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Evaluation of the Effects of Fertilization and Drying Methods on Pre and Post Aflatoxin Infection in Maize under Busogo Climatic Conditions[#]

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ABSTRACT

The research was conducted in the years of 2020-2021 during the growing seasons such as 2021A started from September 2020 till the end of January 2021 and 2021 B started from February till end of June 2021, in Busogo sector, Musanze District, Northern Province. The purpose of this project was to assess the impact of fertilization and drying methods such as sun drying, dry shelter and kitchen drying on aflatoxin contamination in maize before and after harvesting. The area from which this project was conducted was chosen due to the availability of a lot of number of maize growers who cultivate them not only for consumption but also for trade purpose either processed or non-processed. During this study, a total of twenty-four samples were collected, examined and evaluated for aflatoxin levels in both seasons. In the season 2021A and 2021B sample collection equivalent to twelve in each season was done then dried them within 30 days by using different drying methods such as dry shelter, kitchen drying, sun drying. Randomized Complete Block Design (RCBD) with four treatment of fertilizer combination in four replications was used in this study and all experimental plots were planted with one maize variety (H628) purchased from Rwanda Agriculture and Animal Resources Development Board. Except samples from maize cultivated without fertilizer, level of aflatoxin infection was found to be higher 10 Parts per billion. The East African countries community standard limit of 10 parts per billion which indicate a health risk to the consumer, when comparing the three drying methods employed in this study, the kitchen drying method produced lower levels of aflatoxin (1.6 parts per billion) than the dry shelter (3.2 parts per billion) and sun drying methods (2.9 parts per billion), samples taken from the control plot (T4) stands for no fertilizer application resulted greater levels of aflatoxin with an average of 25.75 prts per billion compared to other treatments which yielded aflatoxin levels of at lower than 10 parts per billion. The use of kitchen drying can be recommended as well as mixture of both organic and inorganic fertilizers when growing maize in order to reduce the level of aflatoxin.

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Introduction

Agriculture sector in Rwanda remains the major sector of the economy where 64.7% of the Rwandan population is involved in Agriculture and it contributed 26% in the GDP of the country as found in National institute of statistics of Rwanda (NISR) Labor Force Survey Trends, May 2021 report, September 2021 (NISR, 2021). However, this sector faces challenges such as an ever-changing climate a concentrate on relatively brief initiatives to solve immediate and starting to emerge challenges including the soil infertility and reduced nutrient due to leaching caused by high density of the population and inappropriate harvesting a well as to remove all debris from the field after harvesting, poor information sharing channels of the agricultural related concerns, non-developed research due to narrow findings and limited variety of crops (MINAGRI, 2017).

Maize is a vital staple crop in most Sub-Saharan African countries 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,) Eastern 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).

For the purpose of helping small scale farmers to produce maize which complies with maximum limit of aflatoxin, the Rwandan government launched Rwanda Agriculture and Animal Resources development Board which helps in Aflatoxin reduction relies on good agricultural methods that help increase production and productivity. Use of resistant cultivars, timely planting, effective fertilizer use, effective control of weeds, use of appropriate pest control, drought and mitigation of nutritional issues as well as harvesting on time are all examples of these agricultural practices. Aflatoxin can also be controlled by appropriate drying 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 mycotoxins upon post – harvest (Niyibituronsa et al, 2020). In 2019, 87 % of the population eaten maize of various types within two weeks of the poll maize was consumed at a rate of 10.8% of total food consumed in Ghana in 1987 with the remaining foods consumed at a rate of 10.3 % by all groups (Wahab et al., 2022).

Maize can also be used to make, baked goods, silage, animal feed, adhesives, and oil, fungus that contaminate maize in storage are able to cause mycotoxins that are dangerous to human and animal health, Aspergillus flavus is found to be the essential fungus of preharvest maize which cause aflatoxin. The mentioned issues favorize to maize to get unfavorable for use and also contribute to the reduction of global economy, he also reported that in Rwanda, aflatoxin found to be the serious health issues in agricultural sector (Nishimwe et al, 2019),the aim of this study was to evaluate the effects of fertilization and drying methods on aflatoxin infection in maize before and after harversting.

Materials and Methods

Study area

Experiments 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 2,221 m above sea level. The annual temperature in Busogo is 17°C- 20°C. The soil is relatively productive based on the overall amount of annual rainfall obtained (Meteo Rwanda, 2020).

Experimental Design

During the research, the experiments were 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 (NPK 17-17-17 + DAP), T2 (Farm yard manure), T3 (NPK 17-17-17 + DAP + Farm yard manure) and T4 (control; no fertilizer application). All experimental plots were planted with one maize variety (H628) purchased from RAB at a rate of 30 kg/ha. The 16 experimental plots were placed in the same location within two (2) agricultural seasons which are season 2021A and 2021 B; plots size was set out at 5 m × 5 m (25 m2) 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.

Collecting data

Data were collected at vegetative stage in the field and at the harvest plant emergence, plant height, leaf area index, stem diameter, plant vigor, harvested plant (number), weight and yield metrics are among the data collected.

Crop emergence: Seeds were sown so that each bed possessed twenty four plant holes, during data collection of plant emergence, we counted newly emerging plants emerged at 30 days after sowing

Height of plant (cm): For measuring plant height, the interval from the surface to the collar of developed upper leaf (three months after sowing) was measured using a meter rule. Centered plants in each bed were selected to collect data three couples were selected randomly means that 6 plants per block were selected. plants in each bed

Diameter of stem (cm): Data on stem diameter were collected by measuring the circumference (c) at two thirds (2/3) of plant height during tasseling (2.5 months after planting) and approximated diameter of stem (d) by means of the formula herewith below

$$d = c/\pi$$

Note that $\pi \approx 3.14$ and c = relates to the diameter of the maize stem; the technique for selecting plants to measure stem diameter was the same as for plant height sampling(Longfei et al 2020),

Leaf Area Index (LAI): During calculation of leaf area index, we used to divide the plant into three equal quadrants lengthwise, using mature leaves on their middle side.

LAI=LWK, Note that L stands for length of the leaves while W stands for the width of the leaves, K is considered as the constant which has the value of 0.75. The way LAI was done is the same way plant height selection was done. Sample selection of the plant used to calculate LAI was done on the harvestable plants by looking on their average per bed and per treatment (Rahul et al, 2021).

Plant vigor plant: The vigor of plants was measured by using three point-point scale where poor vigor was characterized by 1, average vigor was characterized by 2, good vigor was characterized by 3. Stem size, the number of stems bore without holes I considered all leaved including damaged ones when categorizing maize stems. The average weight (kg) of collected cobs was determined using a scale balance upon harvest. For the weight of the maize cobs, three samples were taken per plot. The average weight as sample was recorded for each treatment per block (Longfei et al, 2020).

Drying of maize grains

During this study, we examined the issues of maize grain management as well as their storage, 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, we evaluated the impact of various drying processes on aflatoxin attack levels after various treatments were applied during the tests.

Natural drying

Sun Drying: While conducting this research, we adopted well known but old drying method which is putting maize on natural sun (sun drying). The maize grains produced from the treatments T1, T2, T3, T4 for all replications (4) were put directly under sun on sheeting on the average temperature of 21^{o}c for the prevention of moisture content from the soil ground to reach the maize grains.

Artificial Drying

*Kitchen Drying :*In order for assessing the rate of aflatoxin, we used to adopt the kitchen drying at the average temperature of 28 ^{o}c 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 which drying maize on sheltered infrastructure that allow the air to enter the drying dacility 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. L P (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 parts per billion to 10 parts per billion in a sample and continues to read the results until 50 parts per billion 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 Parts per billion 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 5g of Na Cl 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 as it is shown in the appendix 7. Fluted filter paper was used to filter the extract and collect the filtrate in clean vessel (Neogen, 2018).

Picture 1. Sun drying

Picture 2. Kitchen drying

Picture 3: Image of dry shelter

Sampling

According to Hamed (2016), we adopted the simple random sampling method by collecting and measuring the composite sample equal to the maximum of 1 kg of from each treatment in both seasons (2021A and 2021B) and dividing it into 12 sub samples of roughly 300 g each in each season. For the aflatoxin levels analysis, ground samples of roughly 25g 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 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 Rwanda standard board 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,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 with codes associated with them to affiliate the items in the questionnaire (Noreddine ,2020).

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 min 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 2g/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=0.005 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 ,2017)

Results and Discussion

Impact for methods of drying on aflatoxin infection for the Cropping season 2021A & 2021B

The results of aflatoxin levels following the drying methods have shown the great impact on aflatoxin contamination, 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 3.the lower aflatoxin infection levels were seen when using kitchen drying (1.6Parts per billion) when the maize is grown with fertilization of mixture of organic and inorganic fertilizers compared to sun drying (1.6 parts per billion) and dry shelter drying method (2.2 parts per billion).

The inconsistency ratio test demonstrated a significant difference in the influence of methods of drying all treatments (T1, T2, T3, T4) on aflatoxin contamination in the seasons 2021A and 2021B. The result shows that treatment T4 the aflatoxin levels were 26.70 Parts per billion when adopted the sun drying, whereas aflatoxin results for T3 was 1.4 when adopted the kitchen drying (Table 1).

Impact of fertilizer application on aflatoxin infection before harvesting in season 2021A and season 2021B.

The aflatoxin contamination shown a significant difference between four treatments which are Growing maize with only inorganic fertilizer application(T1), Organic fertilizer (T2), mixture of organic and inorganic (T3) and growing maize without fertilizer application (T4).

The lower level of aflatoxin infection was found in the treatment (T3) stands for mixing the inorganic and organic fertilizer application by 5.2 parts per billion compared with non-fertilizer application (T4) by 13.4 parts per billion, organic fertilizer application (T2) by 8 parts per billion, inorganic fertilizer application (T1) by 5.7 parts per billion of the maize grown in season 2021A.

The lower-level aflatoxin infection was found in the mixture of organic and inorganic fertilizer application (T3) by 6.1parts per billion compared with non-fertilizer application(T4) by 14.7, organic fertilizer application(T2) by 8 parts per billion, inorganic fertilizer application (T1) by 6.2 parts per billion, of the maize grown in season 2021B.

Aflatoxin levels was found to be influenced by fertilizer application across the seasons of study, maize grown in T4 (Control) increased aflatoxin levels at the higher than standard limit 10 Parts per billion, whereas maize grown with a combination of organic and inorganic fertilizers (T3) reduced the level of aflatoxin contamination before harvesting at 5.2 parts per billion, fertilizers application while growing maize have great impact on aflatoxin levels on maize. According to Gary et al (2018) aflatoxin levels in the farms where fertilizers were not applied raised the aflatoxin levels at the range between 34.19-62.05% after storage. again, fertilizer called yaralegum combined with green Ok influenced the reduction of aflatoxin contamination at the range of 37.40%-18.72% in the field before harvesting. The research was done following the findings from the authors mentioned above by assessing the effects that can be caused by Application of only organic fertilizers, inorganic fertilizers or mixture of organic and inorganic fertilizers as well as growing the without any fertilizer application. (Table 2).

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

		Results of in Parts per billion of total aflatoxin in various drying techniques for all treatments					
Treatment	Treatment description	Kitchen drying		Dry shelter		Sun drying	
		2021A	2021B	2021A	2021B	2021A	2021B
T1	NPK +DAP	2.70[ab]	1.60[ab]	2.70[ab]	3.20[ab]	1.70[ab]	2.90[ab]
T2	FYM	3.70[a]	4.90[ab]	3.00[ab]	5.0[ab]	2.60[ab]	1.80[ab]
T3	NPK+DAP+FYM	1.40[ab]	2.60[ab]	2.2[ab]	3.20[ab]	1.60[ab]	2.80[ab]
T4	No fertilizer	11.0[ab]	13.0[b]	10.5[b]	11.7[b]	25.50[b]	26.7[b]
LSD	Least sign. difference	8.119	4.53	3.45	6.20	10.31	10.61

Table 2. Impact of fertilizer application on aflatoxin infection before harvesting in cropping season 2021A & 2021B

Season	Aflatoxin results (Parts per billion)				
	Treatments				LSD
	T1	T2	T3	T4	
2021A	5.70[a]	8.0[a]	5.20[a]	13.40[a]	39.8
2021B	6.20[ab]	8.0[ab]	6.10[ab]	14.70[b]	3.54
Mean	5.95	8	5.65	14.5	21.67

T1:NPK + DAP,T2:Farm yard manure,T3:NPK +DAP+FYM,T4:Control; LSD:Least significance difference

Conclusion

Application of fertilizer to combat aflatoxin attack in maize

Aflatoxin was identified in detectable concentrations in maize plots grown without fertilizer. There had been a significant difference in aflatoxin levels as a result of fertilizer between T4 (control) and other treatments, growing maize without fertilization resulted the significant increase in aflatoxin levels. As a result, from soil nutrient, the usage of a mix of organic and inorganic fertilizers was found to be helpful in lowering pre-harvest aflatoxin contamination. Combination of both organic and inorganic fertilizers during maize cultivation demonstrated to reduce aflatoxin in the field before harvesting compared to other treatments used during the study.

Effects of fertilizer and farm management on aflatoxin contamination development and yield metrics.

Aflasafe is achieved through the use of toxigenic strains of *Aspergillus spp*, which work by competitively excluding toxigenic strains from the substrate, only aflatoxins produced on the farm can be avoided by employing strategies such as effective farming systems and the use of aflasafe. Natural infection can only be regulated after the fact by using post-harvest application processing procedures that limit further infiltration into grains.

The results also shown that fertilizing maize with both organic and inorganic fertilizers is helpful to the plant maize to enhance growth and development which lead to the reduction of aflatoxin contamination in maize.

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