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Evaluation of the Effects of Drying Methods on Post Aflatoxin Infection in Maize[#]

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ABSTRACT

The cultivation and the usage of maize have been significantly increased across all provinces of Rwanda. Nevertheless, the problem of aflatoxin contamination remains a major factor that renders them to be unfit for animal and human consumption. In this research, the effects of drying methods (sun drying, kitchen drying, dry shelter) post aflatoxin infection in maize were evaluated in Northern Rwanda. A randomized complete block design with four replications was used with maize variety (H628) with drying methods as the main plot. Maize samples were tested for aflatoxin using aflatest. In both seasons (2021 A and 2021 B) the aflatoxin results show that the aflatoxin infection levels were lower in maize dried with kitchen drying (1.4 ppb) compared to the samples dried with sun drying (1.6 ppb) and dry shelter (2.2). Also, it was seen that the three drying methods are good for reducing the aflatoxin infection levels lower than 10 ppb as standards limit for East African countries. The aflatoxin infection levels were found to be significant (≤10 ppb) as results of kitchen drying than sun drying and dry shelter methods. This research resulted that proper drying such as drying maize on kitchen fire (kitchen drying) produce the lower level of aflatoxin infection in maize.

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Introduction

Maize is a vital staple crop in most Sub-Saharan African countries including Rwanda producing food that is consumed by the majority of the population in different nature, the small-scale farmers focus on cultivating a lot of quantity of food crops such as wheat, sorghum and potatoes on a large quantity of land, maize is a crop which is very important not only for consumption but also for commercial purpose because Rwanda farmers use to trade them with Tanzania and Uganda (Daly *et al*, 2016). Northern province of Rwanda produces a half of maize produced in Rwanda where many of them (60%) are processed into other crucial products like maize flour, most of people used to trade them informally where 80 % of maize are commercialized in that way with in the neighboring countries. Aflatoxin contamination has resulted in lower nutritional qualities in Rwandan maize output due to a variety of variables that happened throughout before and after harvesting stage (Nishimwe *et al.*, 2019). Aflatoxin can also be controlled by appropriate drying process after harvesting with the appropriate time, insect control which may affect maize during storage after harvesting as well as protecting stores against pests by adopting biopesticides, all of these practices can help to limit aflatoxin upon post-harvest (Niyibituronsa *et al*, 2020). The aim of this study was to evaluate the best drying method which can be adopted to minimize the level of aflatoxin in maize**.**

Materials and Methods

Area for research

Experimental fields have been carried in the farm of the College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda situated in the Northern province, Musanze District, Busogo Sector at 72 km from Kigali as Capital city of Rwanda to the North-West. Latitude of 1° 33' 32" South & 29° 32' 57" of Longitude East with an elevation of 2221 m above sea level. In January, the average temperature in Busogo is 20 °C and in May it is 17 °C. The soil is relatively productive based on the overall amount of annual rainfall obtained (Meteo Rwanda, 2020).

Experimental design

During the research, the experiment was conducted for four treatment of fertilizer combination in four replications using a factorial arrangement in Randomized complete block design (RCBD) assigned at random in each replication. Treatments were arranged as T1 stands for application of only NPK 17-17-17 and DAP, T2 stands for application of only farm yard manure, T3 stands for application of combined NPK 17-17-17, DAP and farm yard manure and T4 stands (control): No fertilizer. All experimental plots were planted with one maize variety (H628) purchased from Rwanda Agriculture and animal ressources developmant Board at a rate of 25 kg/ha. The 16 experimental plots were placed in the same location within two (2) agricultural seasons which are season 2021 A and 2021 B; plots size was set out at 5 m × 5 m (25 m^2) in size per treatment and sowing the seed was done with in one day in all plots directly after field preparation where in each hole 2 grains were placed. Spacing of 75 cm between rows and 40 cm with 40 cm between holes. After sowing, 30 tons of organic fertilizer (cow dung) and 100 kgs of DAP were used respectively while urea was applied during weeding (Minagri, 2017).

Drying of maize grains

During this study, issues of maize grain management as well as their storage were examined, in order to evaluate the good drying methods which can favorize small scale farmers, cooperatives or commercial farms to get high quality maize that are free from aflatoxin. During this study, we look out the natural and artificial drying method that are currently used. Finally, different structure of storage and drying methods fluctuating from modest family units to huge commercial units are examined as well as management suggestions for minimizing harm while storing. During this study, the impact of various drying processes on aflatoxin contamination levels after various treatments were evaluated and applied during the tests.

Sun Drying

While conducting this research, sun drying method has been adopted as well-known but old drying method which is putting maize on natural sun. The maize grains produced from the treatments T1, T2, T3, T4 for all replications (4) were put directly under sun on sheeting for the prevention of moisture content from the soil ground to reach the maize corn on sheeting.

*Kitchen Drying:*In order for assessing the rate of aflatoxin, we used to adopt the kitchen drying as the other drying methods which is effective by putting maize grains above fire of the kitchen. Kimario (2021) reported that kitchen drying managed to reduce aflatoxin levels in maize at the rate of 78.5%.

Dry shelter: In this study, dry shelter as one of the drying methods established by the government as post-harvest handling technology was used to evaluate its effect on aflatoxin contamination.

Testing of aflatoxin

With referring to VICAM. LP (1999), we used aflatest (Watertown, MA) of OAC fluorometer. The aflatest was chosen due to its rapidity where with in only 10 min per ton test you get the results, it is also very sensitive consequently providing reading as low as 2 ppb to 10 ppb in a sample and continues to read the results until 50 ppb per sample; it is suitable for using it in the same extract instrumentation for other mycotoxins'test; it is easy to use thus it does not require special. Those testing tools are trusted due to less toxic materials they possess in comparison with conventional methods. This method possess in numerical data in ppb can be used to clean HPLC (High performance liquid chromatography).

The test was carried out by blending a crushed sample of the kernels weighing 25 g of blended maize grains mix with 5 g of NaCl and 125 ml of methanol (70%); 30% of distilled water (H_2O) in a blender jar at high speed for 2 minutes in covered blender jar, fluted filter paper was used to filter the extract and collect the filtrate in clean vessel (Neogen, 2018).

Sampling

According to Hamed (2016), simple random sampling method was adopted by collecting and measuring the composite sample equal to the maximum of 1 kg of from each treatment in both seasons (2021 A and 2021 B) and dividing it into 12 sub samples of roughly 300 g each in each season. For the aflatoxin levels analysis, ground samples of roughly 25 g were utilized for each sub sample and the average level was obtained, the sample collection was done with second -season maize which tend to dry out faster on the farm, the average moisture level in each treatment was 12.5%.

Within the 2 seasons of the study, total number of samples collected and tested were twenty-four (24) with combination produce of each treatment (T1, T2, T3, T4) of all replication. In accordance with the protocol used during sampling, samples were collected in polythene bags and transported them to the RSB Laboratory for aflatoxin testing.

Preparation of the sample

For avoiding the mold formation as well as aflatoxin production, maize grains were dried in order reach the moisture content of no more than 13% within 30 days, when maize are affected by aflatoxin there is a chance of getting pollution and the species causing aflatoxin causes those pollution.

According to Noreddine (2020), to address this problem, the entire 1 kg of the sample was grinded and segmented using only a Bunn and constantly fighting grinder (Mann: Bunn-o-Matic corporation Springfield, Illinois in the United States of America) in order to obtain a combined subsample for representing the overall sample characterized by codes associated with each sub sample to facilitate the identification.

Sample extraction

During sample extraction ground sample of 25 g weight was used, blended for 2 minutes at high speed with the blender covered with 125 ml of 70% ethanol and 5 g of NaCl. after filtering the extract with fluted filter paper and then added 30 % of distilled water, in clean vessel the filtrate was collected (Neogen, 2018).

Dilution of extract

15 ml of filtered was collected using a pipette and transferred to a clean jar where it has been watered by 30 ml of distilled H_2O then thoroughly mixed, a glass microfiber glass filter was used to filter the diluted extract into a glass syringe barrel labeled with 15 ml marking.

Aflatoxin's extraction

During the extraction of aflatoxin, 50 mg of crushed samples was mixed with 250 ml of 65% ethanol (v/v) in a laboratory mixer (IKA.Werker, Germany) and rapidly shaken for 3 minute material was filtering using Whatman No.1 filter paper once it had settled (Whatman International Ltd, Maidstone, UK) (Neogen, 2018).

Quantification of aflatoxin

The samples were analyzed by using an enzyme-linked immunosorbent assay (ELISA) to quantify the total of aflatoxin as indicated by the company's procedures using a Reveal accuscan (Neogen,USA), the ELISA has a quantitative range of 2-150 g total aflatoxins/kg and a lower of detection (LOD) of 2 g/kg (Neogen, 2018).

Statistical analysis

The levels of aflatoxin of four treatments were analyzed in the years of research from 2020-2021. simple descriptive statistics and the SPSS statistical package from 1993 at p=0.05 was used to evaluate data such as mean, range, variance, coefficient of variation and least significant difference. The test statistic was the variance ratio test, the 'F' distribution was used to compare two growth seasons at the same time for significant variations, the selection of it had the advantages of comparing two environments in one period (Mohd and Fadilah, 2017).

Results and Discussion

The results of aflatoxin following the drying methods have shown the great impact on aflatoxin contamination as shown in the Table 1.

The analysis of variance results shows the significant differences in aflatoxin contamination levels within in the drying procedures such as kitchen drying, and drying in two seasons of the study because with a significant level of 0.05, F=17.22 is bigger than F tabulated which is 3.28 meaning that the alternative hypothesis is accepted and null hypothesis is rejected. The results from the same analysis of variance also shows that The effect of drying method on aflatoxin contamination was found to be significant in T2 and T4 groups means in the group of organic fertilizer application and control. between blocks across the two seasons of study which are 2021 A and 2021 B because with F calculated which is equal to 0.67 is less than F tabulated equal to 2.9 at 0.05 as significance level, so, the conjecture hypothesis was accepted and the research hypothesis was rejected. The difference in aflatoxin infection was examined in both season (2021 A and 2021 B) from some treatments following drying methods (dry shelter, sun drying, kitchen drying).

Nethertheless, the level of aflatoxin was found to have significant difference contamination in sun drying methods, kitchen drying and drying shelter methods as it is shown in Table 1. The lower aflatoxin infection levels were seen when using kitchen drying (1.6 ppb) when the maize is grown with fertilization of mixture of organic and inorganic fertilizers compared to sun drying (1.6 ppb) and dry shelter drying method (2.2 ppb).

The inconsistency ratio test demonstrated a significant difference in the influence of methods of drying in all treatments (T1, T2, T3, T4) on aflatoxin contamination in the seasons 2021 A and 2021 B. The result shows that treatment T4 the aflatoxin levels were 26.70 ppb when adopted the sun drying, whereas aflatoxin results for T3 was 1.40 when adopted the kitchen drying.this means that kitchen drying methods reduces the rate of aflatoxin infection in corn in the case organic and inorganic fertilization was conducted.

According to Magembe *et al* (2016), right drying of harvested maize is very crucial, because the ineffective drying methods can increase the growth of fungus as well as reducing the quality of kernels for consumption and reduce the capacity of seeds to germinate for the next season, during harvesting, maize possess the higher moisture content with equivalent to 28-30% and have to be tried up to 12% to prevent fungal growth. According to Zuki et al. (2018), the types of drying methods have great influence on the resistance of maize on fungal contamination which led to the aflatoxin contamination. This is coincided with the actual research assessing the impact of different drying methods currently used in Rwanda on aflatoxin attack on maize, this research has been carried out from the findings of these research mentioned above that both sun drying, kitchen drying, drying shelter drying were good for minimizing the content of moisture of maize to the level which is recommended (≤12%) to get the chances of reducing the intense aflatoxin attack on the kernels. However, kitchen drying method was found to be quick to reduce moisture content of the kernels compared to the sun drying and dry shelter drying methods. This finding was due to the direct exposure of the maize grains above the kitchen fire with short distance from the fire itself and the space where the kernels are put for drying.

Table 1. Aflatoxin levels measured in every treatment resulting all methods of drying within cropping season 2021 A & 2021 B

	Results of in ppb of total aflatoxin in various drying techniques for all treatments					
	Kitchen drying		Dry shelter		Sun drying	
Treatments/Seasons	2021 A	2021 B	2021 A	2021 B	2021 A	2021 B
T1	2.70[ab]	1.60[ab]	2.70[ab]	3.20[ab]	1.70[ab]	2.90[ab]
T2	3.70[a]	4.90[ab]	3.00[ab]	5.0[ab]	2.60[ab]	1.80[ab]
T3	1.40[ab]	2.60[ab]	2.20[ab]	3.20[ab]	1.60[ab]	2.80[ab]
T4	11.00[b]	13.00[b]	10.5[b]	11.7[b]	25.50[a]	26.70[a]
LSD	8.119	4.531	3.453	6.207	10.319	10.612

T1 stands for inorganic fertilizer application, T2 stands for organic fertilizer application, T3 stands for mixture of inorganic and organic fertilizer application, T4 stands for control (no fertilizer application and LSD stands for Least significant difference

Conclusion

The results of the assessment of different drying methods are rather obvious (and confirm previous studies), namely (a) the sun drying (b) sun drying (c) kitchen drying are more effective in reducing aflatoxin contamination of maize. However, the implementation of those good postharvest handling practices (appropriate drying method) requires a close monitoring at the farmer level. It may be interesting to research the constraints by adopting such practices (when farmers are knowledgeable about the problem).The results shown that drying methods have the significant effect on aflatoxin infection, among three (3) drying methods used which are sun drying, kitchen drying and dry shelter, kitchen drying method is the best for reducing aflatoxin contamination in maize.

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