foods, the quantities, and the health (nutritional) value of those foods available for consumption. Dietary data from the United Republic of Tanzania estimated the effect of crop diversification on child growth and projected a positive and significant impact on child nutritional status, particularly for girls and on children's height. A lack of dietary diversity is directly related to levels of mycotoxin exposure. In rural Africa and parts of Latin America, a high percentage of calories come from maize, which is commonly contaminated by aflatoxins and/or fumonisins. In East Africa, aflatoxin exposure has also been directly correlated with reported daily intake of maize, and fumonisin exposure occurs almost entirely from maize. Another major source of exposure to aflatoxin is through the consumption of groundnuts.

Access to a greater variety of foods will lower the risk of exposure by lessening the intake of these commonly contaminated foods. Replacing foods at high risk of mycotoxin contamination with those at lower risk would improve access to foods with better nutritional value. An excellent example of improved health outcomes after a switch from a food source at high risk of aflatoxin contamination to one at lower risk occurred in Qidong, China. A government policy to grow foods that are eaten locally, combined Mengistu Hulluka

with a prohibition on interregional shipments of food products, had forced residents of Qidong County to produce and consume primarily maize for several decades. Liberalization of the transboundary provincial trade policy allowed rice to be imported from other regions of the country, replacing maize as the staple cereal. Since aflatoxin contamination is much lower in rice than in maize, the result was reduced aflatoxin exposure and a precipitous drop in liver cancer incidence.

Food diversity and exposure risk can also be driven by socioeconomic factors. In West Africa, it was reported that the average frequency of maize consumption is 5–7 days per week. Maize is currently the most common cereal staple, having displaced the native cereals sorghum and millet and other sources of starch.

Consumption of groundnuts, another common source of aflatoxins, was positively correlated with household and maternal wealth variables and varied by agro-ecological zone. In Ghana, interesting evidence was shown of an inverse relationship between a woman's income and the level of aflatoxin biomarkers in her blood. This suggested that greater purchasing power may improve the opportunity for diversifying food choices. Changing food preferences where there are no economic constraints can be a matter of social marketing and awareness. However, changing food preferences and access for people living in food-insecure conditions presents an enormous challenge. In 1950, by far the major source of dietary starch in sub-Saharan Africa was sorghum and millet (40%), followed by cassava (30%) and maize (15%). The subsequent shift towards maize is part of a global trend; over the past 50 years, consumption of sorghum and millets has declined by 50% and consumption of cassava by 40%. In turn, this may have had a major role in increasing aflatoxin exposures. In West Africa, for example,

80

aflatoxin concentrations in pearl millet and sorghum were substantially lower than those in maize.

# Genetic resistance to aflatoxin and fumonisin contamination of maize

# A. Aflatoxins

Genetic resistance to aflatoxin and fumonisin contamination exists in maize populations, but it is complex and involves multiple genes, and genetic engineering requires moving resistance genes into agronomically acceptable genotypes.

Resistance to ear-feeding insects is associated with lower levels of aflatoxins and fumonisins. Transgenic expression of *Bacillus thuringiensis* (Bt) toxins reduces insect damage and fumonisin contamination. The effectiveness of Bt in reducing aflatoxin contamination is inconclusive. The new genetic technologies, along with improved breeding populations and phenotyping strategies, have dramatically increased the number of genetic markers associated with resistance to aflatoxins and fumonisins and have identified putative resistance genes and proteins.

Progress has been made in selecting breeding lines of maize with resistance to aflatoxin accumulation that shows high and repeatable resistance under different environments (Flint-Garcia, et al, 2005). In summary, maize hybrids with improved resistance to *Aspergillus flavus* and aflatoxins are being used, but the level of resistance is not yet adequate to prevent unacceptable aflatoxin contamination in some fields.

# **B.** Fumonisins

Many genotypes have been identified with some resistance to fumonisin accumulation (Mesterházy et al., 2012; Santiago et al., 2013), including germplasm lines adapted to Argentina, Central and West Africa, and South Africa, but no hybrids are available with adequate resistance. Heritability for resistance to fumonisin accumulation is higher than that for resistanceto aflatoxin contamination, and a moderate to high genotypic correlation between ear rot and fumonisin content suggests that resistance to the fungus and to fumonisin production may be closely linked. This correlation has allowed selection for resistance to fumonisin accumulation based on ear rot scores, thus making screening quicker and less expensive.

Genome-wide association studies on the maize core diversity panel have identified three novel loci associated with 3–12% of the genetic variation associated with resistance to ear rot. Three putative resistance genes co-localized with the genetic markers. The large number of genetic markers available on the diversity panel is allowing the dissection of complex quantitative traits, such as resistance to mycotoxin accumulation. Fumonisin accumulation is consistently decreased when Bt maize hybrids effectively reduce insect damage. This can make the difference between maize products that are relatively safe and those that are not.

# Genetic resistance to Aflatoxin Contamination of Groundnuts

Genetic resistance to aflatoxin contamination in groundnuts is complex: heritability is low to moderate, there is a poor correlation between fungal growth and aflatoxin contamination, and results from *in vitro* seed assays do not correlate with those from field assays. Germplasm with some resistance is available, but genotypes do not show consistent response across locations, due to large interaction effects between the genotype and environment on aflatoxin contamination. One key environmental effect is drought stress, and many programmes have focused on breeding for drought tolerance as a way to improve resistance to aflatoxin contamination. A field study in West Africa examined 268 genotypes over four locations and confirmed that drought stress intensity increases aflatoxin contamination; however, the investigators did not show a significant relationship between drought tolerance and aflatoxin contamination, possibly due to other site-specific environmental effects. An improved understanding of resistance mechanisms should help improve selection of resistant germplasm. Genome sequences of the two diploid progenitors of groundnut are now available (http://peanutbase.org/browse search), which may facilitate molecular mapping and breeding for disease resistance.

# **Biological Control**

Given the large number of exploratory investigations in Africa, studies are needed to evaluate the impact of the low rate of genetic recombination, which will then inform the deployment of the technology in diverse settings.

#### Sorting

In developed countries, sorting and grain cleaning techniques are required to reduce mycotoxin contamination, notably in grains contaminated by ergot and in nuts. Ergot sclerotia are removed by specific gravity seed cleaning equipment, a practice that has been in place for a long time. In groundnuts, after basic clean-up of the crop by commercial farmers, high-capacity electronic optical sorters are used to remove nuts contaminated by aflatoxin. For maize, normal grain cleaners reduce aflatoxin and fumonisin by 50–60%, far less than the reduction from hand sorting. Soon after the discovery

Mengistu Hulluka

of aflatoxin in 1961, sorting emerged as a regular and effective practice to improve safety for groundnuts. The need for efficient ways to remove aflatoxin-contaminated nuts prompted experiments on the concentrations of aflatoxin in kernels from shells that were not visibly moldy. This revealed that visual sorting was an efficient way to segregate more-versus lesscontaminated kernels in the laboratory. However, parts of some nuts that appeared sound contained substantial levels of aflatoxin.

In the USA, after 3–4 hours of training on visual signs of contamination with *Aspergillus*, people with no prior experience were asked to visually sort samples of groundnuts that had already been classified according to their quality (sound, damaged, intermediate) by federal inspectors according to the official grading procedure. In the best grade of groundnuts, misclassification occurred, which the authors ascribed to mostly false-positives, with some false-negatives and sampling error.

By 1968, another step was introduced into the United States inspection system: examination by the inspectors of the damaged kernels for *Aspergillus*. After training, each inspector was given a folder with two sets of coloured photographs that showed what to look for and what not to look for. Before the development of the current methods, low-technology approach was proven useful .It was demonstrated that visual sorting of groundnuts provided a practical first-action regulatory method. They found that sound mature kernels and sound half kernels contained about 7% of the aflatoxin, with the damaged kernels containing the rest. Studies on grains contaminated with *Fusarium* toxins indicate that these strategies work best where there is ongoing training.

JAD 7 (1) 2017

A study in the Philippines found that manual sorting reduced aflatoxin concentrations in lots of raw groundnuts from 300ng/g to less than 15ng/g (Galvez et al., 2003). Research conducted in Kenya (and Haiti) demonstrated that manual sorting of groundnuts purchased at local markets could reduce aflatoxin concentrations by about 98%. In the case of maize in Africa, manual sorting is moderately effective at the village level for segregating kernel lots for decreased concentrations of aflatoxin. Removing visibly moldy, insect-damaged, and broken grains by hand reduced aflatoxin concentrations by 40%, based on reports from a study in Benin. Studies in South Africa and the United Republic of Tanzania have demonstrated that hand sorting of maize kernels by local farmers by removing the visibly infected or damaged kernels reduced fumonisin concentrations by 20%.

The willingness to hand sort grains and nuts has been shown to depend on the available supply. A study in Ghana found that household income and agricultural training increased the quality of the nuts consumed. In South Africa, the effectiveness of hand sorting on fumonisin reductions has been documented by biomarkers. In developed countries, sorting of contaminated grains is the primary tool used to reduce mycotoxin contamination in grains and nuts after harvest and can be effective at all scales of production.

# Nixtamalization

In Latin America, nixtamalization has been shown to reduce exposure to aflatoxin and fumonisin. A knowledge translation package based on factors known to reduce fumonisin in the residual masa would be beneficial.

In Mexico and Central and South America, nixtamalization has been the usual practice for millennia. Hydrolysis of fumonisin during commercial production of masa virtually eliminates fumonisin. Masa is made by boiling maize meal with the addition of lime, which is then washed out. The ratio of maize to lime to water used and the boiling, soaking, and rinsing practices vary. In the USA, fumonisin concentration is low in commercial tortillas from major companies. In contrast, in the USA, masa products from artisanal production facilities often contain some fumonisin. Where there is sufficient washing of the lime-treated product in the traditional process before consumption, concentrations of fumonisin and aflatoxin are lowered. In Latin America, variability in the process means that there can be residual parent fumonisin in the tortillas that leads to fumonisin exposure.

# Post-harvest Storage Intervention Strategies to Reduce Aflatoxin and Fumonisin Exposure

Mycotoxin contamination of crops can occur in the pre- and postharvest agricultural system due to inadequate agricultural practices. Fungal growth and toxin production can occur in the field (e.g. fumonisin, aflatoxin), in storage (aflatoxin), or in both. High humidity (> 85%), high temperatures (> 25 °C), insect and rodent activity, improper drying of crops, and water infiltration in the storage structure will result in the growth of A. flavus and Aspergillus parasiticus and aflatoxin accumulation. Most developing countries are located in the world's tropical zones and are subjected to monsoons and high temperature and humidity levels, which contribute to large post-harvest crop losses. Inadequate storage practices account for 20-50% of these losses. Despite being a major United Nations priority since 1946, such losses remain a global problem, increasing the risk of food insecurity (food availability, hunger, and nutritional value) and poverty. The double burden of both chronic exposure to mycotoxins and food insufficiency increases both mortality and morbidity, especially in children. Adequate post-harvest measures that are practical, economic, and culturally

acceptable will therefore address food safety and security and improve public health.

In subtropical climates, maize in the field is typically infected by *A.flavus*, and unless it is dried very quickly, aflatoxin concentrations increase after harvest. The stored post-harvest crop ecosystem is therefore an integral part of mycotoxin prevention strategies.

Most of the conditions associated with the post-harvest period can be controlled, unlike those affecting the pre-harvest phase. Strategies to reduce mycotoxin levels during storage mainly consist of: adequate drying of crops before storage; using clean, dry, and enclosed storage facilities; proper water drainage; well-aerated stores; and eliminating insect activity and other pests such as rodents and birds. Before storage, harvested field crops should be dried as soon as possible to reduce fungal growth; safe moisture levels recommended for cereals are 10–13% and for oilseeds are7–8%.

Common storage practices for crops include: on the field; on the floor in homes; on top of or under the roof of houses; in jute or polypropylene bags, wire cribs, pits, and metal bins; and in conical structures or other constructed structures, with or without roofing, made from wood, bamboo, thatch, or mud.

Evidence-based post-harvest storage intervention strategies among subsistence farmers are limited. A field study was conducted among groundnut farmers in West Africa (600 volunteers from 20 villages) to reduce aflatoxin exposure by implementing a specific intervention package, and to assess the impact of the intervention by monitoring aflatoxin B1 (AFB1) levels in groundnuts and blood aflatoxin–albumin adducts (AF–alb) as a measure of exposure. The intervention package included hand sorting of kernels (with removal of damaged kernels), drying kernels on natural fibre mats, estimating the completeness of a sun drying period, storing kernels in natural fibre bags, supplying wooden pallets to store the bags on, and using insecticide (acetilite). Significant reductions in both AF–alb in blood (58% reduction) and groundnut contamination levels (70% reduction) were observed. This is the only study of its kind that showed the reduction of aflatoxin exposure in the groundnut-consuming population.

In Africa, maize is matured under dry conditions and is commonly left in the field to dry on the stalk, whereas in South-East and East Asia, maize is sometimes harvested wet and piled in stacks and left on the field to dry for a period of time. Maize may also be shelled, and this, together with drying practices, increases aflatoxin levels. However, crops dried adequately away from the field and off the ground are less susceptible to insect damage and fungal growth.

Sun-drying of maize and groundnuts is common practice in Africa and, together with the use of platforms, has been shown to reduce the growth of toxigenic fungi such as *Aspergillus*, *Fusarium*, and *Penicillium*.

In Ghana, the method of inverted windrowing of groundnut pods after harvest ensures exposure to direct sunlight and circulating air. This costeffective method dries the pods rapidly and sufficiently to ensure reduction of aflatoxin levels. For groundnuts, drying on raised surfaces or on mats to a kernel moisture content of 8% is required to reduce the risk of aflatoxin contamination. The use of dryers proved to be protective against insect damage, reduced mould and aflatoxin contamination, and had no effect on the grain germination potential. They also were shown to be highly effective in eliminating crop loss due to insect damage. Additional benefits included the reduced need for insecticides to protect the crop, the extension of crop storage duration by 1.8–2.4 months, the improvement of availability of food by more than 1 month, and an increase in jobs and income.

A suggested replacement for sun-drying is the use of solar dryers, because they dry crops faster and more efficiently and provide a controlled environment that offers improved sanitation. The lack of success of using solar based drying among rural commercial farmers has been attributed to the cost, complicated operational procedures, and the reluctance to change from traditional methods. Small-scale farmers require solar dryers that are more affordable to purchase or construct and need little maintenance. Of the solar drying technologies available, including the active (forced-convection) solar dryers and the passive (natural-circulation) types, the use of a ventilated greenhouse dryer has been suggested for rural small-scale farmers, due to its low cost, simplicity, and on-site construction and operation.

The use of hermetically sealed storage bags, such as those of the Purdue Improved Crop Storage project, is apparently effective for insect control, increasing insect mortality by 95–100% in stored maize. The efficiency of hermetic technologies to prevent fungal growth and consequent mycotoxin contamination seems to be dependent on the type and specific characteristics of the crop. Storage of groundnuts in Super Grain Bags (bags made of multilayer polyethylene that have a two track zipper and are sealed using a zipper slider) reduced the growth of aflatoxin producing fungi during an experimental study. It has been shown that groundnuts stored in polyethylene bags were 7–13% more contaminated than samples stored in polypropylene and jute bags. Jute bags are considered more feasible compared with polyethylene and polypropylene only if crops are properly

dried before storage; polyethylene and polypropylene bags are poorly aerated and do not absorb moisture. The use of natural fibre jute bags has been suggested to be more suitable to maintain crop quality.

Strategies to improve post-harvest storage of crops should be an urgent research priority. Ideally, technologies should be economically feasible, require low labor intensity, be practical and sustainable, reduce the need to use chemicals, and be convenient, widely available, and easy to transport. The interventions should also be developed for both rural small scale and commercial farmers.

In sub-Saharan Africa, 80% of farms are smallholder; mostly subsistence farms. A distinction should be made as to what technologies are feasible for commercial versus small-scale farmers in rural areas. The cultural acceptability of a proposed intervention in the different agricultural systems is also important. Therefore, post-harvest strategies in developing countries should be comprehensively field-tested and validated to assess their efficacy, economic feasibility, cultural acceptability, and sustainability. To ensure compliance, it will be important to monitor large-scale implementation.

Apart from the lack of feasible and inexpensive strategies, other obstacles to improving post-harvest storage of crops include the absence of governmental commitment and the shortage of trained personnel, such as agricultural extension workers. Establishing strategies to safeguard crops during storage will inevitably require cooperation and communication between governments, research entities, nongovernmental organizations, other stakeholders (market agencies, farmers' and consumer groups), manufacturers, and the farmers. In Africa, farmers' awareness of the health risks associated with aflatoxin and how to reduce exposure is influenced by their socioeconomic status, education, farm size, extension participation, market orientation, economic motivation, and perceptions.

The role of women in rural agro-ecological zones in developing countries should also be considered, because they play an important role as mothers, educators, and businesswomen, managing household nutrition, farming, and the selling of smallholder crops. Women in certain areas of Ghana and Nigeria were able to produce less maize compared with men. This was due to a lack of access to fertile soil and new technologies or innovations. In Ghana and Nigeria, women have less influence on decision-making compared with men. In South Africa, the situation is different; women head 60% of the rural households in the Eastern Cape Province and manage the farms. More research on gender and mycotoxin management is needed to properly develop education campaigns and ensure equitable access to information by both men and women.

Post-harvest interventions to reduce mycotoxin exposure should include education programmes and awareness campaigns that will facilitate best practices. By working in rural South Africa with 260 smallholder farm households, it was found that few people understood the health risks associated with mycotoxins. This was also the case in a much larger study (sample size, 2400) in Benin, Ghana, and Togo. Sustainability of appropriate agricultural practices will be more effective through continuous campaigns. The emphasis should be on crop quality rather than on productivity in domestic markets. The following is a summary of recommended procedures using evidence-based, culturally acceptable, and validated prevention strategies.

- Develop knowledge translation modules in partnership with farmers, area agricultural extension workers, traditional leaders, church groups, health workers, and civil society, with a focus on women.
- Be prepared to implement prevention strategies on a larger scale.
- Use recommended procedures in chronic-exposure situations as an ongoing intervention package.
- Apply as a multifactorial intervention package to include hand sorting, rapid and proper (elevated) drying, proper storage, elevation of stored crops, and insect control.
- Consider that designs for solar or biomass dryers and storage structures preferably built from locally available materials are urgently needed.

# **Interventions Useful in Emergencies**

A distressing number of cases of acute aflatoxicosis have occurred, notably in the past decade. Those who are most likely to consume foods contaminated with aflatoxins suffer the most severe effects, including disease and death after acute exposure. Thus, feasible interventions and therapies to diminish human and animal exposure to aflatoxins during aflatoxin outbreaks are imperative. Numerous strategies to sequester aflatoxins in the gastrointestinal tract and reduce their bioavailability have been evaluated for their potential as practical, cost-effective, and sustainable solutions to the aflatoxin problem. Aside from avoiding ingestion of contaminated food, none of these primary intervention strategies provides complete protection. However, a refined calcium montmorillonite clay (NovaSil [NS]) and chlorophyllin have been widely studied in animals and JAD 7 (1) 2017

humans for safety and efficacy, with promising results. Similar research is underway to evaluate the efficacy of other enterosorption strategies, including various bacteria and indigestible carbohydrates such as glucans, glucomannans, cellulose, and peptidoglycans.

#### Aflatoxin enterosorbents

Studies, describing materials that can tightly adsorb aflatoxins onto internal and/or external surfaces causing a reduction in toxin uptake and bioavailability, have been recently reviewed. The technical feasibility, costs, and efficacy of various mitigation strategies (including the use of enterosorption and trapping agents) have also been reported. It has been suggested that inclusion of toxin enterosorbents in the diet can decrease morbidity and mortality during outbreaks of acute aflatoxicosis. The most common materials used as toxin enterosorbents and trapping agents are discussed briefly below.

#### Chlorophyll/chlorophyllin

Chlorophyll and chlorophyllin are naturally occurring constituents of the human diet that have been shown to be effective anticarcinogens in several animal models. They are hypothesized to act as interceptor molecules by trapping carcinogens, such as AFB1, thereby diminishing bioavailability by impeding their absorption. In a 4-month clinical trial in China, ingestion of 100 mg of chlorophyllin at each meal led to an overall 55% reduction in median urinary levels of aflatoxin–N7-guanine adducts compared with placebo. In a crossover study among four human volunteers in the USA, data suggested that chlorophyll or chlorophyllin consumption may limit the bioavailability of aflatoxins, as shown in animals. Prophylactic therapy with chlorophyllin or supplementation of diets with foods rich in chlorophylls

may represent a practical measure to reduce the likelihood of developing aflatoxicosis.

# Clays

The use of clay-based products as enterosorbents for aflatoxins is a frequent strategy to reduce aflatoxin exposure in animals. Dioctahedralsmectite clays (especially montmorillonite) are the common sorbents used for this purpose. Earlier studies showed that inclusion of a calcium montmorillonite clay (NS) in animal feed reduced the adverse effects associated with aflatoxin exposure in multiple animal species and decreased the level of aflatoxin M1 (AFM1) in milk from lactating dairy cows and goats (Phillips et al., 2008). Equilibrium adsorption isotherms, molecular modelling, and in vivo studies have

been used to demonstrate that NS binds AFB1 and fumonisin B1 in the gastrointestinal tract, thereby reducing systemic bioavailability. Initial human trials in Ghana and in Texas (USA) showed no adverse health effects in humans. Based on animal and human studies, NS clay does not significantly alter the levels of vitamins and minerals. Overall, use of NS clay during outbreaks of acute aflatoxicosis appears to be a safe and practical strategy for vulnerable populations at high risk for exposure. Other aflatoxin-sequestering materials that have been investigated include lactic acid bacteria.

The young of all species are the most vulnerable to aflatoxins; thus, children are the most likely to suffer the consequences of aflatoxin outbreaks. The trials reported to date have been in adults, and there is a knowledge gap in emergency strategies for protecting infants and children. Further studies are warranted to assess the effects of aflatoxin dose and duration of exposure on efficacy and the safety of NS clay and chlorophyllin in the vulnerable, including malnourished infants, children, and pregnant women. Other research needs include: determining the effects of mixtures of NS, chlorophyllin, and other enterosorbents; assessing the effectiveness of combinations of aflatoxin enterosorbents and chemoprotectants; identifying sustainable and effective delivery strategies to treat acute aflatoxicosis; and conducting phased clinical trials.

# **Chemoprevention Studies**

#### **Dithiolethiones** (oltipraz)

Oltipraz, a substituted 1,2-dithiole-3-thione, was originally developed by the pharmaceutical industry as a possible treatment for schistosomiasis and was extensively evaluated in clinical trials in the early 1980s. Subsequent studies demonstrated that oltipraz and some structurally related 1,2-dithiole-3thiones were potent inducers of enzymes associated with the maintenance of reduced glutathione pools, as well as enzymes important to carcinogen detoxification, in multiple tissues of rats and mice. Aflatoxin biomarkers were used as intermediate end-points in a phase IIa chemoprevention trial of oltiprazin Qidong, China. This was a placebo-controlled, double-blind study in which participants were randomized to receive placebo, 125 mg of oltipraz daily, or 500 mg of oltipraz weekly. In participants receiving the 500 mg weekly dose, urinary AFM1 levels were reduced by 51% compared with the placebo group. Median levels of aflatoxin-mercapturic acid (a glutathione conjugate derivative) were elevated 6-fold in the 125 mg group but were unchanged in the 500 mg group. Increased aflatoxin-mercapturic acid levels reflect induction of aflatoxin conjugation through the actions of glutathione S-transferases. The apparent lack of induction in the 500 mg

group probably reflects masking caused by diminished aflatoxin-8,9epoxideformation for conjugation through the inhibition of CYP1A2 seen in this group. This initial study demonstrated for the first time that aflatoxin biomarkers could be modulated in humans in a manner that would predict decreased disease risk.

# Sulforaphane

Although the oltipraz clinical trial demonstrated the proof of principle for increasing pathways leading to aflatoxin detoxification in humans, the practicality of using a drug-based method for prevention in developing countries is limited. Fortunately, oltipraz is not the only agent that affects enzyme changes through the Nrf2-Keap1 pathway. Many foods have high levels of these enzyme inducers. A beverage formed from hot water infusions of 3-day-old broccoli sprouts, containing defined concentrations of glucosinolates as a stable precursor of the anticarcinogen sulforaphane, was evaluated for its ability to alter the disposition of aflatoxin. Sulforaphane has been extensively examined for its chemopreventive properties and is a potent activator of the Nrf2-Keap1 pathway, leading to increased expression of carcinogen-detoxifying enzymes. In a study in Qidong, China, 200 healthy adults drank infusions containing either 400 µmol or less than 3 µmol of glucoraphanin nightly for 2 weeks. Urinary levels of aflatoxin-N7guanine adducts were similar between the two intervention arms. However, the measurement of urinary levels of dithiocarbamates (sulforaphane metabolites) indicated striking inter-individual differences in bioavailability. This outcome may reflect individual differences in the rates of hydrolysis of glucoraphanin to sulforaphane by the intestinal microflora of the study participants. Accounting for this variability, a significant inverse association was observed for excretion of dithiocarbamates and aflatoxin-N7-guanine

adducts in individuals receiving broccoli sprout glucosinolates. This preliminary study illustrates the potential use of an inexpensive, easily implemented, food-based method for secondary prevention in a population at high risk of aflatoxin exposure. A follow-up intervention seeking to minimize the inter-individual variability in the pharmacokinetics of the glucoraphanin precursor is currently in progress.

#### Green tea polyphenols

Many studies have demonstrated that green tea polyphenols (GTPs) inhibit various chemically induced cancers in experimental animals. Qin et al. (1997) studied the effects of GTPs in drinking water for 2 or 4 weeks to protect against the development of AFB1-induced hepatocarcinogenesis in the rat. The data on GTPs in experimental animals provided the impetus to translate this strategy to human clinical trials. In an initial study in an aflatoxin-exposed high-risk group in Guangxi, China, the effects of GTPs were assessed in urine samples collected from a randomized, doubleblinded, placebo-controlled phase IIa chemoprevention trial. All participants tested positive for AF-alb and took GTPs capsules daily at a dose of 500 mg or 1000 mg, or a placebo, for 3 months. Analyses were performed on blood and urine samples collected during this clinical trial to evaluate the efficacy of GTPs in modulating aflatoxin biomarkers; reductions in AF-alb and urinary AFM1 levels were observed. After the 3-month trial, both of the GTPs intervention groups were found to have reduced AF-alb levels compared with the non-intervention controls.

This research has established that chemoprevention with the above mentioned agents is effective in relevant animal models and that the mechanism applies in humans. Similar plant polyphenols and sulforaphanes occur in several plant species found in developing countries that are affected by aflatoxin. Research is needed to determine which locally grown and consumed plants contain sufficient levels of these naturally occurring chemopreventive agents to induce protection from aflatoxin exposure, and to conduct experimental trials.

# References

- Bennet, JW, and Klich, M. 2003. Mycotoxins. Clinical Microbiology reviews: 16(3): 497 516
- Carnaham, J. 2015. 2015. Is toxic mold exposure the cause of your symptoms? <u>https://www.ji//carnahan.com/2015/02/08/toxic-mold-exposure-cause-symptoms/</u>
- European Commission on Food Safety. 2010. Commission Regulation No. 1881 2006.
- Flint-Garcia, SA. 2005. Using genetics, genomics, and breeding to understand diverse maize germlasm. USDA – ARS. <u>http://imbgl.cropsci.illinois.edu/school/2014/4\_SHERRY\_FLINT-GACIA.pdf</u>
- Harper D. Toxin. Online etymology dictionary
- <u>https://www.blacktoxicmolds.com/mycotoxins\_mold.php</u> Mycotoxins Black toxic mold (Stachybotrys).
- https://www.iarc.fr/en/publications/pdfs-online/wrk/wrk9/IARC\_WGR9. Intervention strategies to reduce human exposure to aflatoxins and fumonisins.
- http://peanutbase.org/browse searchPeanutBase
- https://www.thefreedictionary.com/mycotoxin Mycotoxin definition of mycotoxin by The Free Dictionary
- King, B. 1979. Outbreak of ergotism in Wollo, Ethiopia. Lancet: 1411
- Kumar, M. et al. 2018. Mycotoxins and pesticides: Toxicity and applications in food and feed. In Patia J. Das G. Shin, HS (eds). Microbial Biotechnology, Springer, Singapore. <u>https://doi.org/10.1007/978-981-10-7140-9\_11</u>
- Mesterhazy, A. and Reid, LM. 2011. Breeding for resistance to ear rot caused by *Fusariumspp.* in maize A review. Plant Breeding 131: 1 19

- National Treatment Centers for Environmental Disease (ntced.com). <u>http://www.ntecd.org/diagnosing-mold-exposure/mycotoxins-and-fungus-chart/</u>
- Peraica, M. et al.1999. The effects of mycotoxins in humans. Bulletin of World Health Organization: 77 (9): 711 784
- Philips, et al. 2008. Intake and digestion of wheat forage by stocker calves and lambs. J. Animal Sci. 86 (9): 2424 - 2429
- Richard, JL. 2007. Some major mycotoxins and their mycotoxicoses an overview. Int. J. Food Microb.119 (1-2): 3-10
- Robbins, CA et al. 2000. Health effects of mycotoxins in indoor air: a critical review. Appl. Occup.Environ. Hyg. 15(10): 773 84
- William, JH. et al. 2004. Human aflatoxicoses in developing countries: a review of toxicology, exposure, potential health consequences and interventions. The American Journal of Clinical Nutrition. 80 (5): 1445 1446