Socioeconomics
Discussion Paper Series

Series Paper Number 43

On-farm Assessment of Post-harvest Losses: the Case of Groundnut in Malawi

Taku W Tsusaka, Charles Singano, Anitha Seetha, Nelson Kumwenda
ICRISAT, Lilongwe, T.Takuji@cgiar.org.

6/5/2017

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This work has been funded by the CGIAR RESEARCH PROGRAM ON Policies, Institutions, and Markets Led by IFPRI and mapped to CGIAR RESEARCH PROGRAM ON Grain Legumes

Acknowledgements

The authors thank CRP (CGIAR Research Program) PIM (Policies, Institutions and Markets) led by IFPRI (International Food Policy Research Institute) for funding the study. Appreciation is extended to CRP GL (Grain Legumes) for administrative support provided for the implementation of the study. The opinions expressed here belong to the authors and do not necessarily reflect those of ICRISAT, DARS, CRP PIM, IFPRI, or CGIAR System Organization. Any remaining errors are the sole responsibility of the authors.

Affiliations:

Taku W Tsusaka, Anitha Seetha, Nelson Kumwenda: ICRISAT, Lilongwe.

Charles Singano: Department of Agricultural Research Services (DARS), Ministry of Agriculture, Irrigation and Water Development, Malawi.

Acronyms

ADD	Agricultural Development Division
AEDO	Agricultural Extension Development Officer
AEDC	Agricultural Extension Development Coordinator
AFB1	aflatoxin B1
ALP	alkaline phosphate system
BTB	bromothymol blue
CRP GL	CGIAR Research Program on Grain Legumes
CRP PIM	CGIAR Research Program on Policies, Institutions and Markets
CTI	Compatible Technology International
DADO	District Agricultural Development Office
DAES	Department of Agriculture Extension Services
DARS	Department of Agriculture Research Services
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Extension Planning Area
EU	The European Union
FAO	Food and Agriculture Organization of the United Nations
FGD	Focus Group Discussion
hr	hour
HCl	hydrochloric acid
HH	household
HPLC	high pressure liquid chromatography
HTH	hypertrehalosemic hormone
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFPRI	International Food Policy Research Institute
KADD	Kasungu Agricultural Development Division
LADD	Lilongwe Agricultural Development Division
ng/g	nanogram per gram
MoAIWD	Ministry of Agriculture, Irrigation and Water Development
MSCE	Malawi School Certificate of Education
NaOH	sodium hydroxide
PBS	phosphate buffered saline
PH	post-harvest
PHL	post-harvest loss(es)
PNC	penicillinase system
rpm	revolutions per minute
SSA	sub-Saharan Africa
USD	United States dollar

Abstract

An on-farm measurement was conducted in 2015 of groundnut post-harvest loss (PHL) in Central Region of Malawi, aiming to assess the PHL in quantity and quality along the post-harvest processes at the farm level. A total of 15 voluntary farmers from Mchinji, Lilongwe, and Kasungu districts participated in the on-farm assessment using the count and weigh method. The assessment began in April and was forced to end in August due to an unexpected change in funding availability. The close monitoring through resident enumerators revealed that during lifting, drying, stripping, and transport to homestead, an average weight loss of 133.6 kg (shelled nuts equivalent) per hectare was incurred, which is equivalent to 13.7 % of the harvest without post-harvest losses, translating into a value loss of USD 189.7 per hectare. In particular, the lifting process suffered an average loss of 57.3 kg per hectare, due to such factors as hoe damage, weed infestation, and theft. For on-field drying after lifting, 13 % of the farmers practiced the Mandela cork, the best-bet drying method for controlling aflatoxin, while the rest of the farmers dried on ridges or in small drying rounds. During the drying and stripping processes, farmers experienced a mean weight loss of 73.9 kg per hectare, due to factors including attacks by rodents, spillage by children, and biting by workers. As a means of transporting nuts from the field to homestead, farmers used ox-carts (47% of farmers), bicycles (33%) and walking (20%). The mean weight loss during this transportation was 2.4 kg per hectare, due to use of torn sacks and direct loading onto ox-carts without use of sacks. Regarding quality loss, aflatoxin diagnosis was conducted on nuts sampled at two points in time: after drying and after one month of storage. The average contamination level was 0.87 ng/g after drying and 0.88 ng/g after one month of storage. Although the overall level seemed stable, the individual-level changes were large, and so were the district-level and individual-level variances. Seven percent of the farmers registered a level greater than 4 ng/g, which would not be accepted by major international markets such as the European Union. Mitigation measures at each stage of post-harvest operations and methodologies for assessing post-harvest losses in groundnut are discussed.

Keywords: post-harvest loss, food loss, food security, groundnut, Malawi, count and weight method, aflatoxin

JEL classification: N57, J23, C18

Contents

1. Introduction

1.1 Significance of Post-harvest Loss

It is estimated that nearly 1.3 billion tons of food is globally lost or wasted per year along the post-harvest (PH) chain (Gustavasson et al., 2011), which accounts for over 30 % of total crop production (Foresight, 2011; Gustavsson et al., 2011; Lundqvist et al., 2008). In sub-Saharan Africa (SSA), the annual grain PHL is estimated at USD 4 billion in value, which is enough to feed 48 million people for a year (World Bank, 2011). To boot, the PHL is estimated to be equivalent to 6-10 percent of human-generated greenhouse gas emissions (Vermeulen, et al. 2012; Gustavasson, et al. 2011). Since crop farming contributes significant proportion to smallholders' household income in sub-Saharan Africa, reduction in post-harvest loss (PHL) especially up to farm gate level can directly increase the real incomes of small-scale producers (World Bank, 2011).

Despite the significance of the issue of PHL, however, 95 % of the research investments during the past decades have focused on efforts to raise productivity, whereas only 5 % were directed toward minimizing PHL (WFLO 2010; Kader 2005; Kader and Rolle 2004). To sustainably achieve food security, food availability needs to be increased also through reductions in PHL at farm, wholesale, retail, and consumer levels (Kimatu et al., 2012). Several experts suggest that investing in PHL reduction can be an effective intervention to attain food security in SSA (GIZ, 2013).

PHL can be defined in both qualitative and quantitative terms along the supply chain, from harvest to consumption (Hodges et al., 2011). The quantitative loss is the decrease in weight or volume, whilst the qualitative loss is the reduction in nutrient value and unwanted changes in taste, coloir, texture, and cosmetic features of food (Buzby and Hyman, 2012), which affects the price (i.e., income) and health. PHL arises from a number of factors including improper management of harvest and post-harvest handling, and storage conditions and facility, among which storage is regarded as particularly important (Kaminski and Christianensen, 2013).

While consistent and reliable measurement of PHL is a necessary step toward reaching the goal of reducing PHLs, currently there is no single established definition of PHL nor are there any agreed upon methodologies for consistent measurement (Affognon et al., 2015; Aulakh and Regmi 2013). For example, cracked grains may be sold in the market place, though at cheaper rates. They are not totally losses since they have alternative uses, often at lower prices. This aspect is often neglected. Affognon et al. (2015) argue that in SSA, PHL information is disjointed and quantitative figures are obtained from inadequate data sets. Besides, the majority of the assessments have concentrated on farm-level grain storage, which is considered to be a critical stage along the supply food chain. Unfortunately, many of the available PHL estimates are based on the anecdotal stories with few actual measured or estimated numbers. For example, methodologies used for the PHL estimates in FAO's Food Balance Sheet (FAO, 2017a) vary by commodity and country. These numbers, in turn, feed into estimates of food availability which are widely used in food security assessments and policy analyses. Thus, legitimate accounting of PHL based on a coherent methodology would provide more reliable information for analyses and policy making.

Despite the dominance of agrarian economy and unstable food security in sub-Saharan Africa (SSA), past studies on PHLs largely concentrate on developed countries and Asia. Moreover, the relevant literature for SSA focuses on maize, the staple crop in Eastern and Southern Africa, though there are other important crops as well in the region, of which groundnut is one. While groundnut is the second most important crop for smallholders in

Malawi in terms of both area planted and income generation (Tsusaka et al., 2016), literature on PHL in groundnut largely focuses on qualitative loss in the form of aflatoxin contamination. More detail of the literature is discussed in Section 2.2.

1.2 Groundnut in Malawi

Groundnut is the important and growing income source for smallholder farmers in many countries such as Malawi, Zambia, Mozambique, Uganda, Senegal, Nigeria, and Sudan. In Malawi, groundnut production is dominated by smallholder farmers, and the crop is considered to be one of the most important food crops as well as cash crops (Tsusaka et al., 2016). The grain is consumed in diverse forms: raw, roasted, salted, boiled unshelled, etc. Nuts are ground into coarse flour and mixed with leafy vegetables as part of the traditional diet (Freeman et al., 1999). It is also used for oil extraction and butter production, while the residual cake is processed into animal feed as well as human consumption. Furthermore, groundnut is the second income earner in Malawi for smallholders after tobacco, and is a source of foreign currency for the country's agro-based economy (Msere et al., 2015). However, the export market potential has not been utilised adequately due to all sorts of constraints, such as unpredictable weather conditions, pests and diseases, lack of access to improved cultivars adapted to different agro-ecological zones, poor soil fertility, and cultural practices.

Furthermore, the importance of groundnut is added to by the way women are involved in workload and decision-making in its farming processes, particularly in post-harvest operations (Orr et al., 2016). It is a "women's crop" with high relevance to the issue of gender and rural development.

One critical challenge faced by the groundnut subsector is contamination by aflatoxin. Approximately a half (49 %) of groundnut sold at local markets in Malawi were found to have aflatoxin levels exceeding those considered safe for human consumption (Emmott & Stephens, 2014). The issue of aflatoxin affects not only health but also income. It causes income loss to smallholder farmers as well as reduced foreign exchange earnings for groundnut exporters. Standards for acceptable levels of aflatoxin have been established by various authorities (Matumba et al., 2014b; Monyo et al., 2012). In particular, the European Union markets have established quality standards which prohibit import of groundnut with more than 4 ng/g of aflatoxin, while Japan demands nuts with no aflatoxin (Otsuki et al., 2001). South Africa followed suit recently (Diaz Rois, et al., 2013), though most low-income countries lack aflatoxin regulation and enforcement policies (Cullen and Newberne 1993; Williams et al. 2004; Sowley, 2017). This problem has impeded farmers' access to export markets and most smallholders are confined within the local markets. Besides, even in local markets, producers receive reduced prices when the quality of nuts appears to be poor, resulting in income loss.

1.3 Objective of the Study

Given the significance of the issue and the importance of the crop, it is imperative to identify the extent of PHL for groundnut and the predisposing factors along the post-harvest chain. The objective of this study is to conduct an assessment of on-farm post-harvest loss for smallholder groundnut production in Malawi by way of close observation of farmers′ practice

through resident enumerators. In this study, the processes between lifting and storage were considered.[1]

2. Literature Review on Post-harvest Loss

2.1 Methodologies for Loss Assessment

There are a few methodologies adopted in assessing PHL to date.

Household surveys and focus group discussions (FGD), using questionnaires and checklists as instruments, are among the common methodologies for assessing PHL along the PH chains (Hodges et al., 2013; Behera and Swain, 2013; Kaminski and Christianensen, 2013; Saint et al., 2010). The advantages of this method are that it is less resource demanding and it can be applied to adequate numbers of respondents to achieve representative samples. On the other hand, the method is prone to measurement errors in PHL estimates as it relies on farmers' statements of perceptions, where physical measurements are absent. It is also difficult to formulate detailed mitigation strategies.

Another methodology often adopted is the count-and-weigh method (Gwinner et al., 1996), which has been used widely to assess the quantity losses in grains (Utono et al, 2014; Tefera et al., 2011). The method has been applied to the storage stage for groundnut and other leguminous crops. The disadvantage of this method is being costly and time consuming.

The cost and time associated with the count-and-weigh method can be reduced by employing visual scales and standard conversion graphs. This method has been adopted in SSA for assessing PHL at specific stages in the PH chain for cereals such as maize and sorghum (Lingle et al., 2013; Cantin et al., 2011; Ali et al., 2011). To date, however, visual scales and standard conversion graphs are missing that can be used for legume crops including groundnut.

Aflatoxin contamination is a form of qualitative losses that is commonly found in food crops in SSA. In Malawi, the issue is particularly pronounced with maize and groundnut. Levels of aflatoxin contamination can be assessed by laboratory analyzers. The major methods include HPLC (fluorescence high-performance liquid chromatography), immuno-affinity column and reversed-phase liquid chromatography with post-column photochemical derivatization and fluorescence detection (Matumba et al., 2014a), and ELISA (indirect competitive enzyme-linked immunosorbent assay). ELISA detects aflatoxin B1 (AFB1) by analyzing collected samples of crop grains or products (Waliyar et al., 2015; Reddy et al., 2001). More detail of ELISA is described in the methodology section (Section 3.4.6). The disadvantage of these methods is the investment requirement. Recently, lateral flow immunoassay devices are being developed for field testing of aflatoxin contamination in crops (Santos et al., 2017; ICRISAT, 2016; Huang et al., 2016).

[1] This was due to the unexpected change in funding during the season. The result of this study will provide information that can complement the survey-based study conducted in the following season.

2.2 Factors Causing Post-harvest Loss in Groundnut

Existing studies on factors causing PHL in groundnut concentrate on assessment of qualitative loss at storage. There are three remarkable factors causing the loss in quality of groundnut: namely, environmental conditions, pest attacks, and mycotoxin contamination.

High temperature and high humidity are known as conditions that aggravate the quality loss in groundnut kernels at storage (Wagacha and Muthomi 2008). The best storage condition for unshelled groundnut is reported to be with 7.5 to 8.0 % kernel moisture content at 10 °C temperature and 65 % relative humidity, under which unshelled nuts can be stored without incurring significant quality losses for 10 months (Saint et al., 2010; Patee and Young, 1982). Krishnappa et al. (1998) found that groundnut seeds stored at 7 % moisture content in polyvinyl bags registered the highest germination rate after a storage period of one year. Serious losses in grinding quality are caused when the kernels are stored at too low temperature for long (Hammad, 2001). The environmental conditions also matter at drying of the lifted groundnuts. In developing countries, groundnut is usually dried in field, and the moisture content in kernels and haulms is largely affected by weather conditions. The prevailing drying condition in SSA is high temperature (> 40 °C), which adversely affects the seed viability and oil quality. Excessive drying leads to losses in grinding quality as well. (Hammad, 2001).

Another crucial factor for storage loss is pests such as insects, rodents, birds, and termites, which cause damage to the nuts by eating as well as contaminating it with waste matter and urine, especially when the nuts are not dried to below 8 % moisture content level (Saint et al., 2010). While pod storage tends to be affected by the environmental conditions of storage structures, kernel storage is more prone to insect pests (Ranga Rao et al., 2010).

The most prominent factor for quality deterioration in crop produce is contamination with mycotoxin (Strosnider et al., 2006). Globally, 25 % of food crops are contaminated with mycotoxins (Wild and Hall, 2000) in particular aflatoxin, the carcinogenic fungus Aspergillus flavus which infests the pod and kernel (Nautiyal, 2002). Contamination commonly occurs during post-harvest rather than pre-harvest (Boutrif and Canet, 1998). Aflatoxin levels in food rise especially during storage (Kaaya and Kyamuhangire, 2006). In Malawi, aflatoxin contaminates various crops, particularly maize and groundnut due to hot and humid storage conditions that promote fungal growth (Hell et al., 2000). Apart from storage, on-field drying is an important process for grain preservation which removes water from the grain. Yet, inappropriate methods of drying can exacerbate the contamination with aflatoxin (Hell and Mutegi, 2011). Low-cost technologies such as drying platforms, drying outside the field, and drying on mats have potential for aflatoxin mitigation (Hell et al., 2008l ICRISAT, 2016). In the most common drying method of small rounds, haulms were placed upside down so that the pods were exposed to the sun light, intended for direct and fast drying. On the other hand, the Mandela Cork method is being promoted by the Department of Agricultural Extension Services (DAES), ICRISAT, and various other stakeholders as the best-bet method for drying haulms as a measure of controlling moisture and sunburn, which contributes to aflatoxin mitigation (Hoeschle-Zeledon et al., 2015; SAFE Project, 2010).

In addition, Rahmianna et al. (2007) show that practices such as lifting at appropriate timing, stripping pods swiftly after harvest, rapid drying, and ventilation at storage are recommended measures for aflatoxin mitigation.

As for quantitative loss, the techniques used for harvesting and PH handling, as well as labour availability, have major impacts on PHLs. Saint et al. (2010) point out that lifting (harvesting) can cause quantity loss in groundnut.

2.3 Estimates of Post-harvest Loss in Groundnut

Again, existing studies on PHL estimates in groundnut concentrate on assessment of qualitative loss with aflatoxin contamination.

Roya et al. (2012) report that in Mali, groundnut at storage contained 423 ng/g of aflatoxin, as well as betelnut with 30.6 ng/g, lentils with 21.2 ng/g, and red chili powder with 420 ng/g, all of which exceeded the US regulatory limit of 20 ng/g. In Malawi, locally processed skinned and de-skinned roasted nuts were found to have aflatoxin in the range of 500-2,500 ng/g and 600-36,900 ng/g, respectively (Matumba et al., 2014), which were much higher than what was found in Mali. Recently, Kachapulula et al. (2017) found in Zambia that groundnut and maize produce had 39 ng/g and 16 ng/g of aflatoxin, respectively, exceeding the allowable level in Zambia (10 ng/g).[2]

Aflatoxin infests not only kernels but also butter paste. Waliyar et al. (2015) found in different locations in Mali that contamination levels were significantly higher in groundnut paste than in groundnut kernels, and that AFB1 levels rapidly increased during storage, exceeding the permissible level of 20 ng/g. In Malawi, locally processed peanut butters were found to have aflatoxin levels in the range of 34,200-115,600 ng/g, overwhelmingly exceeding the EU maximum level (100 ng/g), while regionally imported peanut butter had it in the range of 200-4,300 ng/g (Matumba et al., 2014).

Of late, quantitative losses in groundnut in Malawi were examined by Ambler et al. (2017) using the survey method. Their result translates into the average loss of 59.8 kg per hectare, equivalent to 12 % of the harvest.

3. Methodology

3.1 Conceptual Framework

At farm level, grain travels along the post-harvest chain from harvesting to wholesaling. Losses occur at each stage along the chain and contribute to the total PHL. Adapting the USDA's general framework, we divide the chain into major segments: harvesting (lifting), on-field drying, transport to storage, threshing (stripping), storage, dehulling (shelling), winnowing, grading, marketing (to cooperatives, traders, and briefcase buyers). Through the measurement, the relative importance of a particular stage or factor toward contributing to total PHL will be determined.

The total PHL along the value chain is expressed as below:

$$Total\ PHL = \sum_i S_i = \sum_i \sum_j f_i(X_j) \quad \text{.....Eq. (1)}$$

where S_i stands for the losses at each critical stage of the value chain; X_j stands for different factors affecting losses at each step and i represents critical stages. Different measurable factors which impact the losses are explored.

It would be useful if the PHL can be expressed in monetary terms. The value loss at each stage i is incurred from quantitative loss and quality loss through the farm-gate price, expressed as below:

$$PHL\ Value_i = QTL_i \times P_i + \sum_s QLL_{is} \times (P_i - P_s) \quad \text{.....Eq. (2)}$$

[2] Eastern Zambia and Central Malawi form the so-called groundnut belt where groundnut production concentrates. The groundnut producing areas in Malawi and Zambia share similar production environments as well as the socio-economic characteristics of the farmers.

where QTL stands for lost quantity, P_i refers to price at stage i, QLL stands for quantity of nuts that undergoes quality deterioration, P_s is the reduced price given after deterioration, s is for different quality deterioration patterns involved at the same stage. The first term on the right-hand side refers to value loss caused by quantity loss, and the second term refers to value loss due to quality loss. The linkage between Equations (1) and (2) is established by Equation (3).

$$S_i = QTL_i + \sum_s QLL_{is} \quad \text{..... Eq. (3)}$$

Nevertheless, as mentioned earlier, this study covers the post-harvest processes for groundnut from lifting (harvesting) to one month after storage. The study also focuses on the PH chain for grains, since other products such as flour, seed, butter (or paste), cake, and oil account for a negligibly small share in the total flow of groundnut produce.

Lastly, Figure 1 gives a graphical illustration of the value loss (i.e., Eq. (2)) incurred at farm level. While qualitative loss is examined by aflatoxin assay, this paper only valuates the value loss caused by quantitative losses with a breakdown into different PH operations.

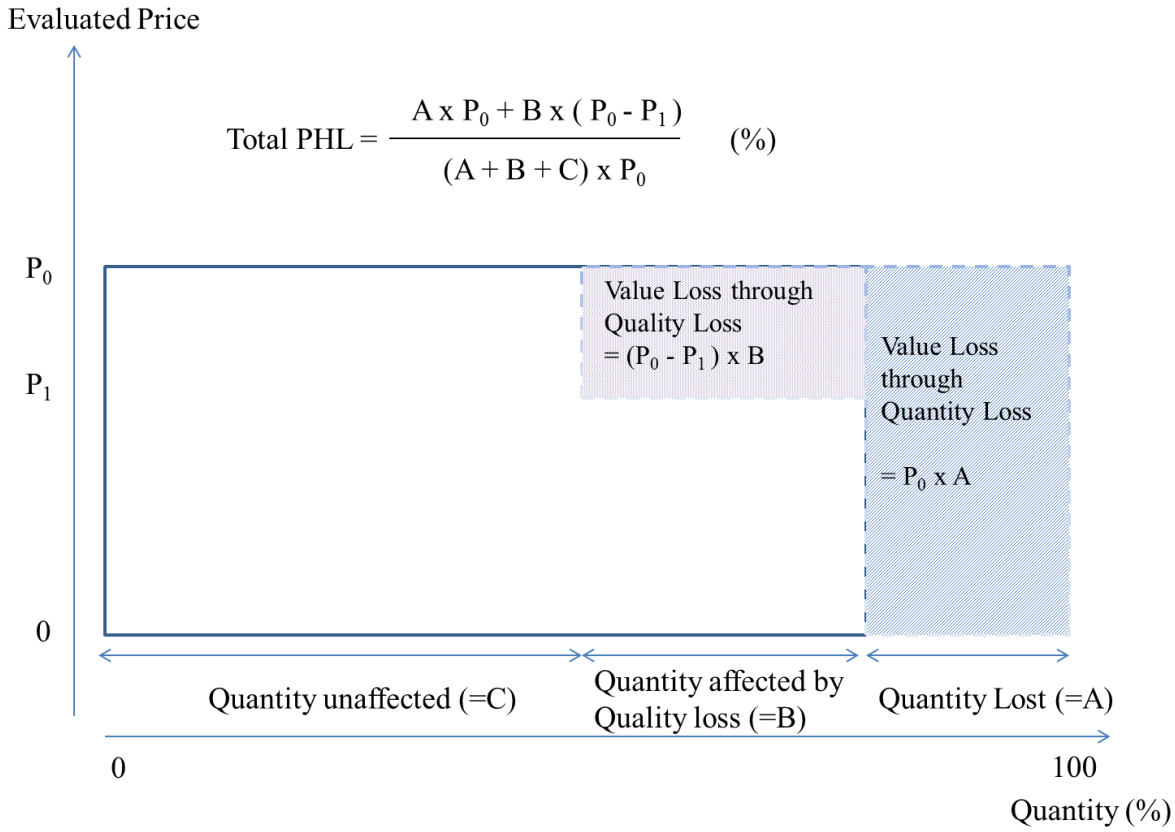


Figure 1: Illustration of post-harvest losses in quantity, quality, and associated value.
Source: Authors

3.2 Choice of Measurement Method

This study employs the count-and-weigh method for quantitative loss assessment,[3] while aflatoxin assay for qualitative loss assessment was performed at the ICRISAT Laboratory in Chitedze, Lilongwe.[4]

3.3 Site and Farmer Selection

Due to the nature of on-farm close monitoring, the number of sample farmers could not be so large. The available budget enabled us to select 15 farmers to assist resident enumerators for the field evaluation process. The three districts of Lilongwe, Mchinji, and Kasungu in the Central Region were selected on the grounds of volume of groundnut production. Lilongwe district is under Lilongwe Agricultural Development Division (ADD) whereas Mchinji and Kasungu districts both belong to Kasungu ADD. The 2013/2014 groundnut production was 137 kilo tons in Kasungu ADD and 130 kilo tons in Lilongwe ADD, which together accounted for 70 % of the national groundnut production of 381 kilo tons (MoAIWD, 2013). From each district, one extension planning area (EPA) was selected based on groundnut production volume. Further, from each EPA, one section was selected on the same grounds.

The following EPAs were selected through discussions with the legume officer at each EPA: Nyanja EPA (Lilongwe), Chipala EPA (Kasungu), and Kalulu EPA (Mchinji). Likewise, three sections were chosen as follows: Kalumbu Section (Nyanja EPA), Nkhuza West Section (Chipala EPA), and Chisewa Section (Kalulu EPA).

Finally, five groundnut producers were selected from each section on the basis of area planted to groundnut. The selection was conducted in March 2015, one month before the estimated timing for harvesting. Since our focus was on those producers participating in the value chain, it was confirmed that all the 15 selected farmers showed an intention to sell groundnut and had allocated greater than one acre of field to groundnut production. The selected farmers received briefing by the EPA AEDC (Agricultural Extension Development Coordinator) and the section AEDO (Agricultural Extension Development Officer) on the objective and protocol of this exercise. As an incentive scheme, it was promised that 10 kg of certified CG7 seeds would be provided before the next planting season (i.e., December 2015 to January 2016), conditional on successful cooperation during the post-harvest processes (i.e., April to August 2015). Among the 15 participating farmers, three (20 %) were female and 12 (80 %) were male. The participants were all smallholder farmers and the area sown to groundnut was in the range of 0.4 to 2.3 hectares per farm household, averaged at 0.7 hectares.

3.4 Procedure of Measurement

Most of Malawian farmers are endowed with a single crop season annually, which runs approximately from December to April. In this regard, the on-farm PHL assessment was designed to be conducted from April 2015 onward. The assessment began at the time of

[3] Section 2.2. implies there was a choice between the count-and-weigh method and the survey method for assessment of groundnut PHL. However, the research coordinator (CRP PIM Flagship Leader) had not communicated the methodology on time. Therefore, the research team had to proceed with the method proposed in their approved proposal, which was the count-and-weigh method, in order not to miss the single post-harvest season in 2015, while waiting for further communication.

[4] The lateral flow device had not been launched at the time of the measurement.

harvesting, and was intended to cover the following activities: lifting, drying, stripping, transportation to homestead, storage, shelling, and marketing. It must be noted, however, the assessment had to wind up in August because of an unexpected reallocation of budget.

The enumerators were instructed to record information on a daily basis. It was emphasised that the enumerators were not allowed to influence farmers' practice, so that the measurement would truly capture PHL associated with their usual practice. On the other hand, however, to maintain cordial relationship, the enumerators were allowed to assist the farmers with their activities according to the farmers' direction.

One acre of each farmer's field was sampled for the study. The sampled field was demarcated into 10 equal *sections* (5m-by-5m each) and they were numbered from 1 to 10 for identification. The detail of the measurement and assessment is presented in subsections 3.4.1-3.4.5.

3.4.1 Lifting (Harvesting)

The pods were sampled from each and every portion of the sampled field. The collected pods were placed in separate sampling bags, labeled with information, i.e., farmer name, EPA, date, portion number, enumerator, weight including the bag, number of pods. The recorded data included the followings:

1) Location and size of groundnut field lifted daily, variety of groundnut lifted, number of people involved in lifting, way of lifting, equipment used for lifting.
2) Condition of the field lifted, e.g., weedy, wet, etc.
3) Treatment of lifted haulms: how soon the pods were stripped after lifting, weight of the pods.
4) Amount of pods left unlifted in the soil and on top of ridges during lifting by hand hoe.

3.4.2 Drying

Some farmers dried pods on field, while others did so at homestead. In either case, it was confirmed that the sampled pods were dried the same way as were non-sampled pods. At this stage, the enumerators visited and monitored the process every two days. The recorded data included:

1) Date for the start of drying.
2) The drying site: homestead or field.
3) Method of drying: on the haulms, bare ground, raised platform, matt, on house roof top, etc.
4) Weather conditions and specific days.
5) For aflatoxin assay in the laboratory, samples weighing over 1 kg each were collected after the drying period. The samples were labelled: name of farmer, name of EPA, date of collection, name of enumerator, weight of pods. These samples were carried in mini polypropylene bags of 5 kg capacity each. At Chitedze Research Station, the samples were kept in a deep freezer to prevent further multiplication of fungi prior to analysis.
6) At this stage, the observations focused on monitoring pests including livestock, wild animals, and human beings (theft).
7) Special attention was paid to development of moulds on the pod.

3.4.3 Stripping

For the stripping activity, the enumerators recorded information as follows:

1) Date for the start of stripping.
2) Number, gender, and age of people engaged in stripping.

3) Method of stripping: hand or stripper.
4) After stripping, weight measurement was conducted, for which three farmers (two in Lilongwe and one in Kasungu) were selected.[5] From each one's field, one out of the ten *sections* was randomly chosen.
    a. Pods that had been left on haulms during stripping were collected.
    b. The collected pods were sent to Chitedze, where they were shelled and the kernels were weighed.

3.4.4 Transport to Homestead

Stripping was followed by transporting the pods from field to homestead. The record keeping procedure is as follows:

1) Weight: During the period between stripping and transport to home, all the pods were weighed on a daily basis.
2) Means of transport: e.g., ox-cart, bicycles, and people's head.
3) Timing: the date of transport to homestead.
4) Cost of transport: in the case of hired transport.
5) Container used for transport: e.g., baskets, polypropylene bags, etc.
6) Other observations along the way: e.g., consumption of nuts by those conducting the transport, spillage onto the road, etc.
7) Weight: the pods were weighed upon arrival at homestead prior to next activities.

3.4.5 Storage

Due to unexpected budget limitation, data recording during storage was limited to the first month of the storage stage. The kind of information recorded is as follows:

1) Storage facilities: e.g., polypropylene bags, traditional granaries, etc.
2) Form of storage: whether shelled or in-shell.
3) Place of storage: whether inside the dwelling houses or outside.
4) Quality of nuts: pest infestation.
5) Withdrawal: use of nuts such as consumption, sales, and gift giving.
6) Any relevant activity implemented by the farmers during the period.

At this stage, the enumerators visited the farmers regularly, once a week on average.

3.4.6 Aflatoxin Assay

After one month of storage, groundnut samples of 1 kg each were collected for aflatoxin contamination assay in the ICRISAT laboratory at Chitedze. In compliance with the sampling protocol (Appendix 2), the samples were placed in mini polypropylene sampling bags, labelled, and placed in a deep freezer to prevent multiplication of fungi before the assay.

The assay was performed using the direct competitive ELISA. In general, ELISA detects and quantifies the presence of an antigen in a sample by an enzyme labelled-toxin and the antibodies specific to the antigen. In the aflatoxin assay using the direct competitive ELISA, the antibody is coated onto the wells of the ELISA plate (Maxisorp or equivalent), whereby the test sample and the enzyme-labelled aflatoxin B1-BSA are added to the wells. If no toxin is present in the sample, the enzyme-labelled toxin binds to the captured antibody coated onto the wells. If toxin is present, it competes with the labelled toxin over binding to the antibody.

[5] Due to resource limitation, not all the sampled farmers were selected for certain measurements.

By washing procedures, any unbound labelled enzyme is washed away. By adding a substrate, a colour emerges, of which the intensity is proportional to the quantity of AFB1-BSA-enzyme bound to the well; the colour intensity decreases with increasing concentration of the toxin. For the detail of the direct and indirect ELISA, refer to Devergne et al. (1981) and Lu et al. (2012) as well as Appendix 2.

Sample Extraction

The sampled nuts were shelled and the kernels were ground into powder. The powder was triturated in 70 % methanol (viz., 70 ml absolute methanol in 30 ml distilled water) containing 0.5 % KCL (the proportion used is 5 ml for 1g powder) in a blender for two minutes. The extract was transferred to a conical flask, shaken for 30 minutes at 300 rpm (revolutions per minute), filtered through Whatman No. 41 filter paper, and diluted at 1:10 in the detergent PBS-T (phosphate buffered saline Tween®). Each well of an ELISA plate was coated with 150 µl of AFB1 antiserum diluted at 1:80,000 in the coating buffer. The extract was incubated either for 1 hr (hour) at temperature 37 ^{o}C or overnight at 4 ^{o}C. The plate was washed with PBS-T, and 100 µl of AFB1 standards was added at concentrations ranging from 100 ng to 0.09 ng, covering the upper two rows of the plate. The samples (100 µl) diluted to 1:10 were added into the lower part of the plate. Two replicates were prepared per sample.

Alkaline Phosphate System (ALP)

v/v 10 % diethanolamine was prepared with its pH adjusted to 9.8 with concentrated hydrochloric acid (HCl), and was stored in a dark colored bottle. Substrate para-nitro-phenylphosphate was added at the rate of 1 mg/ml buffer before use. 50 µl of AFB1-BSA-labelled with ALP (Alkaline Phosphate) was added at a dilution of 1: 2000, incubated at 37 ^{o}C for 1 hr, and later washed with PBS-T. Finally, 150 µl of ALP substrate was added and the plate was kept at room temperature in the dark.

Penicillinase System (PNC)

On the penicillinase system (PNC), 15 mg of bromothymol blue (BTB) was dissolved in 100 ml of 0.01 M sodium hydroxide (NaOH). The alkali was neutralised by adding 0.1 N hydrochloric acid (HCL) drop wise until the pH of the solution became 7.2. Sodium penicillin-G at 0.5 mg/ml (w/v) concentration was added, and 50 µl of AFB1-BSA labelled with PNC was added at a dilution of 1:10,000. The samples were incubated at 37 ^{o}C for one hour, followed by washing with distilled water-Tween®. Then 150 µl PNC substrate was added and the plate was kept at room temperature for half an hour. The detailed analytical procedure for aflatoxin assay is presented in Appendix 2.

3.5 Training

A two-day training course was organised on 28th and 29th April 2015 for the 15 enumerators and three supervisors on the concept of PHL, implementation approach, and the information to record and observe in field. The focus area was data collection from the time of lifting until marketing. The training covered both theoretical and practical aspects of field demarcation and crop weight measurement. In addition, both the enumerators and supervisors received checklists (Appendix 1) on what to do, as a guide through the data collection exercise.

At the end of the session, each enumerator was provided with a weighing scale of maximum capacity of 500 kg, a tape measure of 50 meters length, 10 polypropylene sampling bags of 5 kg capacity each, a hard cover notepad for data recording, and a ballpoint pen.

Within a week of the training session, formal briefing meetings were organised in each of the three sites, involving the participant farmers, enumerators, and supervisors.

4. Result

4.1 Farming Practices

In total, six groundnut varieties were grown by the participants: CG7, Nsinjiro, Chitembana, Kakoma, Chalimbana 2000 and the traditional variety referred to as Kamlomo. The most popular variety was Chalimbana 2000 grown by 46 % of the farmers, followed by CG 7 produced by 40%. All the farmers planted groundnut between the end of December 2014 and the end of January 2015 due to the late on-set of rainfall in the season. All of them practiced mono-cropping for groundnut. The participants also produced other crops such as tobacco, maize, sweet potato, and cassava.

Labour for most of the field activities for groundnut and other crops was provided by family members including children. Hired labour was sourced only for plots of larger than one acre during the labour demanding processes of lifting and stripping. The participants reported that groundnut production was for multipurpose of consumption, income, and seed recycling. Much of the consumption was enjoyed in the forms of boiled fresh nuts, roasted nuts, and flour used for seasoning relish.

4.2 Post-harvest Loss

Both quantitative description and qualitative statements are presented to describe the post-harvest losses and their observed causes.

4.2.1 Lifting (Harvesting)

According to the participating farmers, the timing for lifting was determined based on the following factors: suggested maturity period (i.e., number of days from the day of planting) specific to the variety, change in skin colour of the kernels (presence of dark markings on the skin of the kernel), and leaf fall.[6] The participants emphasised that the timing of lifting was of paramount importance in maintaining the grain quality and minimizing crop losses, and that both early and late lifting can contribute to PHL.

According to the enumerators, lifting was done using hand hoes by all the participating farmers, and none of them used any type of machinery, which is in line with the finding by Saint et al. (2010).[7] The lifting activity started almost at the same time in the three districts as the participants in the study had planted within the same period.[8] Nonetheless, One third of the participants had to delay the lifting process due to field work on other crops such as maize harvesting. This tendency was observed and deemed as a factor contributing toward PHL. Some pods were damaged during the lifting process, which is regarded as another contributing factor, since such damage (cuts) can act as entry points for infestation agents

[6] Leaf fall may not be a reliable indicator since it can be a result of disease attacks.

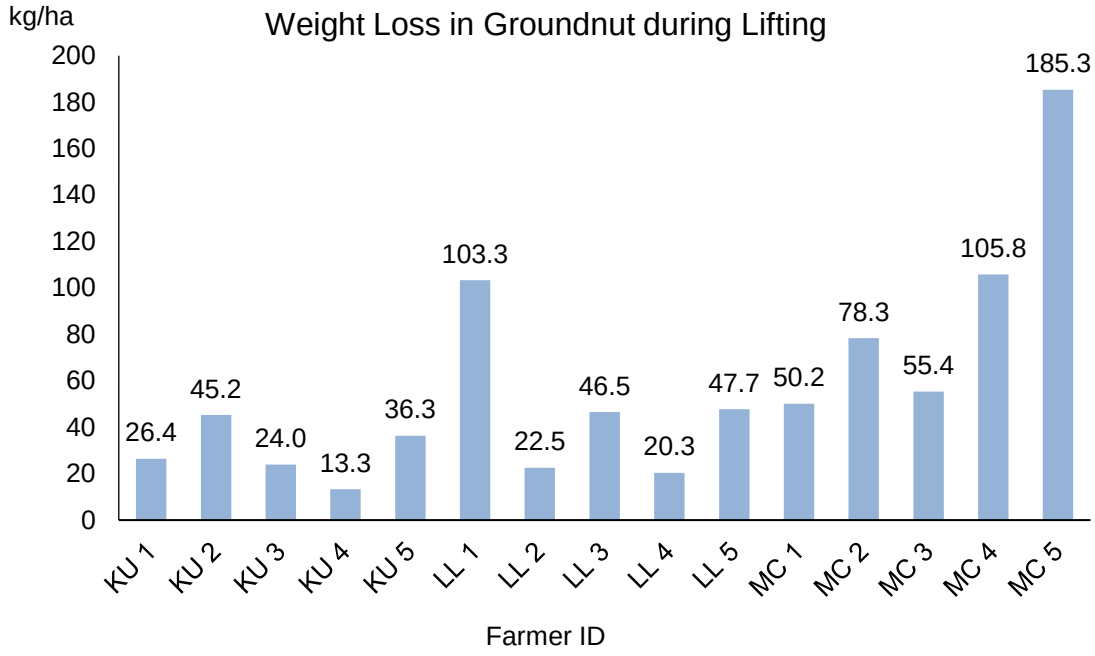
[7] In Malawi, there is an initiative recently launched by CTI (Compatible Technology International) and ICRISAT to develop and introduce small-sized equipment for lifting, stripping, and shelling of groundnut in order to mitigate drudgery and improve efficiency, funded by the McKnight Foundation.

[8] Before lifting in quantities, some farmers had conducted informal harvesting for home consumption purposes. Nonetheless, the largest size of field where informal harvesting took place was 0.0088 acre.

such as pests and diseases. It was observed with one farmer in Mchinji that some pods were shed off in the soil and were sprouting.

After lifting, the haulms were left on the ridges for two to three days to reduce moisture before transfer into small rounds (batches) for further drying. Meanwhile, large pests such as rodents and birds started attacking the crop. The damaged pods were piled at one place. In the fields of a quarter of the participants, the situation was worsened by weeds as weeds provided conducive environments for multiplication of rodents.

At this point, the weight measurement was conducted of the pods collected from the field, which indicated an average weight losses of 29.2 kg, 47.9 kg, and 94.9 kg per hectare in Kasungu, Lilongwe, and Mchinji districts, respectively[9] (Figure 2), with the overall average being 57.3 kg per hectare. All the weights presented in this study are shelled weight equivalent for easy comparison of losses between different stages.[10]



The weight is equivalent to shelled nuts' weight.
KU = Kasungu district; LL = Lilongwe district; MC = Mchinji district
e.g., KU 1 indicates farmer number 1 in Kasungu district

Figure 2 Weight loss in groundnut during lifting (kg per hectare; shelled weight equivalent)

The higher weight losses recoreded during lifting were mainly attributed to the presence of weeds. In adition to attracting rodents, the presence of weeds also caused workers to fail to lift some of the haums. Two thirds (65 %) of the total fields under study had presence of weeds at the time of lifting, and in particular 27 % were infested with plenty of them. Interestingly, missed haulms were found more frequently where hired labour was used for lifting, which is in line with the theory of princial-agent problem or moral hazard (Hölmstrom,

[9] The weights are based on shelled nuts equivalent (i.e., downstream of the post-harvest chain).

[10] The conversion rate of unshelled and shelled weights is 3:2.

1979; Frooman and Pouryousefi, 2017). When lifted, some haulms had no pods, implying that the pods were unintentionally stripped and left into the soil. The incidence of unintentional stripping in the soil depended on the variety as well as the timing of lifting. Moreover, some pods were consumed on the spot by those involved in lifting. Ridges adjacent to foot paths tended to have no haulms, as they were stolen by passers-by, as is a common issue in this part of the world (Emmott and Stephens, 2012). Lastly, two thirds of the labour used for lifting groundnut was provided by men, while women provided the one third.

4.2.2 Drying

It was observed that all the farmers except one in Kasungu dried their nuts under the sun in the field where lifting had taken place.[11] The haulms were dried by way of (1) leaving on ridges, (2) placing in forms of small rounds (Figure 3), or (3) constructing the Mandela cork (Figure 4) which was practiced by 13 % of the farmers under study. On average, the drying period lasted from four to five weeks for all the methods adopted, which is in line with Mestres et al. (2004) who found that field drying of groundnut normally took up to four weeks.

Figure 3: Small round drying method, practiced by Mr. Godfrey Gonekeni, a farmer in Kasungu, standing beside the small round of groundnut haulms being dried in the field

[11] The one farmer in Kasungu dried his nuts mainly at homestead in fear of thefts in field. This farmer left the haulms in the field only for two days and transported them home, where the haulms were stripped first and then the pods were dried on the ground.

Figure 4: Mandela cork drying method, practiced in the studied field in Kasungu

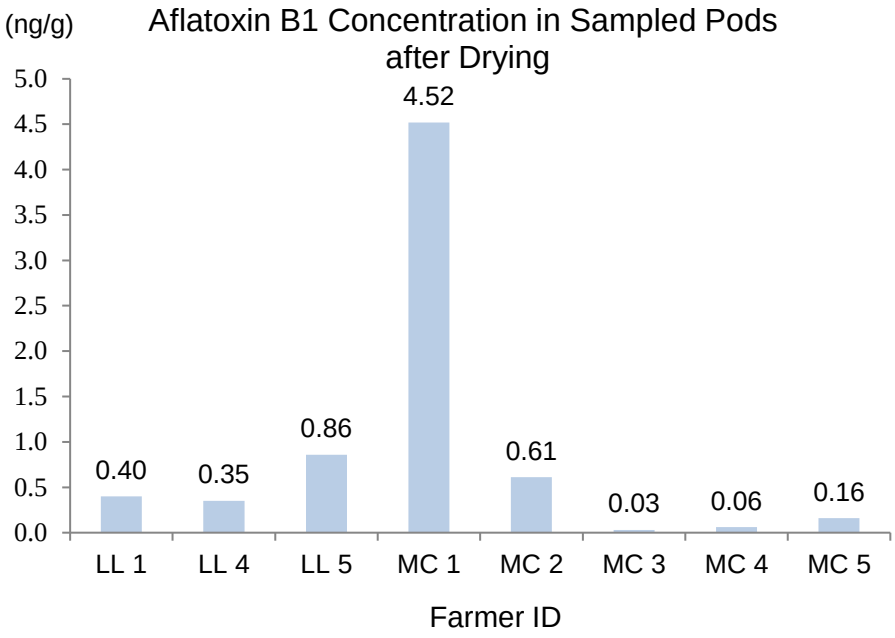
The Mandela cork and small rounds methods were used not only for drying but also for facilitating monitoring thefts and loss caused by humans and pests such as rodents, ants, livestock, and wild animals (e.g., monkeys and mice). The farmers checked for such incidents through deformation of cork and rounds as well as presence of destroyed pods within and around the sites. Although the enumerators confirmed this practice, it was difficult to distinguish the damage caused by hand hoes during lifting and by pest attacks. In addition, some pods were casually picked and eaten by passers-by including school children.

To determine the progress of drying, farmers reportedly shook the pods in hands and checked the cracking sound in order to judge the level of moisture content appropriate for storage.

After drying, some haulms were left uncollected from the drying schemes (e.g., small rounds), resulting in the increase in losses during drying. This tendency was pronounced when children were performing the activity who lacked attention to the task.

It was observed that some fungal had developed on the pods during the drying period, which was confirmed by the aflatoxin assay with the pods sampled from the five sites in Mchinji and three sites in Lilongwe at the end of the drying process (Figure 5).[12]

[12] Not all the sites were included due to the resource limitation.



LL = Lilongwe district; MC = Mchinji district
e.g., MC 1 indicates farmer number 1 in Mchinji district

Figure 5: Aflatoxin B1 contamination levels in groundnut pods sampled at the end of the drying process before stripping

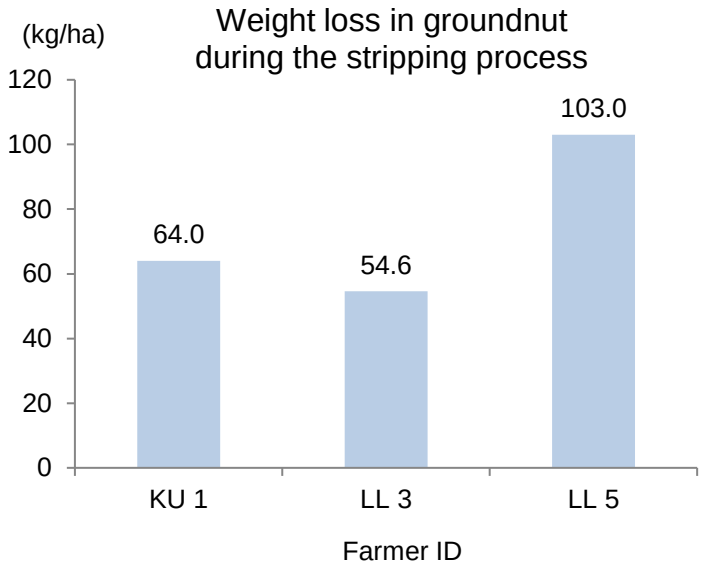
Nonetheless, the detected aflatoxin levels were below the critical limits except for one sample from Mchinji for which the farmer mentioned termite infestation as a factor for aggravating the contamination. Termite infestation arises from different factors including deforestation, soil degradation, and overgrazing, and it is difficult to predict an attack beforehand. The farmer in question applied cypermethrin and HTH to dispel the termites.

Furthermore, there was a local shower during the drying process in part of Mchinji, by which his haulms became wet: another factor for multiplication of aflatoxin. In Malawi, raining after harvesting is rare. The association between PH rainfall and aflatoxin contamination has been mentioned in existing studies such as Matumba et al. (2009) and Monyo et al. (2012).

4.2.3 Stripping

Stripping was conducted in the field by all the farmers except one who did it at homestead. Some farmers delayed the start of stripping due to other activities such as maize harvesting and stooking. All the farmers practiced hand stripping engaging family or hired labour, whereby children and adults of both genders were involved, with the age ranging from 8 to 65. In the majority of fields, the process began by gathering several small drying rounds to one point. Apparently, during this process, some haulms were spilled and lost. These spilled haulms were never collected. Besides, some of the workers were found to be consuming groundnut kernels while stripping.

At the end of the stripping, the weight measurement was conducted of the sample collected from two farmers in Lilongwe and one in Kasungu[13], indicating the loss per acre ranging from 22.1 kg to 41.7 kg during the stripping process (Figure 6).[14]



The weight is equivalent to shelled nuts' weight.
KU = Kasungu district; LL = Lilongwe district
e.g., KU 1 indicates farmer number 1 in Kasungu district

Figure 6 Weight loss in groundnut during the stripping process (kg per hectare; shelled weight equivalent)

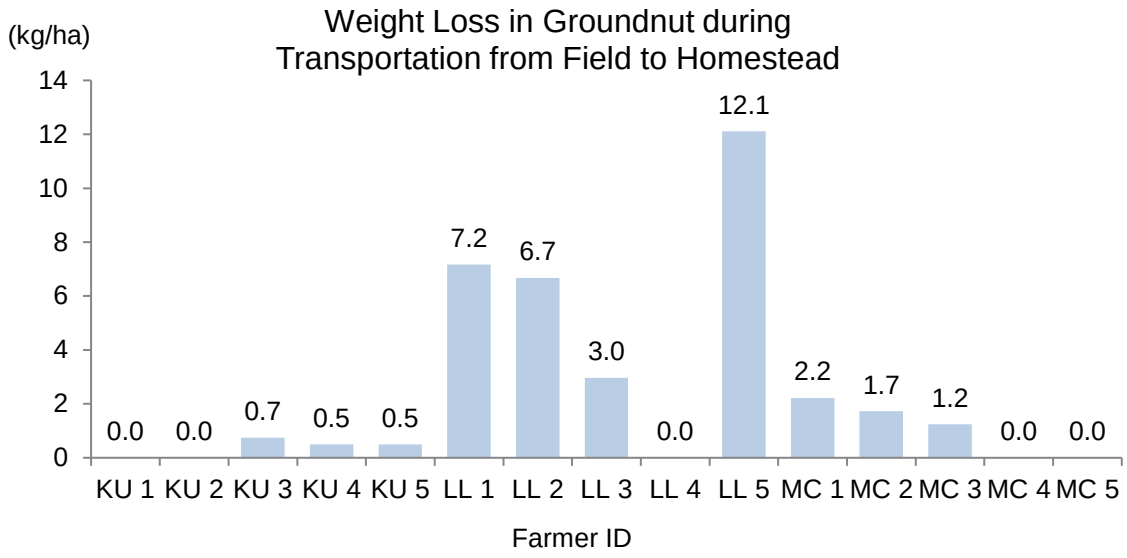
4.2.4 Transport to homestead

Ox-carts (47% of the farmers), bicycles (33%), and walk (20%) were the three modes of transport used to deliver groundnut pods from field to homestead. In the case of walk, the pods were carried on workers' top of head. The farmers filled polypropylene bags (sacks) with the pods before loading in transport. The losses incurred during transportation were primarily due to spillage as some polypropylene bags were old and torn. To a much lesser extent, spillage occurred due to the pods directly loaded in ox-carts without using polypropylene bags, depending on the condition of the ox-cart.

Figure 7 presents the result of the measurement of weight loss incurred during the transport from field to homestead. The highest weight loss per hectare in the respective districts was 0.7 kg (Kasungu), 12.1 kg (Lilongwe), and 2.2 kg (Mchinji). Five farmers (two from Kasungu, two from Mchinji, and one from Lilongwe) incurred no weight loss during transportation.

[13] Not all the sites were included due to the resource limitation.

[14] The weights are based on shelled nuts equivalent (i.e., downstream of the post-harvest chain).



KU = Kasungu district; LL = Lilongwe district; MC = Mchinji district
e.g., KU 1 indicates farmer number 1 in Kasungu district

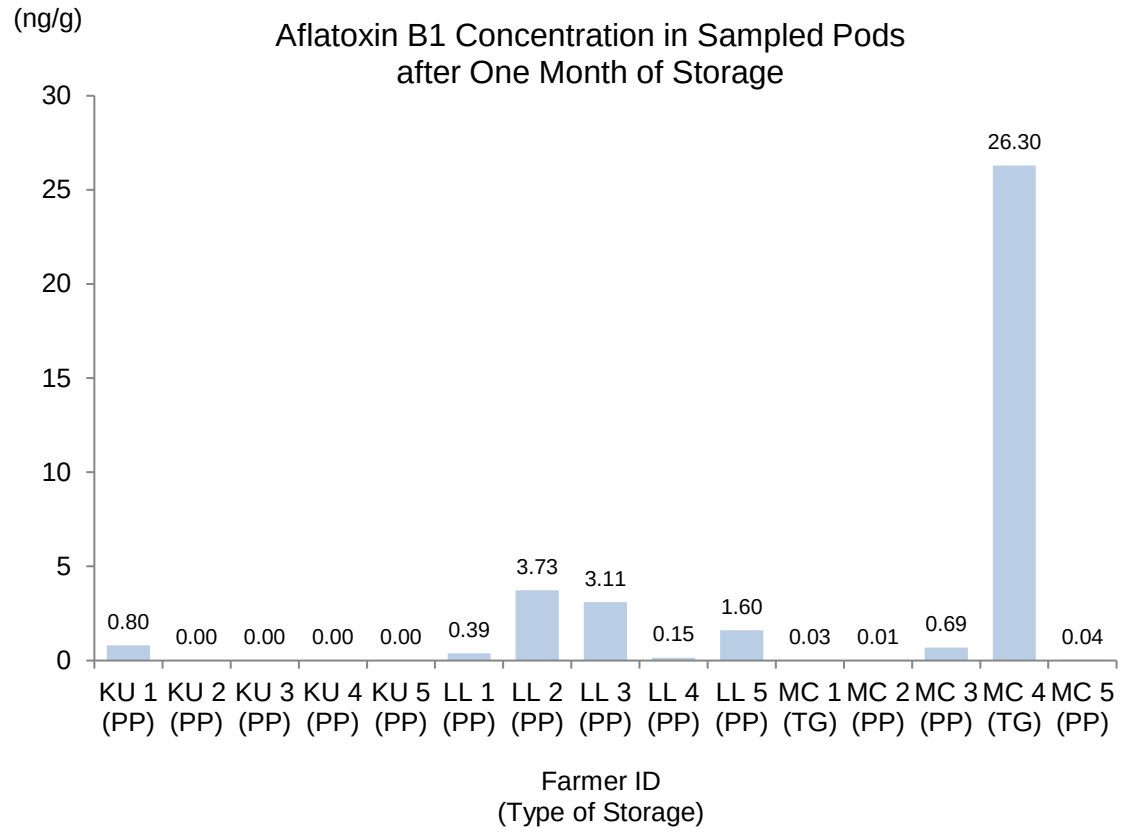
Figure 7 Weight losses in groundnut during transportation from field to homestead (kg per hectare; shelled weight equivalent)

4.2.5 Storage

None of those who dried the groundnut in field further dried the pods at homestead after transporting from the field. One farmer temporarily kept the pods in a metal drum before storage, while another farmer used a traditional granary in preparation for storage inside the house. The polypropylene bags filled with the pods were carried into corrugated or grass thatched houses, where the sacks were placed on bricks or planks as pallets. The storage room kept other materials such as hoes, empty tins, and empty sacks. There was rodent damage to the pods, in particular to the ones stored in the drum.

The practice of the sampled farmers contrasted with what Saint et al. (2010) observed several years ago in Kasungu, i.e., 60 % of the households stored groundnut in woven granaries smeared with mud, whereas the other households adopted the practice we observed this time.

After one month of storage, the second round of aflatoxin assay was performed. The result indicates that the contamination levels ranged from 0.00 to 3.73 ng/g except one farmer (Figure 8). Although the overall level of contamination seemed stable since the measurement after drying, the individual-level changes were large, and so were the district-level and individual-level variances. In fact, the average contamination levels decreased in Mchinji and increased in Lilongwe.



KU = Kasungu district; LL = Lilongwe district; MC = Mchinji district
e.g., KU 1 indicates farmer number 1 in Kasungu district
TG = Traditional granary; PP = Polypropylene bag

Figure 8: Aflatoxin B1 contamination levels in groundnut pods sampled after one month of storage.

None of the participating farmers sold their groundnuts during the first month of storage in anticipation for better prices towards the end of the year, which is consistent with the finding in Lilongwe and Kasungu by Saint et al. (2010). Delays in selling can result in PHL occurring during storage due to aflatoxin, rodents, and other pests (Bhattacharya and Raha, 2002; Abass et al., 2014).

4.2.6 Summary of the Losses

Table 1 summarises the quantitative losses mentioned in Subsections 5.1-5.5, indicating that the overall average loss was 75.4 kg per hectare, which is equivalent to 15.2 % of the harvest without PHL. The majority of the losses occurred during the lifting operations. Area-wise, Mchinji incurred a relatively large loss during lifting, whereas Kasungu suffered a large loss during drying and stripping. The table also presents the value loss due to the quantitative loss. On average, smallholder groundnut farmers incurred a loss of USD 107.1 per hectare.

Table 1 Summary of Post-harvest Losses in Groundnut in Mchinji, Lilongwe, and Kasungu districts in 2015.

	Mchinji	Lilongwe	Kasungu	Average

Quantity Loss (kg per hectare, shelled equivalent)				
Lifting	94.9	47.9	29.2	57.3
Drying & Stripping	n/a	59.3	103.1	73.9 [1)]
Transport to homestead	1.0	5.8	0.3	2.4
Sum	169.8 [2)]	113.0	132.6	133.6
% of Quantity Loss [3)]	15.5	12.0	14.7	13.7
Value loss (USD per hectare) [4)]	241.1	160.5	188.3	189.7

1) The average over Lilongwe and Kasungu
2) For the drying & stripping, the average value was used.
3) The denominator for this percentage is the sum of the reported average groundnut yield (i.e., with PHL) and the PHL found, for each district.
4) The price applied is USD 1.42, which is twice the unshelled price in 2015 (FAO, 2017b). The price for shelled groundnuts is assumed to be double the price for unshelled nuts, which is common in Central Malawi.

5. Discussions

On average, the studied farmers incurred the quantitative loss of 133.6 kg per hectare of groundnut, accounting for 13.7 % of the harvest without PHL, which is equivalent to the value loss of USD 189.7 per hectare.[15] Given that the majority of smallholders in Malawi live below the poverty line and that the losses incurred from storage and beyond were not included in the calculation, the PHL in groundnut translates into huge lost economic opportunities for smallholder farmers. Our on-farm measurement based result is comparable with the result of the perception based study by Ambler et al. (2017) that the average groundnut farmer in Malawi incurs 12 % of the harvest. Our interpretation is that both studies somewhat underestimated the losses because the former did not cover the entire PH chain, whereas the latter relied on perception, i.e., farmers could not perceive invisible part of the losses.

Part of the PHL that occurred can be avoided through provision of proper techniques and technologies for groundnut PH management. During lifting, the use of hand hoes contributed to PHL as damaged pods tended to be exposed to pests and diseases. One way to mitigate this loss may be an animal-traction lifter that was shown to cause much less pod damage has recently been developed in Malawi (Spieldoch et al., 2013), though not yet widely disseminated. Since the majority of the PHL occurred during lifting, interventions at this stage of the PH would be particularly effective.

Despite the government's extension efforts through DAES, relatively few farmers adopted the best-bet drying method of the Mandela cork, leaving great potential for aflatoxin mitigation by diffusing the proper drying method. Besides, Waliyar et al. (2015) recommends, as part of aflatoxin management, drying groundnut pods until the moisture content comes down to 8 % before storage. During drying, termite infestation stroke one of the studied farmers. Apart from application of chemicals as practiced by the farmer in question, some cultural measures can also be considered such as fumigation, digging mound, flooding, and removal of the queen (Taye et al., 2013). Saint et al. (2010) also argue that drying of haulms provides a stage for attacks by rodents and birds, as the crop is gathered in one place and tends to be placed upside down. Use of the Mandela cork in combination with monitoring of pests is recommended to minimise the loss. For the losses caused during the recollection of haulms

[15] Groundnut is both a food crop and cash crop. Smallholders allocate the harvest for consumption first, and the surplus is sold. It is therefore assumed that the PHL affects the surplus, i.e., income, not consumption, up to the extent that the surplus becomes zero.

from the drying schemes, which was often undertaken by children, engaging adults or adopting labour saving technologies would mitigate this type of loss.

There are three major limitations in this study. First, due to the unexpected pause in funding, the study did not cover the entire on-farm PH chain, and hence longer-term storage, shelling, winnowing, grading, transportation to the market, and marketing were not covered. Second, the qualitative losses during storage were not calculated due to the unavailability of a rapid assessment methodology for groundnut with visual scales similar to the ones used in maize. Third, because of the resource demanding method used, the study worked with a small sample of farmers which may lead to an estimation bias, though the participants were carefully chosen to avoid outliers and the result was consistent with the existing study on a representative sample.

Future research is expected to include the remaining stages to generate a complete picture of the PHL occurring at the farm level, and also develop a rapid method for assessing PHL in groundnut using visual scales similar to the ones used for maize. Furthermore, it is desirable to develop a method that can easily be applied to other agricultural commodities in developing countries where value chain efficiency (e.g., infrastructural and economic levels) is not particularly high. The improved estimates of PHL can then be used by modelers to produce global food security projections in more reliable manners.

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Appendices

Appendix 1 Checklist used by the enumerators as a guide for collecting information during the entire study period

No.	Information recorded
1	Name of farmer
2	Name of district
3	Name of extension planning area (EPA)
4	Name of section
5	Age of farmer (Years)
6	Gender of farmer
7	Village and Traditional Authority
8	Area (Acres) under groundnut production
9	Groundnut variety grown
10	Planting dates
11	Dates of implementing every activity
12	Gender of Supervisor for every activity
13	Age of supervisor
14	Number of people involved per activity
15	Age of people involved in every activity and their aged
16	Area harvested (acres) on daily basis
17	Tools used per activity
18	Method used per activity such as lifting, stripping etc
19	Observation noted during every activity
20	Quantity of groundnut harvested
21	List of other crops grown
22	Use for the planted crops

Appendix 2 General Aflatoxin Analysis Methodology using ELISA

ELISA FOR THE ESTIMATION OF AFLATOXINS

AFLATOXINS

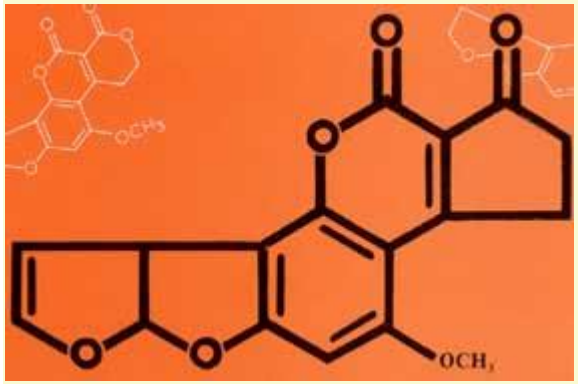
Agricultural products are often contaminated with fungi that can produce toxic metabolites referred to as “mycotoxins”. Among these, aflatoxins have assumed economic importance because of their influence on the health of human beings and livestock and on the marketability of agricultural products. Aflatoxin is a Group 1 carcinogen proven to cause liver cancer and also suppresses the immune system. In most developing countries limited or no facilities exist for monitoring these toxins in foods and feeds. They are based on phisicochemical methods as TLC and to a limited extent high performance liquid chromatography (HPLC) are used for the estimation of aflatoxins. Immunological methods are preferred over analytical methods because of their simplicity and cost-effectiveness. However, commercial kits based on immunological methods are expensive, and may be difficult to import them. To develop cost effective and simple technologies for the estimation of aflatoxins, United Kingdom Department for International Development (DFID) granted a project in 1998 (project no R7083) to ICRISAT and SCRI. This funding helped in the development of immunochemical methods. High quality antibodies were produced for aflatoxins and the methodologies developed to use antibodies for aflatoxin estimation in different agricultural commodities. The results were comparable with those of HPLC. Costs for performing this test procedure were compared with those of TLC and HPLC and found to be the least expensive of all the procedures and permitted analysis of up to 200 samples per day. The method developed is therefore simple, robust and cost-effective. Constant monitoring of food and feed will contribute to improvement of health of humans and livestock and will

DEFINITION OF TERMS

Antigen: A substance which can elicit production of antibodies when introduced into warm blooded animals.
Antibodies: Glycoproteins that are produced as a result of an immune response following introduction of antigens leading to the production of a specific antigen-antibody complex.
Conjugate:A compound molecule prepared by linking two molecules.
AFB1-BSA:A conjugate consisting of aflatoxin molecules linked to bovine serum albumin. This is required to induce antibodies.
AFB1-BSA-Enzyme: AFB1-BSA attached to an enzyme molecule (alkaline phosphatase or penicillinase or horseradish peroxidase).
Enzyme-Linked Immunosorbant Assay (ELISA): Detecting and quantifying the presence of an antigen (aflatoxin) in a

PROCEDURE SUMMARY

also enhance export potential leading to increased income for poor farmers in developing countries.



sample using an enzime labelled toxin and antibodies specific to aflatoxin Direct competitive ELISA: The antibody is coated on to the wells of the ELISA plate (Maxisorp or equivalent). The test sample and the enzyme-labelled aflatoxin B1-BSA are added to the wells. If no toxin is present in the sample, the enzyme labelled toxin will bind to the capture antibody coated to the wells. If toxin is present in the sample, it will compete with the labelled toxin for binding to the antibody. During washing procedures any unbound labelled enzyme will be washed away. On the addition of substrate, a colour will develop the intensity of which is proportional to the amount of AFB1-BSA-enzyme bound to the well; i.e., the colour intensity decreases with increasing concentrations of the toxin in the sample.

DIRECT COMPETITIVE ELISA

Coat each well of an ELISA plate by using 150 µl of AFB1 antiserum diluted at 1:80,000 in coating buffer

SAMPLE EXTRACTION

Incubate 1 h at 37 ºC or overnight at 4 ºC

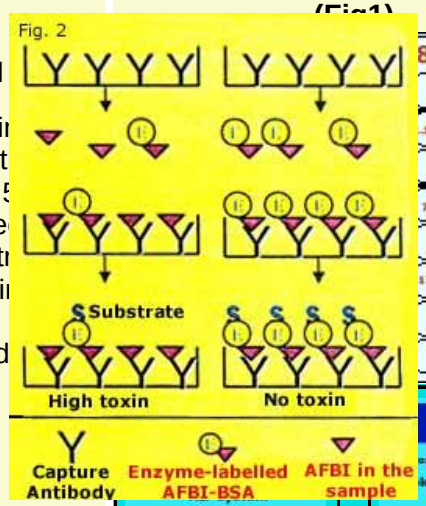
30 ml distilled water) containing 0.5

Wash the plate with PBS-T

blender for 2 min. Transfer the extr

Add 100 µl of AFB1 standards at concentrations ranging from 100 ng to 0.09 ng. This cover upper two rows of the late. Add samples (100 µl) diluted to 1:10 in the lower part of the plate Use two replicates per sample (see Fig.1)

ELISA PLATE FORMAT



Coating buffer

Na_2CO_3	1.59g
$NaHCO_3$	2.93g
Distilled H_2O	1 L

Phosphate buffered saline Tween (PBS-T)

Na_2HPO_4	2.38g
KH_2PO_4	0.4 g
KCl	0.4 g
NaCl	16g
Tween20	1 ml
Distilled Water	2 L

PBS-T-BSA

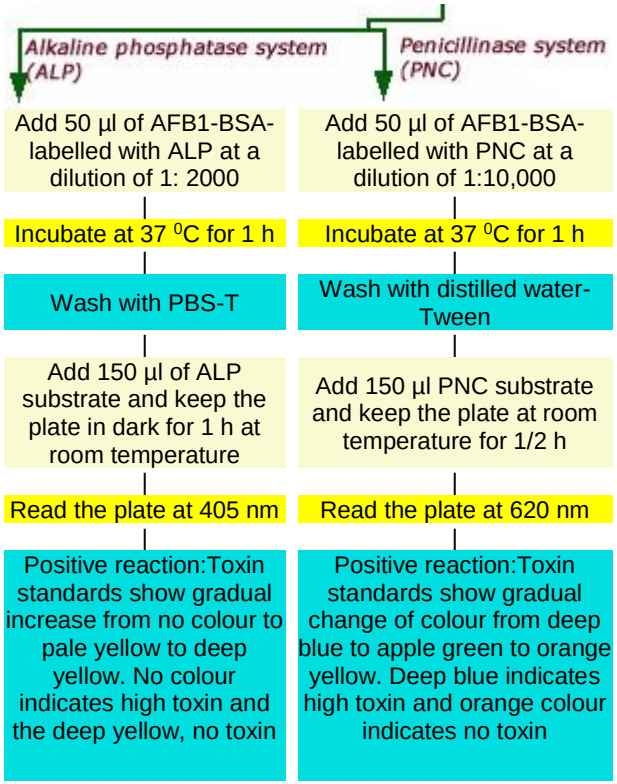
Dissolve 200 mg BSA in 100 ml PBS-T

Distilled water Tween

Distilled Water	2 L
Tween20	2 ml

SUBSTRATES

- **ALP system**
  Prepare 10% diethanolamine (v/v) in distilled water and adjust pH to 9.8 with conc. HCL. Store this in a dark coloured bottle. Add substrate para-nitro-phenylphosphate at the rate of 1 mg/ml buffer before use.
- **PNC system**
  Dissolve 15mg bromothymol blue (BTB) in 100 ml of 0.01 M NaOH. Neutralise the alkali by adding 0.1 N HCL drop wise until the pH of the solution is 7.2. Add sodium penicillin-G at 0.5 mg/ml (w/v) concentration.



Calculations:

Using the OD values obtained for AFB1 standards draw a curve, taking AFB1 concs. on the X-axis and OD values on the Y-axis.

AFB1(µg/kg) : (AxDxE)/G

A= AFB1 concentration in sample extract (ng/ml)

D= Times dilution with buffer

E = Extraction solvent volume used (ml)

G = Sample weight (g)

For more information

Dr Anitha Seetha
s.anitha@cgiar.org
http://aflatoxin.info