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Prevalence and distribution of aflatoxin contamination in groundnut (*Arachis hypogaea* L.) in Mali, West Africa

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ABSTRACT

Groundnut is a major source of livelihood for the rural poor in Mali. However, the crop is prone to pre- and post-harvest aflatoxin contamination caused by *Aspergillus flavus* and *Aspergillus parasiticus*. Therefore, to minimize health related hazards from exposure to aflatoxin contaminated food, information on the prevalence and distribution of aflatoxins (AFB1) in the groundnut value chain in Mali is needed for timely interventions. To this end, a study was undertaken in three districts (Kayes, Kita and Kolokani) to assess aflatoxin contamination in the field and storage. Ninety pod samples in each district were collected from fields (30 villages/district and 3 samples/village) during 2009 and 2010. Pre-harvest contamination was estimated at harvest, whereas samples for post-harvest contamination were collected from granaries of the same farmers at a monthly interval for 3 months. The villages in each district were categorized into safe, acceptable, moderate risk and high risk areas based on pre-harvest AFB1 levels. Kayes recorded more pod samples (77%) within 20 μg/kg of pre-harvest aflatoxins followed by Kolokani (55.6%) and Kita (45.6%) based on 2009 and 2010 mean values. Toxin concentrations at harvest were comparatively less in Kayes during both years. Further, Kayes had more villages under safe and acceptable limits when compared to Kolokani and Kita. Overall, 46 out of 90 villages in the three districts had acceptable pre-harvest toxin limits. Further, 12 villages in Kolokani were in the high risk category. An increase in toxin levels was noticed with period of storage during both years. Comparatively, toxin levels after storage were least in Kayes during 2009. Kayes also recorded less AFB1 levels in 2010 after Kita. Our results indicate that Kayes is relatively safe over Kita and Kolokani in pre-harvest aflatoxin contamination. The reasons for district-wide variations in pre-harvest contamination; and the reasons for post-harvest flare up of the problem are discussed. Further, proper storage of pods at farmers' granaries in Mali is suggested to overcome the problem from reaching alarming levels.

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1. Introduction

Food safety apprehensions are increasing globally, particularly in developed countries where food safety issues are heavily influenced by consumer perceptions and policies with respect to food production, processing, handling and trade. As developing countries are increasingly involved in international trade, they must meet specific and more stringent food safety standards of target trading partner countries. Further, the concept of globalization has enabled developing countries to benefit from knowledge from other parts of the world, resulting in equally stringent policy contexts for various food safety concerns such as mycotoxins in food and animal feed. However, these tighter policy contexts have mostly not led to a decrease in aflatoxin levels in food products in local markets. Importantly, due to poor management of on and off-farm operations, risks of ingestion of aflatoxin contaminated food are much greater in the developing than in the developed world.

Aflatoxin contamination in groundnut (*Arachis hypogaea* L.) is a major concern posing a significant threat to the health of rural people, and affects the economy of poor farmers who rely on

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groundnuts for nutrition and income generation. For example, 42% (by volume) of groundnut export from Malawi to European markets was rejected due to aflatoxin contamination in 2005 (Diaz-Rios and Jaffee, 2008). Other important commodities in Sub-Saharan Africa (SSA) currently known to be contaminated with aflatoxins include oilseeds, cereals (especially maize), legumes, tree nuts and spices (Bankole and Adebanjo, 2003; Bankole et al., 2006; Bandyopadhyay et al., 2007; Wagacha and Muthomi, 2008). Some of these commodities are important in the diet of many poor people and are also common components of livestock feeds. Due to environmental factors and poor management of on and off-farm operations, risks of ingestion of aflatoxin contaminated food are much greater in the developing than in the developed world. Geocarpic nature of groundnut makes the pods vulnerable to toxigenic molds and its subsequent associated health risks on consumption.

Aflatoxins are a group of 20 closely related secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Liu and Wu, 2010; Snigdha et al., 2013). These fungi are ubiquitous, air-borne and soil-inhabitants, that are also found in crops and foods, including food storage facilities (Waliyar et al., 1994, 2003a; Jaime-Garcia and Cotty, 2004; Williams et al., 2004; Azziz-Baumgartner et al., 2005; Lewis et al., 2005). Aflatoxins exhibit a wide array of biological effects on humans and have been confirmed to induce liver cancer in persons with Hepatitis C (Liu and Wu, 2010). Consumption of high doses of aflatoxins is fatal (acute aflatoxicoses), while small quantities of chronic exposure may lead to liver cancer, and liver cirrhosis (Williams et al., 2004; Strosnider et al., 2006). These also act as growth retardants especially in children (Gong et al., 2004) and are also reported to be potential immune-suppressors (Sahoo and Mukherjee, 2001). Aflatoxin related health issues may be more severe in the rural poor, who often survive on simple and unprocessed legume- and cereal-based diets.

Most developing countries lie in the tropics, where temperatures and relative humidity favor mold growth and subsequent aflatoxin contamination. Infection by *Aspergillus* spp. and aflatoxin production in groundnut occurs at pre-harvest, during harvest, post-harvest, in storage, as well as during processing and transport along the value chain (Waliyar et al., 2007). Drought stress (mid or late in the season) increases risk of *Aspergillus* spp. infection and has been correlated with high amounts of aflatoxins (Nigam et al., 2009). Mid-season drought results in reduction of seed moisture content, over-maturity, increased risk of insect and other pod damage and decreased plant vigor (Craufurd et al., 2006; Bruns, 2003; Waliyar et al., 2003b). A number of soil-inhabiting pests, including pod borers, millipedes, mites, white grubs, termites and nematodes have been implicated in *Aspergillus* spp. infection of groundnuts at the field level (Umeh et al., 2000; Bruns, 2003). Mechanical damage to pods at the time of harvest can also lead to seed invasion by *Aspergillus* spp. and the geocarpic nature of groundnut makes the pods vulnerable to toxigenic molds.

Subsistence farmers in Mali usually harvest groundnut, dry in the field, strip and transfer pods to granaries for storage. When *A. flavus* infected groundnut pods are harvested and kept for drying, they are subjected to rapidly changing environmental conditions which cause shifts in the dominant and sub-dominant fungal species on and within the pods. Lifting and drying the pods under high moisture conditions may further result in considerable seed invasion by *A. flavus* and other fungi that have already gained entry into pods. The main factors that contribute to post-harvest aflatoxin contamination are moisture and temperature (Hell and Mutegi, 2011). *A. flavus* grows at a moisture content of >9% and at a temperature of 25–30 °C, and a water activity of 0.99 with a minimum of $0.83a_w$. For optimum production of aflatoxins, a temperature of 25 °C and $0.99a_w$ with a minimum of $0.87a_w$ is required (Ribeiro et al., 2006).

Important information gaps remain concerning the prevalence of aflatoxin contamination at various stages in the groundnut value chain in Mali. This study was therefore designed to assess the aflatoxin contamination levels at harvest and in storage at the farm level. Our goal was to evolve a practical management strategy for the aflatoxin problem in groundnut through a thorough understanding on the prevalence and distribution along its value chain. The specific objective of the study was to assess the occurrence, spread and level of toxin (AFB1) contamination in major groundnut growing areas of Mali.

2. Materials and methods

2.1. Selection and details of sampling sites

Purposive sampling was adopted to select the three districts/regions used for this study based on groundnut production and agro-ecology. The districts of Kayes, Kita and Kolokani, were selected that represent the three key agro-ecological zones of Mali. Kita falls under the wettest zone which receives an average annual rainfall of 1100 mm, whereas the other two were in semi-arid zones with rainfall ranging from 700 to 750 mm annually. These three districts also represent the major groundnut growing areas in Mali where the crop is grown predominantly under rain-fed conditions by subsistence farmers. Soils in the selected districts are mainly of sandy loam texture. In each district, 30 villages were randomly selected, with at least 30 km apart from each other. A total of 270 farmers, three from each village, were selected in consultation with village heads based on farmers' willingness to accept new technologies. Sampling was done at harvest and from granaries as described in the subsequent section.

2.2. Groundnut sampling at harvest

Pod sampling was done for both 2009 and 2010 sown crops. In each district, pod samples were drawn from 90 farmers' fields in 30 villages (3 farms/village). After crop harvest and drying, pod harvesting was carried out in the 2009 and 2010 crops during November from the previously identified plots (5–10 m^2). Pods were pooled, mixed thoroughly and a 1 kg pod sub-sample was taken to the laboratory for aflatoxin analysis. Pods were shelled manually by hand and a 500 g kernel sub-sample was drawn for aflatoxin analysis. The kernels were brought to uniform moisture content ≤7% by natural air drying. The dried samples were later serologically assayed for AFB_1 within one week. Results of sample analysis were categorized based on toxin levels as follows: 0–4 μg/kg; 5–10 μg/kg; 11–20 μg/kg; 21–35 μg/kg; 36–100; 101–500 μg/kg and >500 μg/kg. Percentages were computed for frequencies of occurrence of the groups.

2.3. Groundnut sampling from farmers' granaries

Pod samples were collected from farmers' granaries for both the 2009 and 2010 harvested crops separately. The same 270 farmers where the field sampling was done in the districts were also used for sampling in the granaries. From each district, 90 pod samples were obtained (3 samples from each village) at monthly intervals up to February 2010 and February 2011 for crops harvested in November 2009 and November 2010 respectively. Pod samples were taken at three levels at the top, middle and bottom of the storage structure and mixed. Finally a 1 kg pod sub-sample was taken from each granary, shelled manually by hand and a 500 g kernel sub-sample was used for aflatoxin estimation.

2.4. Mapping of risk and sensitive areas for aflatoxin contamination

The mean pre-harvest aflatoxin levels were estimated at harvest from the 2009 and 2010 crops as described above. Risk and sensitive villages were mapped using Geographical Information System (GIS) at ICRISAT, Patancheru, India. All the 90 selected villages in Mali were categorized into different zones as safe (aflatoxin range of 0–4 μg/kg); acceptable (4.1–30 μg/kg); moderate risk (30.1–100 μg/kg); and high risk (>100 μg/kg).

2.5. Data collection and analysis

Aflatoxin content (AFB1) in kernels was estimated in the pod samples from the field and granaries using the indirect competitive enzyme linked immunosorbent assay (ELISA). The methodology involves immobilizing the antigen on the surface of an ELISA plate, followed by competition for antibody binding between the AFB1 present on the surface of the plate and AFB1 molecules present in the sample or standard. Later, the enzyme labeled secondary antibodies were used to detect the aflatoxin specific antibodies (Reddy et al., 2001). The standard error of means for the aflatoxin contents were calculated for the 2009 and 2010 crops at harvest and monthly during storage. The data were log transformed and analysis of variance (ANOVA) was carried out using GENSTAT statistical package (version 10.1; Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK).

3. Results

3.1. Pre-harvest aflatoxin contamination from farmers' fields

The mean aflatoxin contamination for groundnut samples from farmers' harvest during 2009 and 2010 is given in Table 1. In general, the majority of samples in all the three districts were in the 0–4 μg/kg category. Kayes recorded 41.1% samples, whereas Kita and Kolokani recorded 36.7% and 34.4% of samples, respectively, under this category. Overall, the samples within 20 μg/kg toxin levels were highest in Kayes (77.7%), followed by Kolokani (55.6%) and Kita (45.6%). The samples within the range of 101–500 μg/kg were least in Kayes (10%), followed by Kolokani (17.8%) and Kita (18.9%). Kolokani was the only district that recorded samples (5.6%) with >500 μg/kg (Table 1).

3.2. Mapping of risk and sensitive areas for aflatoxin contamination

Based on GIS data, seven villages in Kayes (Kersquare, Medine, Hambidedi, Counda, Maloum, Papara and Kamankole) were in the safe category (0–4 μg/kg). Further, 13 villages in this district were within acceptable limits (4.1–30 μg/kg) and there were eight villages with moderate risk (30.1–100 μg/kg). The details of villages under each category are depicted in Fig 1a. The villages Dyaabougou and Saliambougou fall under high risk zone (>100 μg/kg).

In Kita district, four villages, Diangola Kita, Kodafara, Makodji and Keniekola were safe with toxin limits <4 μg/kg (Fig 1b). However, eight other villages (Kouroutkot, Kabe, Sananfara, Delikebala, Bambana, Karaya Toumoumba, Thierou and Madyla) had acceptable pre-harvest contamination. The details of 16 other villages that were under moderate risk are given in Fig 1b. Sibikily and Diatala villages were the high risk villages in Kita with a mean pre-harvest aflatoxin contamination of >100 μg/kg.

In Kolokani district, Touzona, Guihoyo and Bamabougou villages were designated as safe. Twelve other villages were in the acceptable zone with toxin levels in the range of 4.1–30 μg/kg. The villages Somambougou, Doribougou, Farabougou and Tiambougou were in the moderate risk zone. A total of 11 villages were identified as high risk areas with a toxin limit >100 μg/kg. The details of villages that fell in different categories are depicted in Fig 1c.

3.3. Post-harvest aflatoxin contamination in farmers' granaries

Post-harvest aflatoxin accumulation was noticed in groundnuts stored in farmers' granaries during both 2009 and 2010 in all the three districts under study (Figs. 2 and 3). An increase in aflatoxins was observed during storage in all the three districts. In Kayes, pods after storage recorded 163.5 μg kg^{-1} of toxin during 2009 and 34.3 μg kg^{-1} in 2010. Similarly, for Kita, at three months after storage, the toxin levels were up to 310.9 and 28.4 μg/kg during 2009 and 2010, respectively. For Kolokani, toxin levels were up to 270.3 and 61.4 μg kg^{-1} in 2009 and 2010, respectively. In general, the toxin levels after storage were less for the 2010 crop compared to 2009. The log transformed aflatoxin data for the 2009 crop indicated no significant differences between districts and months. However, for the 2010 crop, significant differences were observed between districts only.

4. Discussion

Our results indicate the prevalence of aflatoxin contamination at both pre- and post-harvest stages in Kayes, Kita and Kolokani districts of Mali. An increase in toxins was noticed with an increase in storage period during both years. These results imply that the Mali groundnuts are heavily contaminated with AFB_1 at both pre- and post-harvest stages, and pose a serious threat to human and animal health. Comparatively higher pre-harvest aflatoxin (AFB1) levels in pods from Kolokani over Kita and Kayes both during 2009 (172.1 μg/kg) and 2010 (44.8 μg/kg) were recorded in our present study (Figs. 2 and 3). This could be attributed to the fact that Kolokani is a drought prone area with an annual rainfall of 700 mm. Further, this situation could have been exacerbated by intermittent drought spells during the crop season. Infection by *A. flavus* and aflatoxin concentration in groundnut can be related to soil moisture stress during pod-filling when soil temperatures are near optimal for fungus growth (Craufurd et al., 2006).

Our results also highlighted the prevalence and distribution of AFB1 in granaries above acceptable limits. This implies that farmers in Mali would not be able to access high value markets in developed countries including Europe, Japan and the United States without significant improvement in storage practices. The high prevalence of AFB1 in granaries in the present study highlights the food safety risks faced by the rural poor in Mali and the need to adopt improved storage practices. Similar trends in post-harvest aflatoxin contamination in Malawi, East Africa was noticed from pod samples collected from homesteads, local markets, warehouses and shops. The AFB1 content in samples was 21% in 2008 and 8% in 2009

Table 1
Pre-harvest groundnut aflatoxin contamination in different districts of Mali, West Africa as estimated at harvest during the years 2009 and 2010.[a]

Aflatoxin range (μg/kg)	% of groundnut samples in each category/district[b]		
	Kayes	Kita	Kolokani
0–4	41.1	36.7	34.4
5–10	26.7	2.2	15.6
11–20	10.0	6.7	5.6
21–35	3.3	11.1	8.9
36–100	8.9	24.4	12.2
101–500	10.0	18.9	17.8
>500	0.0	0.0	5.6

[a] Aflatoxins were estimated at harvest for kharif (rainy season) sown groundnuts.
[b] Values are means of two years (2009 and 2010).

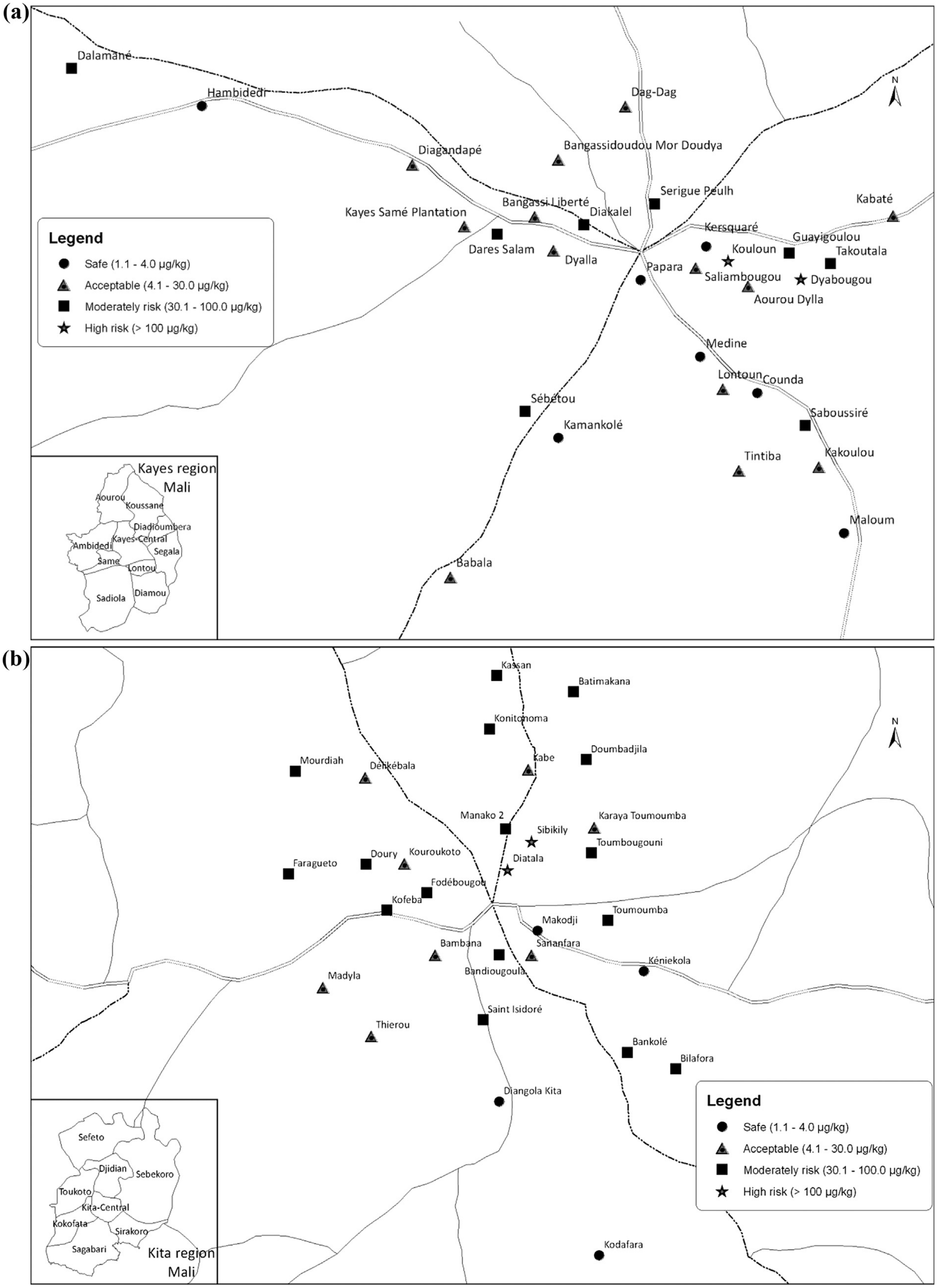

Fig. 1. (a). Mapping of different villages of Kayes, Mali based on pre-harvest aflatoxin contamination in groundnut for the years 2009 and 2010. (b). Mapping of different villages of Kita, Mali based on pre-harvest aflatoxin contamination in groundnut for the years 2009 and 2010. (c). Mapping of different villages of Kolokani, Mali based on pre-harvest aflatoxin contamination in groundnut for the years 2009 and 2010.

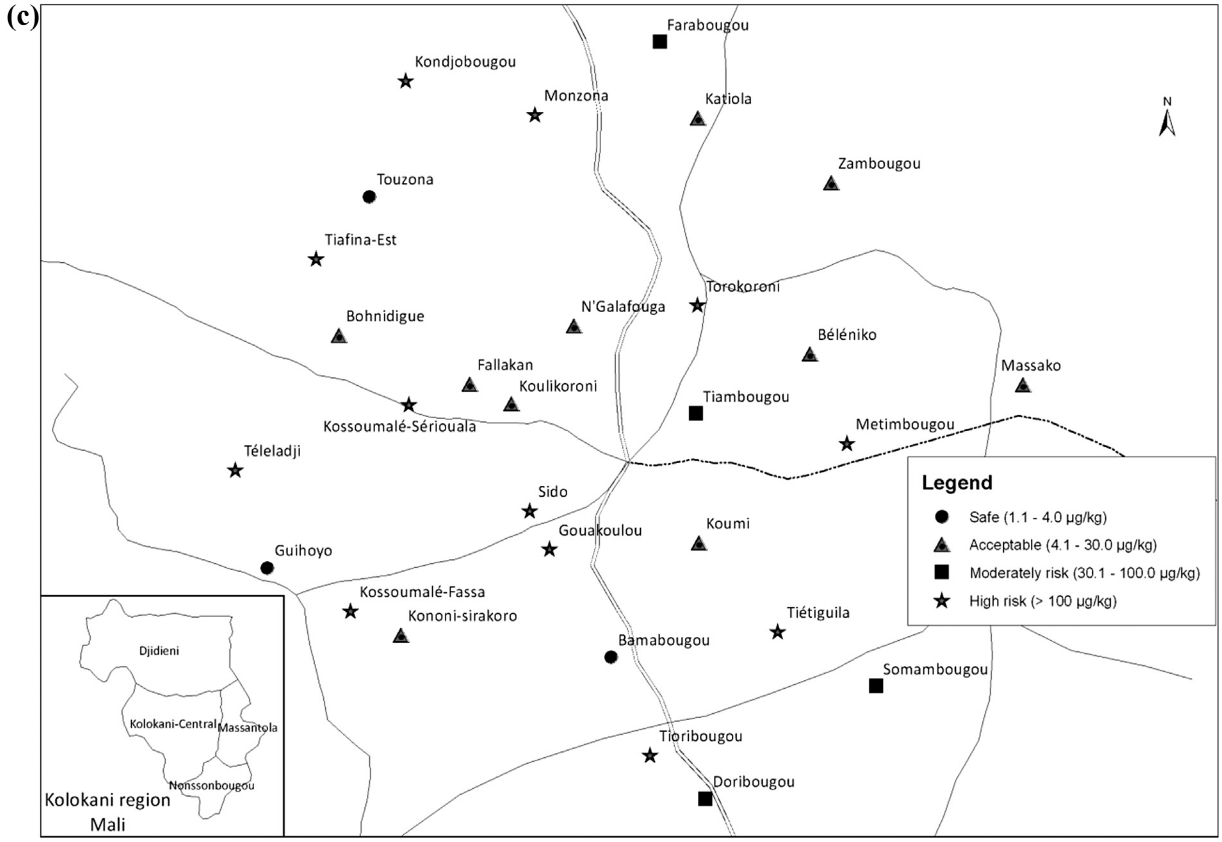

Fig. 1. (*continued*).

(Monyo et al., 2012). Aflatoxin concentration in food above a certain limit is considered hazardous and a threat to food security (Lewis et al., 2005). The safe limits of aflatoxins for human consumption in various countries range from 4 to 20 µg/kg. For India, up to 30 µg/kg is the permissible limit (FAO, 2004). However, in many developing countries including Mali, there are no AFB1 safe limits and there is lack of skills and resources to detect contaminations and enforce regulations (Williams et al., 2004; Wild, 2007; Wild and Gong, 2010).

In our present study, AFB1 accumulation in granaries can be attributed to poor storage conditions that favor the mold growth. Further, pest damage and ignorance of ideal storage methods were found to be important in increasing AFB1 contamination during storage. Since Kayes, Kita and Kolokani are the major groundnut producing districts of Mali, the phenomenal aflatoxin contamination during post-harvest storage is of concern. Since 95% of the groundnut produced in West Africa is consumed locally, this situation can pose

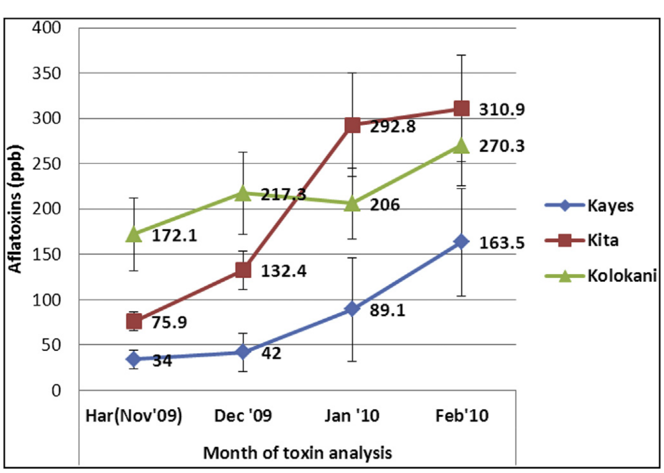

Fig. 2. Extent of post-harvest aflatoxin contamination in groundnut (2009 rainy season crop) in farmers' granaries during three months of storage from harvest in different districts of Mali, West Africa. Aflatoxins were estimated at harvest and monthly intervals using indirect ELISA. Values are means of 90 samples collected from 30 villages (3 samples/village).

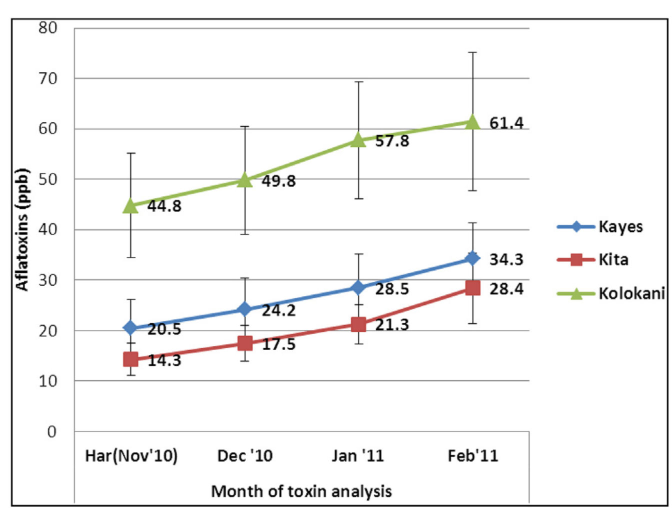

Fig. 3. Extent of post-harvest aflatoxin contamination in groundnut (2010 rainy season crop) in farmers' granaries during three months of storage from harvest in different districts of Mali, West Africa. Aflatoxins were estimated at harvest and monthly intervals using indirect ELISA. Values are means of 90 samples collected from 30 villages (3samples/village).

critical health risks to all consumers in Mali when compared to export losses. Several factors contribute to mold growth in groundnut during storage. Majorly, seed moisture and temperature will influence aflatoxin contamination (Klich, 2007; Hell and Mutegi, 2011). Other important factors that need attention in this regard are rain water seepage, moisture absorption, condensation, and insect infestation (Hell et al., 2000a, 2000b; Umeh et al., 2000; Bankole and Adebanjo, 2003; Craufurd et al., 2006; Klich, 2007; Boken et al., 2008). Thus it is possible to minimize toxin contamination to a large extent or to acceptable levels by adopting suitable production, harvest, process and storage technologies.

In our studies, Kayes is identified as relatively safe with more villages (n = 14) in the safe zone and with acceptable limits of pre-harvest aflatoxins when compared to Kita and Kolokani. More number of villages identified in the present study in the high risk zone under Kolokani district (n = 14) is of concern. Since, the selected districts are from different agro-climatic zones, devising location specific management practices to mitigate pre-harvest contamination and proper and improved storage practices are the proposed strategies for preventing aflatoxins from reaching alarming levels. Creating awareness among the rural poor in Mali on the importance of proper cultural practices at the field level and ideal storage conditions for checking mold growth is also essential. The variations observed in this study can be partly due to the way the smallholder farmers manage their crop at and/or after harvest. Due to labor constraints at the time of harvest, there is a tendency for farmers to keep the harvested crop lying in the field and thus exposing it further to invasion by *A. flavus* fungi and subsequent contamination. Sample size per village in each district could have contributed to variation in our present study. However, this variation will not negate our findings at the district level.

Improper pod drying after harvest also contributes to varying levels of aflatoxin accumulation in farmers' granaries. Bulk storage of in shell groundnuts requires a moisture content of <10% to prevent mold growth (Diener and Davis, 1977). Variations during storage at farmers' granaries can also be attributed to the possible differences in moisture content of groundnut pods prior to storage due to improper drying practices. The types of granaries are also a source of contamination if poorly ventilated and unprotected from humidity. Since collection of weather data during the crop growth period and storage from fields/villages/granaries was a difficult task, climatic conditions prevailing in the respective districts were taken into consideration in the present study. Though Kita is in the wettest zone with more mean annual rainfall (911.6 mm) than Kayes (632.9 mm) and Kolokani (700 mm), untimely and uneven distribution of rainfall might have contributed to higher pre-harvest aflatoxin levels. Similarly, edaphic factors and population dynamics of toxigenic *A. flavus* strains in farmers' fields in these three districts might also have contributed to the differences. Devising area-targeted management strategies and training farmers in both pre-and post-harvest handling of groundnuts is hence crucial for minimizing aflatoxin contamination.

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