QUANTIFICATION OF AFLATOXINS IN STORED MAIZE GRAINS FROM MUSANZE DISTRICT OF RWANDA FOR BIOSAFETY AND FOOD QUALITY ASSURANCE

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Abstract
Maize grains are cereals widely cultivated and consumed in Rwanda. Unsafe maize grains and derived products contaminated by aflatoxins are harmful and can cause cancers to consumers. The main objective of this study was to detect and quantify aflatoxins in maize found in Musanze District using ELISA testing kit (Stat Fax 4700 Microstrip Reader, 05/2012). The maize grains were randomly collected from four sectors such as Musanze, Kinigi, Cyuve and Nyange of Musanze Disrict of the Northern Province of Rwanda. These maize grains samples were analyzed for physical characteristics, total aflatoxins content and Aspergillus presence detection and prevalence. The findings indicated the presence of Aspergillus species and aflatoxins contamination of maize grains in all samples collected from four different sectors of Musanze District. The physical matters found in stored maize gains were 13.2% in Nyange, 11.1% in Musanze, 10.29% in Kinigi, 8.5% in Cyuve sectors of Musanze District; while the total aflatoxins were 14.49 ppb, 13.26 ppb, 8.085 ppb, and 8.28 ppb for samples collected in Musanze, Cyuve, Kinigi and Nyange sectors respectively. These results are above international standards of aflatoxins content in maize grains (about 20 ppb). Thus, stored maize grains were unsafe for consumption. However, the moisture content was slightly below 13% in all samples of four sectors during dry season and this met the standards set by the Rwanda Standardisation Board. Normally the moisture content collerates with Aspergillus concentration in stored maize grains and consequently influenced by the relative humidity during rain season just after post-harvesting. Aflatoxins found in maize grains may be due to the bad storage conditions during rainy season. Under favorable conditions, maize grains can be contaminated by Aspergillus species and there is a growing urgency to train farmers about safety and hygiene. In this case, it is recommended to develop a strategic plan for the prevention and reduction of aflatoxins in harvested grains.

Key words: Aflatoxin, *Aspergillus*, ELISA, ppb, maize, moisture, mycotoxin

Received: 26.07.2020 Reviewed: 04.12.2020 Accepted: 21.12.2020

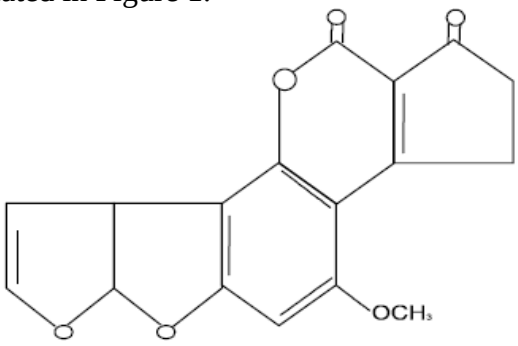
INTRODUCTION

The presence of mycotoxins in cereals and their products is harmful for human and animal health and can generate cancer after long exposure to these mycotoxins. Among mycotoxins, aflatoxins are most prevailing in food, feed grains and seeds [1, 2, 3].

Aspergillus flavus and *Aspergillus parasiticus* have been associated with cereal products stored in prohibited conditions of uncontrolled humidity during rainy season [4, 5].

Strategic plans were taken by different countries to prevent the contamination of cereals in trade with mycotoxins through food safety regulation and development of food standards. [6, 7]. In the research that have been conducted in Rwanda shows that, aflatoxin appeared to be the most dominant mycotoxin group with an overall mean of 144.04 µg/Kg occurring most frequently in maize (85%) than in other commodities leading to cancer generation [7, 8, 9].

The chemical structure of Aflatoxin B1 is as indicated in Figure 1.



B_1

Figure 1: Chemical structure of aflatoxin B_1

Aflatoxins B1 and B2 produce an intense blue fluorescence visible approximately 450 nm, when exposed to long-wavelength (365 nm) ultraviolet light. This property has been useful for developing variety of qualitative and quantitative analytical methods for aflatoxin detection [1, 9].

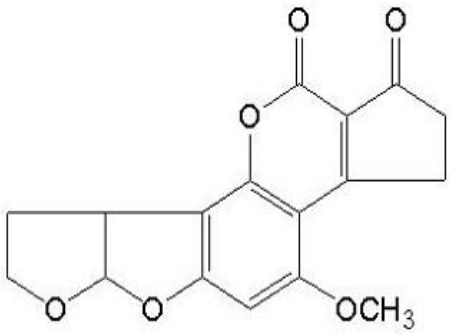
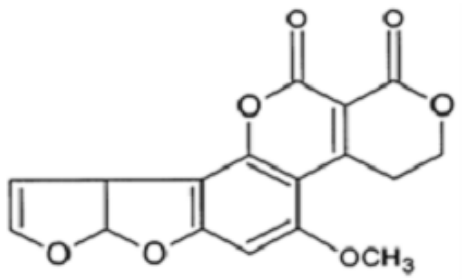


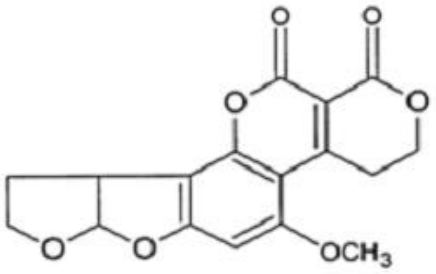
Figure 2: Chemical structure of aflatoxin B2.

The chemical structures of aflatoxin B2 is shown in figure 2. It is produced by *Aspergillus* species growing in cereals and cereal flours stored in rooms having a high relative humudity.

The presence of aflatoxin G yellow green fluorescence of the relevant structures under UV-light and aflatoxin G1 and G_2 are produced exclusively by *A. parasiticus* imply a significant risk in consumption of aflatoxins G2 is the dihydroxy derivatives of G1Aflatoxin G: produced by *Aspergillus parasiticus* G1 & G_2 in foods .They developed a method for removing the pigments from solvent extracts by treatment with insoluble basic copper carbonate. Production of abundant amounts of yellow pigment by A. *flavus* **[5, 6]**.



Aflatoxin G_1



Aflatoxin G_2

Figure 3: Structure of aflatoxins of G1 and G2

The chemical structures of aflatoxins are shown in fig.3 [6].

The distribution of aflatoxin on a maize cob or in maize grain lot is very heterogeneous with large quantities of the toxin concentrated in just a few or a small percentage of the kernels. The highest concentrations of aflatoxin usually are found on damaged kernels [8].

Problem statement

Quantifying the baseline levels of exposure and the associated burden of disease in developing countries is essential for determining the efficacy of interventions intended to reduce exposure to aflatoxins. Aflatoxin poisoning in the east African region has become an epidemic, particularly in arid and semi-arid areas. Rwanda also is one country in East African region. Chronic aflatoxin exposure can have a negative impact on health and has been associated with liver cancer, growth retardation and stunting in children, and suppression of the immune system.

In Rwanda, there is not many researches that were conducted on aflatoxin contamination in maize grains.This research on evaluation of aflatoxins level in maize grown in Musanze district was intended to fill the knowledge gap by providing data about the levels of aflatoxins and identifying the producing *Aspergillus* species.

The main objective of this study was to quantify aflatoxin and identify its producing *Aspergillus* species in maize grains found in Musanze District market.

MATERIALS AND METHODS

Sample collection

The study was conducted from four sectors including Kinigi, Nyange, Cyuve and Musanze sectors of Musanze District in Rwanda. Furthermore, samples were collected with aseptic condition. Four samples were stored in sealed bags and kept at room temperature in dark and dry place on the moisture content of 13%. Sampling was done at several points (top, middle and bottom of each sack or bag) to give a representative sample. A sample of 500 g of stored maize grains were randomly collected and weighted from each sector of Musanze District in Rwanda.

Sample preparation

The collected samples were ground in a "Romer Mill" which has the capacity to divide the sample into 2 equal portions. The sample was collected according to accepted sampling technique to obtain the representative sample maize 20 g of grounded maize were taken for each sample and putted into different conical flask according to the sample and mixed with 100 ml of 70 ml of methanol and 30 ml of distilled water solution in ground sample blend for 3 minutes at medium speed and samples was allowed to settles, then the top layer of extract was filtered through a filter paper and proceeded to clean the sample extract and 4 ml extract was applied to the glass tube [1].

Analysis of sample by using ELISA test kit

For testing aflatoxins, 13 dilution wells were used and 200 µL of conjugate was put into each dilution well after adding conjugate 100 µL of standards were added in 5 dilution wells and 100 µL of samples were added in remained 8 dilution wells and Mixed wellafter mixing 100 µLof each sample and standard were transferred into antibody-coated wells incubate for 15 minutes, after incubation antibody-coated wells were washed well with distilled water and were dried on absorbent paper towel, after drying 100 µL of substratewas added into each antibody-coated well and were incubated for 5 minutes, after incubation 100 µL of stop solution were added into each well, the result were analyzed using ELISA test with 450 nm [7, 9].

Physical parameters of maize grains

Methodology for physical parameters, 100g of samples were measured and sorted to remove foreign matter, broken grains (pest damaged, rotten and diseased, heat damaged, immature grains) and abnormal odors. Then the separated contaminants were measured in grams.

Quantification of Aspergillus

In the quantification of yeast and *Aspergillus* their methodology, 25g of sample were weighed into a sample bag and added to 225 g of phosphate Buffer saline (PBS). From the bag, 1 ml of the 10^{-1} dilution sample was aseptically transferred to a test tube containing 9 ml of PBS. It was vortexed until homogenized. This was a 10^{-2}. This step was repeated for the 3rd time to get the dilution10^{-3} [1, 6].

Once dilution were done the next steps involved spreading the microorganisms in the food sample in a petri plate so they can be counted upon incubation. To do so, 1000μL of each dilution was traneferred on each plate with the desired agar. Plates were incubated at 5°C, 25°C and 37°C in order to quantify and confirm the presence of A*spergillus.* After incubation, for the final calculation, the number of counted colonies is to be multiplied by the reciprocal or the dilution factor [9].

Statistical analysis of data

Data were statistically analyzed using the Excel software for graphics and tables making.

RESULTS AND DISCUSSION

The results of Aspergillus species growth on culture media in Petri dishes are presented in Figure 4 below:

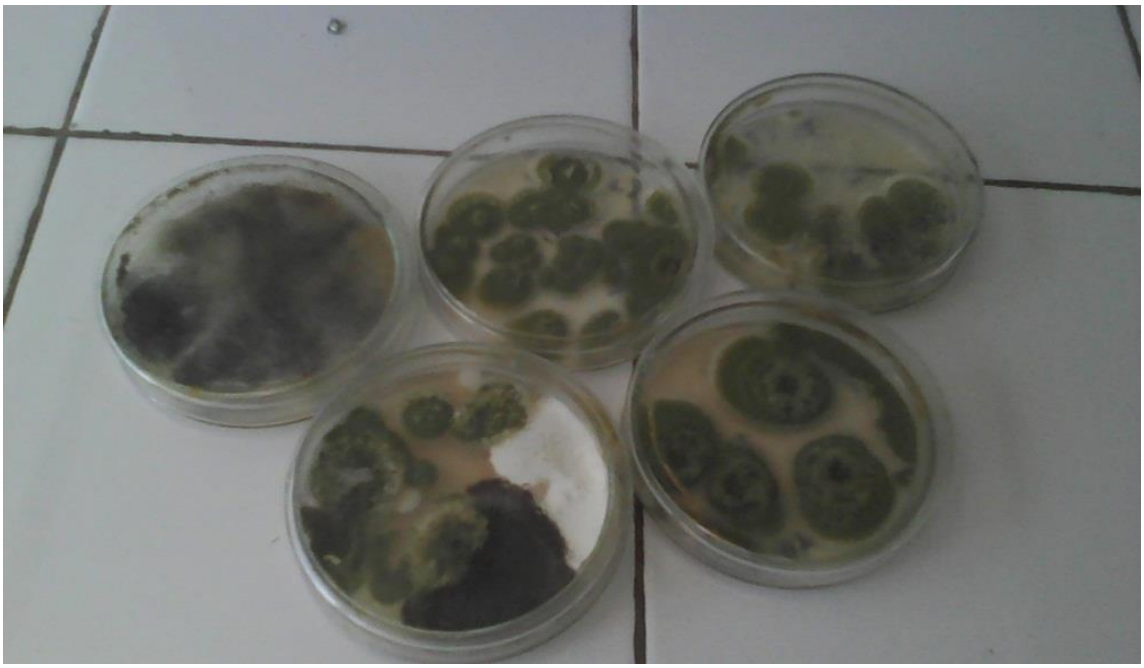


Figure 4: Colony of yeast grown on CYA medium set at 25ºC in incubator

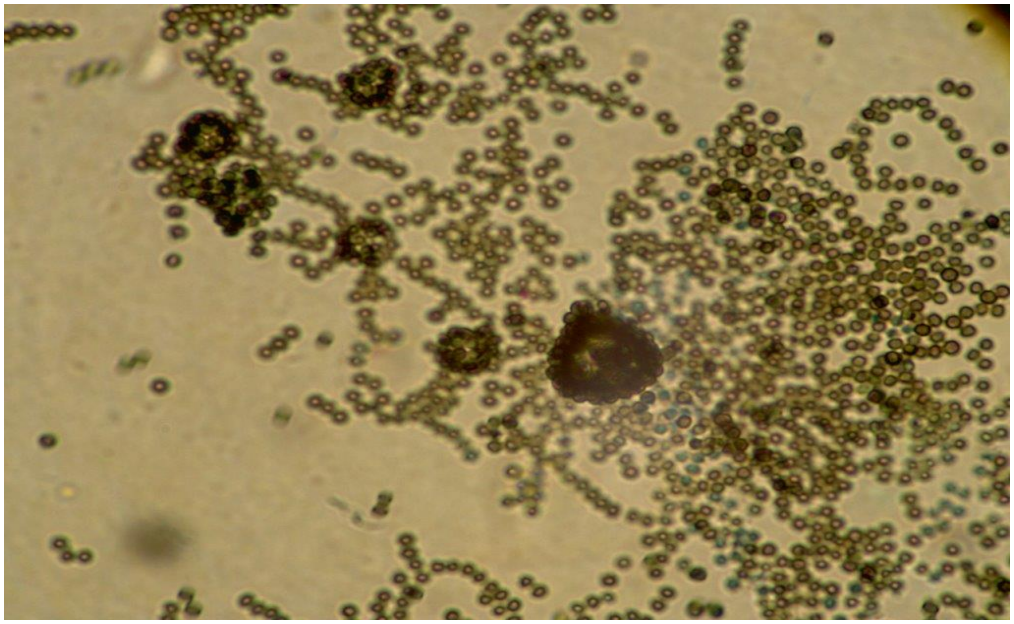


Figure 5: Microscopic view of Aspergillus species showing conidiospores

According to Figure 4, the colonies forming units (CFU/gram) of *Aspergillus* were counted and microscopically examined in order to identify conidiospores of Aspergillus species as presented in below Figure 5:

According to Figure 5, the conidiospores scrutinised and found that they should be generated by *Aspergillus species* responsible for aflatoxins found in stored maize grains of four sectors. Normally the aspergillus multiplication in maize grains are affected by the moisture content and relative humidity found in store. The results of analysed sampled maize grains are presented in below Figure 6:

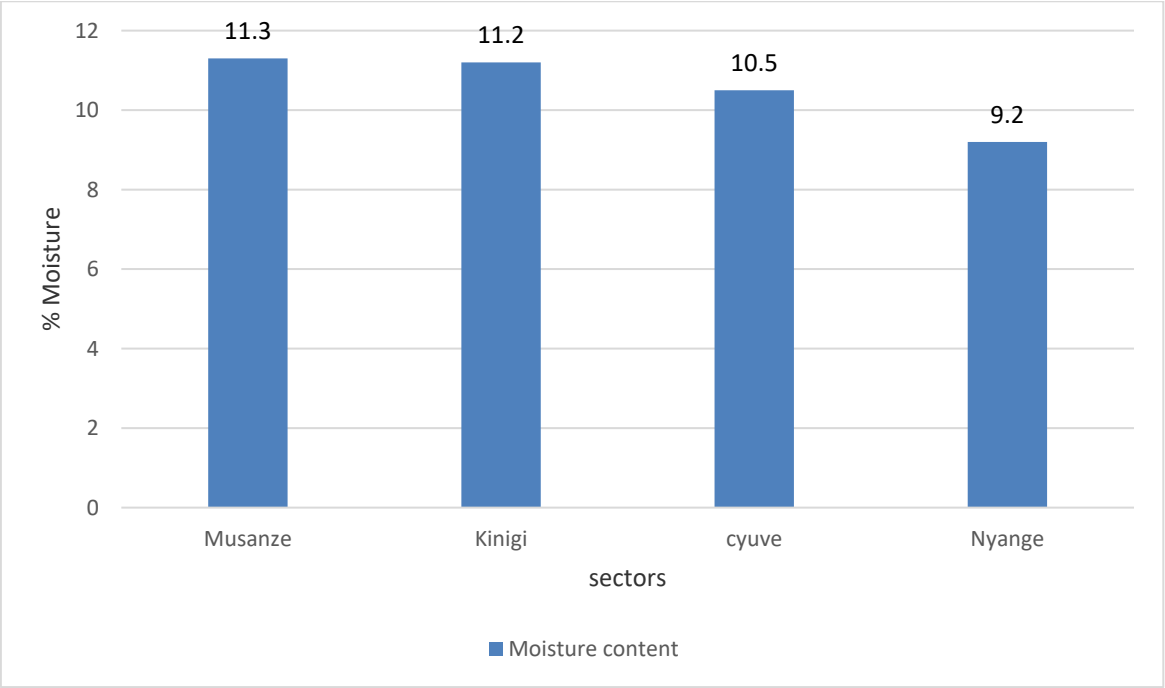


Figure 6: Moisture content determination in maize grains of four sectors

The Figure 6 shows the moisture content measured in samples collected in four sectors of Musanze District whereas the moisture content can vary according to relative humidity of the storage atmosphere. According to Table 1, physical parameters included the total defects of maize grains were: 10.29% for Kinigi, 13.2% for Nyange, 8.5% for Cyuve, and 11.1% for Musanze sector.

In this research work, aflatoxins content in maize grains was evaluated and the results are presented in Figure 7:

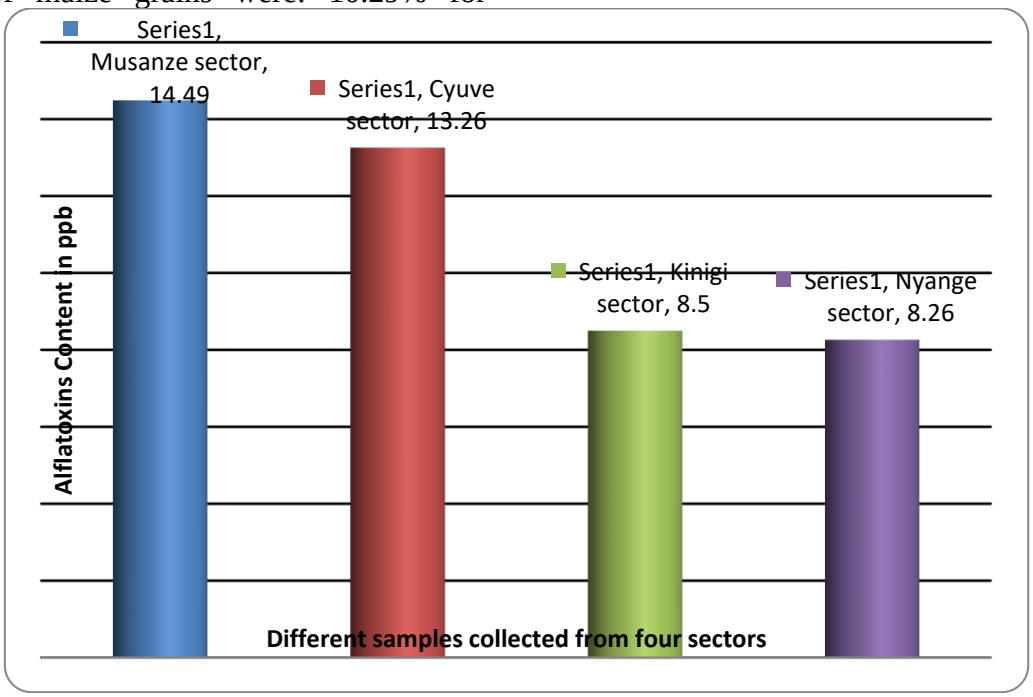


Figure 7

Figure 7 shows that the aflatoxin level is slightly high in Musanze sector flowed by Cyuve sector. The conditions of storage could be defectous in Musanze sector. Where the moisture content in stored maize grains seems to be high. Lower aflatoxin level was observed in Nyange sector maize grains where the moisture content in grains was also lower.

Concerning the enumeration of *Aspergillus species* in stored maize grains, the obtained results of detected Aspergillus species are presented in below Table 2.

These results of Table 2 indicate that Musanze sector sample had the highest colonies forming units (CFU/gram) while Nyange sector sample had the lower *Aspergillus* content in maize grains. The level of *Aspergillus flavus* can be favorised by the presence of physical matters from unappropriate harvesting technology, post-harvesting cross-contamination and prevaling relative humidity during rain season.

Table 1: Physical parameters of stored grains from four sectors of Musanze District

Physical matters	standard specs Max in %	Kinigi sector		Nyange sector		Cyuve sector		Musanze sector	
		Test Results	Decision	Test Results	Decision	Test Results	Decision	Test Results	Decision
Foreign matter	1	0.02	ok	1.6	High (+0.6)	0.4	Ok	0.1	ok
Broken grains	4	0.5	ok	2.7	ok	0.4	Ok	1.6	ok
a.Pest damaged	3	0.3	ok	2.6	ok	0.7	Ok	1.9	ok
b.Rotten and diseased	4	2.09	ok	5.6	High (+ 1.6)	1.9	Ok	3.5	ok
c.Heat damaged	1	3.5	High (+2.5)	3.8	High (+2.8)	1.6	High (+ 0.6)	2.7	high (+ 1.7)
d.Immature grains	2	4.4	High (+2.4)	1.2	ok	4.3	High (+ 2.3)	3	High (+ 1)
Total defects (a+b+c+d)	10	10.29	High (+0.29)	13.2	High (3.2)	8.5	Ok	11.1	High (+ 1.1)
Abnormal odors	Free from	normal	ok	normal	ok	normal	Ok	normal	Ok

Table 2: Enumeration of Aspergillus species from contaminated maize grains

Test	Culture medium	Culture medium and quantity used	Temperature of incubation in °C	Results in different dilution for each sample (CFU/g)
Aspergillus species	Czapek Dox Agar mix yeast extract agar	9.802 g of Czapek Dox Agar and 1grams of yeast extract agarin 200 ml of distilled water	25°C within 5 day	**Musanze:** 93 x 10^{-2}
				Cyuve: 72 x 10^{-1}
				Kinigi: 69 x 10^{-1}
				Nyange: 61 x 10^{-1}

DISCUSSION

The moisture content is below stndards (13% in stored dried cereals) *Aspergillus* species grow is relative humidity is enhanced during rainy season if the storage conditions are not respected. Thus, higher relative humidity should influence the moisture content enhancement that provides water activity for better *Aspergillus* growth and aflatoxin production in stored cereals (Trenholm *et al.*, 1998). Maize grains contamination by *Aspergillus* from rodents, insects, birds and physical matters during harvesting and storage [1,3]. In developing countries, storage conditions are not well respeted due to financial problem [4, 8].

Aflatoxin are contaminants of agricultural commodities in the field particularly in critical temperature and humidity conditions before or during harvest or because of inappropriate stora ge conditions [2, 6].

Maize grains had foreign matters, broken maize grains, and damaged grains from insects, birds and mechanical means, rotten and diseased grains, heat damaging, immature grains. Thus, Table 1 shows the physical parameters considered as total defects of maize grains assessed as follows: 10.29% for Kinigi, 13.2% for Nyange 8.5% Cyuve, and 11.1% for Musanze. Normally, these physical matters could be responsible for cereals (like stored maize grains) contamination by *Aspergillus* leading to alfatoxins production [2, 5, 8].

Aflatoxin was measured and expressed in ppb and according to Figure 4, the results show that Musanze sector had the high level of contamination with *Aspergullus* species than other three sectors. This should be due to the relative humidity influenced by climatic conditions prevaling near volcanic chain. Moisture content should affect aspergillus growth and aflatoxins production if the storage conditions are not well respected [3, 8]. According to Table 2, the enumeration of *Aspergillus species* from contaminated maize grains shows that Musanze sector has a big number of colonies forming units (CFU/gram of sampled and stored maize grains) than three remaining sectors, and this should be influenced by the moisture content of grains during storage [1, 2, 8]. Normally cereals should be stored in appropriate packaging materials in order to avoid the presence of rodents, insects and high relative humidity [4, 8].

CONCLUSION

The aims of this study were to identify and quantify aflatoxin generated by *Aspergillus species* in maize produced in Musanze district, in fact samples were taken in four sectors of Musanze district and the result show that there are two sample that have high quantity of Aflatoxin, while other two has low quantity of Aflatoxin according to the standard, it means that the farmer has not respected the postharvest technology of grains conservation.

RECOMMENDATIONS

Based on these results, the following recommendations can be emitted to stakeholders: Farmers and processors should respect harvesting and storage conditions of cereals. They should avoid the presence of foereign physical matters, insects, rodents in store of cereals. The relative humidity should be ajusted to normal conditions set by ability authority. Consumers shoud be informed about mycotoxins danger in order to avoid cancer outbreaks. The Governemet should construct storage rooms (silos), sensitize and train the farmers and processors about post-harvest technology.

ACKNOWLEDGEMENTS

Many thanks go to INES-Ruhengeri Administration for technical assistance, and supervised student, Mss Jeannine Mukakabera, a biotechnologist for his willing collaboration under the auspices of the Department of Biotechnologies at INES-Ruhengeri. Special thanks go to Reverend Father Dr. Fabien Hagenimana for financial and moral support.

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