# CONTRIBUTION OF PUSH-PULL CROPPING SYSTEM TO MANAGEMENT OF EAR ROTS AND MYCOTOXIN CONTAMINATION IN MAIZE IN WESTERN KENYA

NANCY KARIMI NJERU

(BSc. Microbiology & Biotechnology, MSc. Microbiology, University of Nairobi)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CROP PROTECTION

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2020

## DECLARATION

This thesis is my original work and has not been submitted for the award of a degree at this or any other University.

Nancy Karimi Njeru ---------- Date 27th July 2020

**This thesis has been submitted with our approval as the supervisors**

Prof. James W. Muthomi ---------- Date 28th July 2020

Department of Plant Science and Crop Protection

University of Nairobi

Dr. Maina J. Wagacha ---------- Date 28th July 2020

School of Biological Sciences

University of Nairobi

Prof. Zeyaur R. Khan ---------- Date 27 July 2020

International Centre for Insect Physiology and Ecology (*icipe*)

Dr. Charles A. O. Midega ---------- Date 27th July 2020

International Centre for Insect Physiology and Ecology (*icipe*)

## DECLARATION OF ORIGINALITY

Name of student Nancy Karimi Njeru

Registration number A80/52476/2017

College College of Agriculture and Vetrinary Sciences (CAVS)

Faculty/School/Institute Faculty of Agriculture

Department Department of Plant Science and Crop Protection

Course name PhD Crop Protection

Title of work Contribution of push-pull cropping system to management of ear rots and mycotoxin contamination in maize in western Kenya

**Declaration**

1. I understand what plagiarism is and I am aware of the University's policy.

2. I declare that this thesis is my original work and has not been submitted for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

3. I have not sought or used the services of any professional agencies to produce this work.

4. I have not allowed and shall not allow anyone to copy my work with the intention of passing it off as his or her own work.

5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature ……

Date 27th July 2020…….....

## DEDICATION

I dedicate this thesis to my husband John, my parents Dominic and Tarcisia, my siblings Muthoni, Wangari, Dancun, Njoki, Lilian and Murugi and grandmother Susan for moral support. Lastly and most importantly, I dedicate this thesis to my son Spencer Liam for coming to my life at this time when I just finished the PhD experiments.

## ACKNOWLEDGEMENT

I am grateful to my International Centre for Insect Physiology and Ecology (icipe) supervisors: Prof. Zeyaur R. Khan and Dr. Charles A.O. Midega, first for entrusting this project work to me and secondly for financial, moral and intellectual support during the whole research period. I also acknowledge my University of Nairobi supervisors: Prof. James W. Muthomi and Dr. John M. Wagacha for the training, advice, mentorship and guidance throughout the project. I acknowledge African Regional Postgraduate Programme in Insect Science (ARPPIS) through icipe for funding this project and awarding me a scholarship.

I would also like to acknowledge the technical assistance of Chrispine Onyango and other technologist from push-pull program, icipe – Mbita. I also acknowledge the assistance from the icipe field-based staff: Dickens Nyagol, Isaac Onyango, Joshua Owino, Rodgers Oluoch, Tom Odero, Martin Ogola, Kennedy Anyango and push-pull farmer teachers: Lilian Auma and John Otiep. I would like to acknowledge the cooperation of the farmers who allowed me to collect field data and samples for laboratory analyses from their farms for the three cropping seasons. Finally, I acknowledge the logistical assistance offered by my cousin Samuel Muriithi.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| µl | Microliters |
| ANOVA | Analysis of Variance |
| AEZ | Agro-ecological Zones |
| CAN | Calcium ammonium nitrate |
| CAST | Council for Agricultural Science and Technology |
| CFU/g | Colony Forming Units per gram |
| cm | Centimeters |
| DAP | Diammonium phosphate |
| DON | Deoxynivalenol |
| EC | European Commission |
| ELISA | Enzyme Linked Immuno-Sorbent assay |
| FAO | Food and Agriculture Organization of the United Nations |
| FDA | Food and Drug Administration |
| FYM | Farmyard manure |
| GLC | Gas Layer Chromatography |
| $H_2SO_4$ | Sulphuric Acid |
| HPLC | High-performance Liquid Chromatography |
| HPTLC | High-performance Thin Layer Chromatography |
| icipe | International Centre of Insect Physiology and Ecology |
| IITA | International Institute of Tropical Agriculture |
| ITOC | icipe Thomas Odhiambo Campus |
| JECFA | Joint Expert Committee for Food Additives (UN Food and Agriculture Organization and World Health Organization) |
| KCl | Potassium chloride |
| KEBS | Kenya Bureau of Standards |
| KG | Kilogram |
| $KH_2PO_4$ | Potassium di-hydrogen phosphate |
| $KNO_3$ | Potassium nitrate |
| LM | Lower Midland |
| MG | Milligram |
| $MgSO_4$ | Magnesium sulphate |
| mm | millimeters |
| NAFIS | National farmer information service |
| OD | Optical density |
| PDA | Potato Dextrose agar |
| PBS | Phosphate Buffer |
| SNA | Synthetic Nutrient Agar |
| UK | United Kingdom |
| UM | Upper Midland |

| | |
|---|---|
| UNDP | United Nations Development Programme |
| UV | Ultra-violet |
| WHO | World Health Organization |
| ZEA | Zearalenone |

## GENERAL ABSTRACT

Push-pull is a cereal cropping system that has recently been reported to reduce incidences of ear rots and mycotoxins in maize. However, the effectiveness and mechanism involved is not yet understood. In the current study, the (i) socio-economic and agronomic factors associated with the occurrence of ear rots and contamination of maize with mycotoxins in different cropping systems in western Kenya, (ii) impact of insect management under push-pull cropping system in managing aflatoxin and fumonisins, (iii) role of soil health improvement under push-pull on the population of mycotoxin-producing fungi and (iv) effect of desmodium roots exudates on mycotoxin producing fungi of maize were determined.

A household survey covering 116 farmers who practiced push-pull and 139 farmers practicing other cropping systems was conducted in five counties of western Kenya. At least 10 maize ears were sampled per farm during harvest and analyzed for ear rot fungal pathogens, aflatoxin and fumonisins. Sixty push-pull farms, each with a neighboring control farm were examined for damage due to stem borer, fall armyworm, ear rots, ear rot fungal pathogens, aflatoxin and fumonisin levels. Soil was sampled and analyzed for mycotoxigenic fungi and nutrient content at planting, flowering and at harvest. Dried desmodium roots exudates were extracted with methanol and dichloromethane and tested *in vitro* for growth inhibition of toxigenic isolates of *A. flavus* and *F. verticillioides*.

All the respondents were small holder farmers with over 50% being female. Twenty six percent of the respondents had knowledge on aflatoxin while over 50% had knowledge of maize ear rots. Most farming practices were similar between cropping systems but significantly ($P < 0.05$) lower population of *F. verticillioides* and *A. flavus* were isolated from the maize samples from push-pull farms. All push-pull samples were contaminated with aflatoxin below 10 µg/kg (Kenyan regulatory threshold) while 4.3% of the samples from non-push-pull had levels above 10 µg/kg.

Five percent and 9.4% of the maize from push-pull and non-push-pull farms, respectively, had fumonisin above 1000 µg/kg European Commission regulatory threshold. Knowledge on aflatoxin was 7.5 times higher among elderly aged 45 to 60 years while knowledge of ear rots increased 6 times with level of education ($P < 0.05$) and non-push-pull respondents were 34% more knowledgeable. Fumonisin and aflatoxin contamination in maize increased 3.9 times and by 28%, respectively, with application of diammonium phosphate (DAP) fertilizer during planting ($P < 0.05$). Aflatoxin levels also significantly increased 2 times with stemborer infestation of maize. Stemborer and fall armyworm damage on foliage and ears of maize were significantly ($P < 0.05$) reduced by slightly over 50% under push-pull cropping system. *Fusarium* ear rot was the most common ear rot with mean incidence of 5 and 10% under push-pull and non-push-pull, respectively ($P < 0.05$). Populations of *F. verticillioides* and *A. flavus* were significantly low under push-pull. Aflatoxin levels were not significantly different between cropping systems, but fumonisin were significantly lower by 39% under push-pull cropping system. There was positive and significant correlation among insect damage, ear rot, ear rot fungi and mycotoxin levels in maize. Populations of fungi and nutrients in soils were not significantly different between the cropping systems and did not have significant correlation between them. Methanolic extracts of desmodium roots showed significant reduction in radial growth of toxigenic *A. flavus* by 11-17% and *F. verticillioides* by 53-61% through reduced spore germination and germ tube elongation. The results showed that planting maize under push-pull cropping system indirectly reduced mycotoxin contamination through reduced insect damage. Reduced growth of toxigenic fungi in soils under push-pull by chemicals produced into the rhizosphere by desmodium roots was suggested as a potential mechanism of reducing mycotoxin contamination.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background information

Production of maize (*Zea mays*) in Kenya occupies more land than any other crop and over 70% of it is done by small holder farmers (Keya & Rubaihayo, 2013; Kibet, 2011). Nearly all agricultural households in Kenya grow maize. Maize is used directly or indirectly as food, feed and raw material for industrial manufacture of maize-based products. There are many factors that constrain maize production, thus resulting in deficiency of maize supply. These includes: weeds, insect pests, diseases, environmental factors, climate change, lack of sufficient extension services, soil nutrient deterioration and poor infrastructure (Kibet, 2011). Several strategies have been explored to increase maize production. These include use of fertilizers, use of improved seeds, growing maize in high potential areas and use of credit to obtain inputs (Kibaara, 2005). Despite these efforts, there is still an increasing gap between maize production and consumption.

Weed growth contributes to significant reduction in grain yield (Shrestha et al., 2018). In Kenya, striga weed is one of the biggest constraint to maize production, especially in western region (Atera et al., 2013; Gichana, 2014). Maize farmers may lose between 15 and 90% of their prospective output to the parasitic weed (Atera et al., 2013). The weed siphons nutrients and water from maize and causes serious damage. There exist several maize farming systems and most of these systems are affected by striga (Andersson & Halvarsson, 2011). Several technologies have been employed to control striga such as use of StriAway maize which is herbicide resistant and has innovative seed coat. Other measures include more fallow in crop rotation and push-pull technology in which desmodium crop used as intercrop releases chemicals that cause suicidal germination of striga seeds (Gichana, 2014; Khan et al., 2010).

Maize ear rot is a an economically important disease of maize globally and is characterized by: whitish mycelia at the base of maize ears, white-pinkish kernel coloration, yellowish coloration of the kernels or yellowing and drying of infected ears on green maize plants, depending on the causative fungi (Flett et al., 1998). Maize ear rots are caused by various fungi and most ear fungi: *Fusarium, Aspergillus* and *Penicillium*, are toxigenic and contaminate maize with associated mycotoxins (Dragich & Nelson, 2014). The prevalence of ear rot fungi depends on the climatic conditions of a region during maize growth (Bigirwa et al., 2007). Infection of maize with ear rots has a significant impact on maize grain yield.

Sobek & Munkvold (1999) and Parker et al. (2017) reported that the incidence of ear rots of maize significantly correlates with insect pest damage on the grain. One of the main insect pest for maize are stemborers in class lepidoptera (Midega et al., 2014; Onyango & Ochieng'-Odero, 1994). The amount of damage caused by stemborers depends on the stage of maize at infestation and the population density of the insect pest. The population density of stemborers is influenced by weather and presence of natural enemies of the pest. Stemborer infestation of maize is mainly characterized by batches of stemborer eggs, larvae or pupae on leaves and stems, and presence of holes on the maize parts (icipe, 2013).

### 1.2 Statement of the problem

Maize is one of the main staple source of food and feeds in Sub-Saharan Africa (Ranum et al., 2014). The bulk of maize grain reserve in Sub-Saharan Africa is produced by smallholder farmers. However, the safety of the maize is threatened by mycotoxins, such as aflatoxins, fumonisins, deoxynivalenol and zearalenone (Broggi et al., 2007). Mycotoxins are toxic chemicals produced by some fungi as secondary metabolites (Coker, 1997; WHO, 2018). When mycotoxins

contaminate agricultural products like maize, they become a threat to safety of food. Food that is contaminated with mycotoxins cause various health risks such as suppression of the immune system, worsening of illnesses such as HIV/AIDS and malaria, cancer and death in humans (Lewis et al., 2005; WHO, 2018; Williams et al., 2005). Human beings also get mycotoxin contamination through inhaling and coming in contact with contaminated foodstuffs (Paterson & Lima, 2010). In livestock, poisoning by mycotoxins in feed have been reported to cause degradation of ruminal microflora, reduced feed intake and reduced productivity (Fink-Gremmels, 2008).

Prevalence of aflatoxin and fumonisin in western Kenya has been reported in a number of studies, in various levels ranging from very low to very high (Kedera et al., 1999; Mutiga et al., 2015; Nduti et al., 2017). These studies mostly targeted maize from stores and, therefore, did not involve development of pre-harvest mitigation strategies. Aflatoxins pose the greatest health risk to maize consumers due to their widespread occurrence and their high toxicity to human health (Coker, 1997; Williams et al., 2005). Kenya has experienced several acute aflatoxicosis outbreaks which resulted in sickness and death of up to 331 people and, condemnation of contaminated maize consignments by ministry of public health and sanitation (Lewis et al., 2005; Mutegi, Cotty, & Bandyopadhyay, 2018). More so, sub lethal contamination of maize and maize products with aflatoxin go undetected, especially for subsistence farmers who consume own food.

Various mechanisms have been evaluated for management of mycotoxins in maize including proper drying of the ears before shelling, proper storage conditions, use of antioxidants under different conditions of water activity, temperature and controlled atmosphere, sorting out of contaminated grains, planting resistant varieties and use of biological control agents (Chulze, 2010; JECFA, 2018; Mohamed, 2016). However, cultural method like sorting and drying are post-harvest and have minimal impact on mycotoxins produced pre-harvest. Most of the other methods

like use of biological control agents and acquisition of resistant varieties have a cost attached to them, making it inapplicable to small scale farmers, most of whom are resource constrained. Genetic engineering and breeding are excellent options for mitigation of mycotoxins (Mesterházy et al., 2012). However, there are limited number of commercially available genetically engineered resistant cultivars and there is no legislation on use genetically modified food in most sub-Saharan countries. This creates the need for development and evaluation of an integrated, cost effective and easy to adopt methods for management of mycotoxins. Since mycotoxin contamination of maize primarily starts during growth and continues while in storage, management strategies should be long term, robust, sustainable and most of all be able to manage of mycotoxins in the field during crop growth.

### 1.3 Justification

Research has demonstrated that incidence of ear rots and mycotoxins correlates strongly with insect pest damage, poor soil health, pre- harvest and post-harvest handling of maize (Bowers et al., 2013; Fountain et al., 2014; Mehl et al., 2018; Mutiga et al., 2017; Parker et al., 2017; Parsons, 2008). Some ear rot fungi contaminate maize with associated mycotoxins (Munkvold, 2003; Ogara et al., 2017). Insects' wounds acts a court for entry of ear rot fungi inocula, thus exposing the maize to mycotoxins. Cropping systems as drivers of contamination of maize with mycotoxins, and underlying mechanisms, has not being widely studied. A cropping system that manage insect pests, soil fertility and modify soil chemistry is a potential strategy for the management of ear rots. Such cropping system could also modify soil fungal population and diversity and subsequently mycotoxin contamination of maize. Such a strategy will be more beneficial to resource constrained small holder farmers because there would not have to be established every season and therefore cheaper than use chemicals. Push-pull cropping system has been reported to integrate management

of stemborers (*Buseola fusca* and *Chilo partellus*), fall armyworm (*Spodoptera frugiperda*), striga weed (*Striga hermonthica*) and soil nutrition (Khan et al., 2000; Midega et al., 2018).

The cropping system intercrops cereals like maize and sorghum with a non-food legume as a `push` crop and planting a border `pull` crop around the intercrop (Khan et al., 2000). The 'push' crop is *Desmodium* spp. while the 'pull' crops are *Brachiaria*/Napier grass (*Pennisetum purpureum*). Desmodium leaves and stems produce semio-chemicals that push away the insects while napier and Brachiaria grass pull them (Midega et al., 2014, 2018). The border crop, additionally, produces sticky chemicals that reduce the number of eggs to hatch and the number of larvae to mature (Khan et al., 2000). Desmodium roots induce suicidal germination of striga seeds by producing allelopathic chemicals, thus suppressing the development of the weed (Khan et al., 2003). Furthermore, desmodium fix nitrogen and contributes to availability of phosphorus thus improving soil fertility by mycorrhiza association (Khan et al., 2000).

Most of existing management strategies for management of mycotoxins have limited impact while others are not easily adopted because of high input costs which are also sustainable (Mohamed, 2016; Ndemera et al., 2018). Therefore, the results of the study will contribute to establishment of strategies for mycotoxin management in western Kenya and sub-Saharan Africa (SSA) as a whole. This study would also contribute to optimization of the benefits of push-pull cropping system and increased adoption of the cropping system. As a result, the cropping system would contribute to improvement of livelihoods of small holder farmers through contribution to food and nutritional security, food safety, environmental sustainability and household incomes.

### 1.4 Objectives

The main objective of the study was contribution of push-pull cropping system to food safety through management of ear rots and contamination of maize with associated mycotoxins.

**The specific objectives were:**

i) To establish the socio-economic and agronomic factors associated with ear rots and mycotoxin contamination of maize among push-pull and non-push pull farmers in western Kenya.

ii) To determine the effect of management of stemborers and fall armyworm by push-pull cropping system on occurrence of ear rots and mycotoxin contamination in maize.

iii) To determine the relationship between soil nutrition and population of mycotoxin-producing fungi in soils under push-pull maize cropping systems.

iv) To determine the effects of desmodium root extracts on growth of mycotoxin producing *A. flavus* and *F. verticillioides*.

### 1.5 Hypothesis

i) Socio-economic and agronomic factors of farmers in western Kenya influence mycotoxin contamination in maize.

ii) Reduction of stemborers and fall armyworm by push-pull cropping system lower the occurrence of ear rots and mycotoxins in maize.

iii) Push-pull cropping system improves soil nutrition, which in turn influence the population of mycotoxin-producing fungi in the soil.

iv) Desmodium root extracts suppresses the population of mycotoxin-producing fungi.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Maize production and its contribution to food security in Kenya

Agriculture is the backbone of most Sub-Saharan countries (Keya & Rubaihayo, 2013). In East Africa, the agricultural sector, though mainly dominated by small-scale mixed farmers, has a great contribution to the gross domestic product. Maize is a multi-purpose crop used as both food and feed, with 85% of its production being used as the staple food for millions of people in sub-Saharan Africa (Keya & Rubaihayo, 2013). As food and feed, maize grains are consumed in different forms for their richness in vitamins A, C and E, carbohydrates, essential minerals and protein (Iwouha, 2017). Maize is also rich in fiber and calories. Many daily diets for millions of Africans contain maize either directly or indirectly. The starch from maize can also be processed into additives and ingredients such as dextrin, sorbitol, sorbic acid and lactic acid (Iwouha, 2017).

Maize crop grows under a wide range of agro-ecological zones worldwide and accounts for up to 50 % household expenditure for low-income small-holder farmers in Eastern and Southern Africa (Iwouha, 2017). Maize requires different amounts of water for growth depending on temperature, humidity, wind speed and sunshine of the agro-ecological zone. In Kenya, maize grows under altitude range of 0 – 2200 m above sea level, temperature range of up to 30°C, rainfall range of between 500 and 800 mm and a wide range of soils with a pH of 5.5 – 7.0 (Jaetzold et al., 2009; Paredes et al., 2014). Food security and availability of maize in Kenya are synonymous, with over 90% of the Kenyan population depending on maize for food (Keya & Rubaihayo, 2013). More than 75% of Kenya's local production of maize is provided by small scale, mixed cropping farmers. Small scale farmers produce food for home consumption and sell the surplus to get income (Kang'ethe, 2011).

The demand for maize in Kenya is higher than its local production (Keya & Rubaihayo, 2013). The difference is always met by importing maize from neighboring countries like Tanzania and Uganda. On average, Kenya imports over 20 million 90 kg bags of maize annually (Onyango et al., 2018). Like Kenya, Uganda, Tanzania, Rwanda and Burundi have low maize yield due to limited use of agricultural inputs, limited land, insect pests and diseases (Keya & Rubaihayo, 2013).

## 2.2 Limitations to maize production in Kenya

### 2.2.1 Low soil fertility

Cultivated soils become increasingly depleted of nutrients over time. Nitrogen is known to be the most limiting nutrient in maize production, followed by phosphorus, and their deficiency causes poor yields in maize (Jama & Van Straaten, 2006; Pasuquin et al., 2014). Low fertilizer use by farmers due to unavailability and high costs have also significantly contributed to the decline in soil fertility (Okalebo et al., 2007). Several technologies have however, been tested and recommended for replenishing nitrogen in soils and these include use of green manure, use of animal manure and biological nitrogen fixation, all of which are organic matter based (Jama et al., 2000; Kifuko-Koech, 2013; Ndung'u et al., 2006).

In cereal-legume intercrop cropping systems, legumes have been widely used as a source of nitrogen through biological nitrogen fixation (Ahmad et al., 2014). This nitrogen maybe available to the associated crop in the current cropping season or as residual nitrogen for the succeeding cereal crops in subsequent seasons (He et al., 2003). The potential transfer of nitrogen by legumes is varied among different legume species, depending on root tissue composition and legume population density (Louarn et al., 2015). Some legumes concentrate nitrogen in their pods, hence low soil nitrogen replenishment (Flynn & Idowu, 2015).

Adoption of organic manure for soil amendment is very limited, and this has been attributed to the challenges that come with them. Processing and application of organic waste residues is labor intensive, organic materials are of different quantity and quality, and release of nutrients in soil at different times result to differences in nutrient availability and crop yield (Gachengo et al., 2004). Intercropping maize with desmodium, instead of cereal legumes, has been proven to be a more viable option for replenishing soil fertility, especially of small holder farmers who have limited resources ( Khan et al., 2000; Kifuko-Koech, 2013).

### 2.2.2 Damage by insect pests

There are many insect pests that attack maize and adversely affect its production. The major insect pests of maize are stemborers (Lepidoptera and Crambidae family), European corn borer (*Ostrinia lubilalis*), shoot fly (*Atherigona orientalis*), cutworms (*Agrotis* spp.), aphids (*Rhopalosiphum maidis*) pink borer (*Sesamia calamistis* Hampson) and fall armyworm (*Spodoptera frugiperda* J. E. Smith). Stemborers and fall armyworm are considered one of the most destructive insects that constrain efficient production of maize in Sub Saharan Africa (Kankonda et al., 2017; Midega et al., 2018; Onyango & Ochieng'-Odero, 1994). Stemborers are an increasingly economically important constraint to maize production, causing yields losses of 20-40% of the potential output in the field during cultivation and 30 – 90% post-harvest, depending on pest population density and the phenological stage of the crop at infestation (Frank et al., 2008).

The most common maize stemborer species in Western Kenya are *Buseola fusca* Fuller, *Chilo partellus* and *Buseola segeta* (Calatayud et al., 2014; De Groote, 2002; Khan et al., Midega et al., 2008). First report of fall armyworm in Africa was made in January 2016 on maize plants (Goergen et al., 2016). Lepidopteran larvae cause damage to maize by feeding on the stems, causing holes

and widows. Severe attack by stemborers and fall armyworm may cause the 'heart' to turn yellow and die, making the plant to die (Ajala & Saxena, 1994; Goergen et al., 2016; Van Rensburg, 2001). The insect pests may also feed on the ears. Severe insect pest infestation of maize result in reduction in yield, due to reduced number of ears harvested, and due to tunneling of the maize stalks. Yield losses due to stemborer infestation of maize can also be influenced by the variety of maize planted.

### 2.2.3 Competition from weeds

Weeds limit crop potential by competing for nutrients, water, carbon dioxide and space as well as by harboring insect pests and diseases (Rajcan & Swanton, 2001; Shrestha et al., 2018). Weeds can cause up to 65-100% production losses in maize (Atera et al., 2013; Berner, et al. 1995). Most maize farming systems are affected by weeds, the most important of which is striga (Andersson & Halvarsson, 2011). Striga (*Striga hermonthica* and *S. asiatica*) is a parasitic weed found in Sub-Saharan Africa, whose flower produces between 50000 to 500000 seeds per flower. The weed slows down the growth of the host plant by damaging its photosynthesis function and using its nutrients thus causing a deficit (Berner et al., 1995). Striga seeds only germinate in the presence of a host plant like maize and in the absence of the host plant, the seeds can stay in the soil for more than 20 years (Matusova et al., 2005).

Striga has been associated with up to 100% yield losses in Sub-Saharan Africa (Berner et al., 1995). A study by (Atera et al., 2013; Massawe et al., 2002) showed that striga weed contribute to 18 – 42% maize yield losses in Tanzania. In Sub-Saharan Africa, the problems associated with striga weed are accelerated by factors such as poor farming practices, deterioration of soil fertility, and expansion of agricultural production to marginal lands (Kountche et al., Al-Babili, &

Haussmann, 2016). Several methods to combat striga exist today and they include use of pesticides, planting resistant maize, increasing the fallow duration in crop rotation, intercropping the host plant with legumes and push-pull cropping system (Andersson & Halvarsson, 2011). Improving soil fertility has also been known to reduce striga infestation of cereal crops (Shrestha et al., 2018).

### 2.2.4 Diseases of maize

Diseases of maize are caused by bacteria, fungi, viruses or mollicutes (CIMMYT, 2004; McGee, 1988). The diseases may affect the foliage, stalks, or ears of the crop. Common diseases of maize include head smut (*Ustilago maydis*), bacterial stalk rot (*Erwinia carotovora*), leaf blight (*Exserohilum turcicum*), wilt (*Harpophora maydis*), common rust (*Puccinia sorghi*), grey leaf spot (*Cercospora maydis*) downy mildew (*Peronosclerospora sorghi*), maize lethal necrosis (chlorotic mottle virus and any Potyviridae virus), maize streak disease (maize streak virus) and anthracnose (*Colletotrichum graminicola*) (CIMMYT, 2004; Krishisewa, n.d.; Miano et al., 2011). Symptoms of maize diseases include rotten and soaky internodes, wilting leaves, lodged leaves, hanging ears, premature death of plant, shrunk leaves, undeveloped shrunken kernels, disintegrated sheaths, powderly leave surfaces, malformed tassels, chlorosis, white stripes, stunting, necrosis, twisted leaves, among others (USDA, 2000). Diseases reduce maize grain yield by reducing crop vigor, malformed kernels, premature death of plant and rotting of kernels. Fungal ear rots reduce grain quality by contamination with associated mycotoxins (CIMMYT, 2004).

## 2.3 Ear rot diseases of maize

Maize ear rots are caused by various fungi including *Diplodia*, *Aspergillus*, *Fusarium*, and *Penicillium* spp. However, *Fusarium* and *Aspergillus* ear rots are the most economically important.

Incidence and severity of ear rots is usually related to the incidence and severity of insect pests including stemborers, European corn borer, western bean cutworm or corn ear worm feeding damage (Goergen et al., 2016; Sobek & Munkvold, 1999). Stemborers and fall armyworm have been reported as the major insects pests of maize in Africa, including western Kenya (Calatayud et al., 2014; Kfir et al., 2002; MOA, 2017) The species that causes ear rots are favored by different weather and biotic conditions ( Munkvold, 2003). The insects that feed on maize ears acts as vectors for some mycotoxin-producing ear rot fungi (Dowd, 2003). The open wounds left by insects become possible infection routes by ear rot fungi. *Fusarium* ear rots exist in two forms: *Giberella* ear rots and *Fusarium* ear rots, depending on the species causing the disease (Dragich & Nelson, 2014). *Giberella* ear rot is cause by *Giberella zeae*, the asexual stage of *F. graminearum*, characterized by pinkish-red discoloration of maize ears that starts from the tip of maize ears and grows towards the base. *Giberella zeae* usually infect maize during silk formation, within the first one week (Dragich & Nelson, 2014; Munkvold, 2003).

*Fusarium* ear rot is caused by *F. verticillioides* and produces white, pale pink or pale lavender mycelia (Sobek & Munkvold, 1999). *Fusarium* ear rots are associated with insect infestation of maize and the infection is localized at the point of insect feeding. *Fusarium graminearum* is mainly associated with deoxynivalenol toxin while *F. verticillioides* is mainly associated with fumonisin contamination of maize (Dragich & Nelson, 2014). *Fusarium graminearum* predominates in higher altitudes, above 1800 m above the sea level while *F. verticillioides* predominate in altitudes between 900 and 1500 m above the sea level (Bigirwa et al., 2007). *Giberella zeae* is common in areas with high temperatures of about 30°C while *F. verticillioides* is common in areas with temperatures of 24 - 26°C, in both cases coupled with high moisture levels. Infection of maize by

ear rots contaminates the grains with mycotoxins associated with the fungal species ( Munkvold, 2003).

*Aspergillus* ear rot is caused by several *Aspergillus* spp., mainly *A. flavus* and *A. parasiticus* (Mahapatra et al., J2015). The disease is characterized by production of greenish masses of spores on and between kernels. Infection of maize ears usually occurs near the tip and mostly only a few kernels are infected. *Aspergillus* ear rot is favored by dry weather conditions and drought. The disease contaminates maize with aflatoxins (Schoeman, 2012). *Diplodia* ear rot is one of most destructive fungal disease of maize, caused by *Diplodia zeae* (Clayton, 1927). *Diplodia* ear rot is one of the most common type of maize ear rot (Opande et al., 2017; Owuor et al., 2018). *Penicillium* and *Trichoderma* ear rots are less common types of maize ear rots.

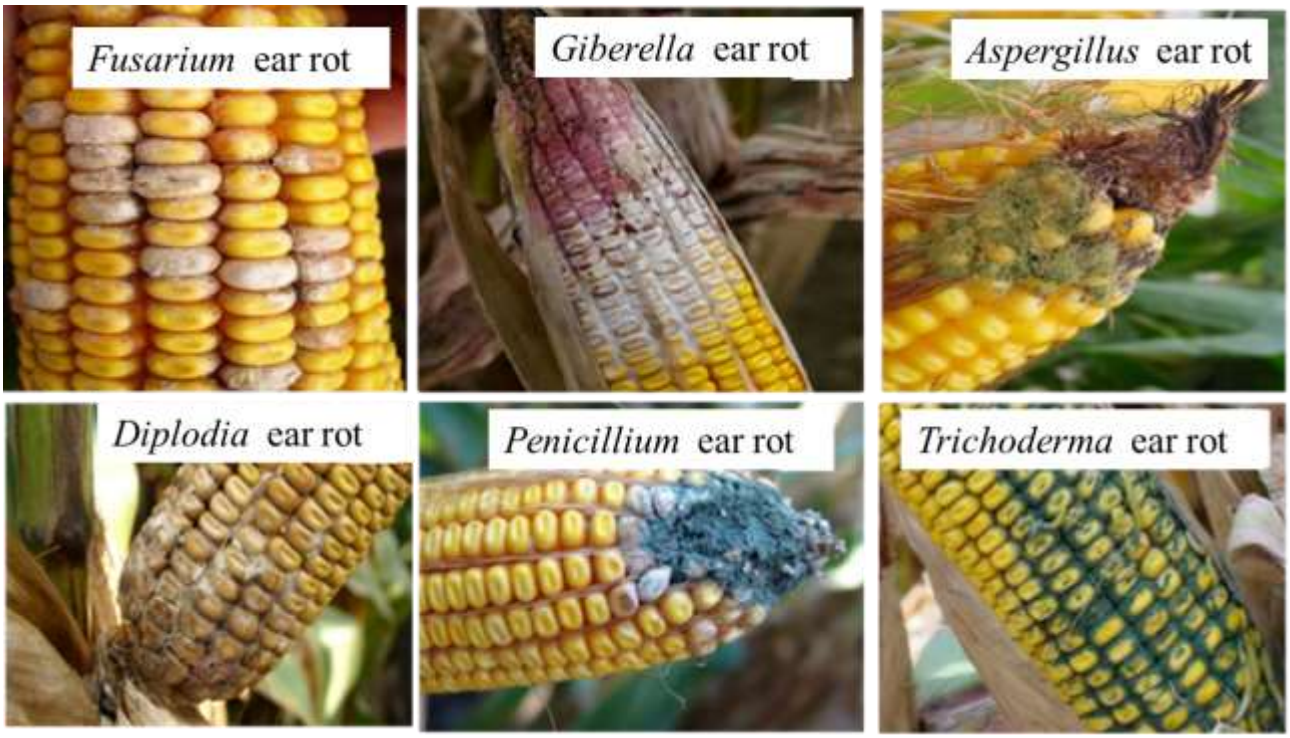


Figure 2. 1**:** Common types of ear rots in maize
(Source: Pioneer 2019)

## 2.4 Mycotoxins associated with maize

Mycotoxins are low molecular weight toxic compounds (Negedu et al., &2011) that are produced by a diverse group of fungi with different morphology, biochemistry and ecological niches (CAST, 2003; O'Callaghan et al., 2006). Mycotoxin production by fungi is a characteristic of their biosynthetic pathway as secondary metabolites, and the quantity of mycotoxin produced depends on the climate, soil nutrition status, the previous growth history of the farm and developmental stage of the fungus. Production of mycotoxin in maize can occur in the field during growth or after harvest (Schmale & Munkvold, 2009; Smith et al., 2012).

Mycotoxins can cause growth retardation, damage vital body organs, suppress immune system, interfere with the reproductive systems and reduce productivity in animals and humans (CAST, 2003). Maize contaminated with unacceptable levels of mycotoxins poses great economic losses and risk to agricultural trade (Leslie, Bandyopadhyay, & Visconti, 2008). It is estimated that about 40% of grain losses in Sub Saharan Africa are due to mycotoxins. Climate change greatly influence the levels of mycotoxins due to excessive precipitation or drought, which in turn influence temperature, moisture and relative humidity (Fountain et al., 2014; Miller, 2008). There are various mycotoxins that contaminate maize grains including fumonisins, aflatoxins, deoxynivalenol and ochratoxins (Kimanya et al., 2012; Koenning & Payne, 2018).

### 2.4.1 Aflatoxins

Aflatoxins are toxic metabolites that contaminate a wide range of food crops, including maize (Bennett & Klich, 2013; Koenning & Payne, 2018; Schmale & Munkvold, 2009). There are four classes of aflatoxins; B1, B2, G1 and G2, but B1 is regarded as the most potent and dangerous worldwide. Aflatoxin intoxication contribute to the disease burden in countries where there is repeated exposure to aflatoxins (Schmale & Munkvold, 2009). Maize characteristics including rate

of maturity, shape of kernel, kernel breakage, kernel texture, and high percentage of damaged maize ears at harvest drive aflatoxin contamination of maize (Mutiga et al., 2014; Mutiga et al., 2017). Physical factors such as warm temperature and erratic weather patterns also influence presence and accumulation of aflatoxin (Cotty & Jaime-Garcia, 2007). Drying time for the maize also significantly influences formation of aflatoxin (Mbuge et al., 2016). These factors favor colonization of maize by aflatoxin producing fungi. Infection can occur at any stage, from pre-harvest to storage. Stress conditions of drought, heat, insect, nematode and fertilizer increase the amount of aflatoxin produced in maize (Koenning & Payne, 2018).

Aflatoxins are produced by fungi under *Aspergillus* section *Flavi*, which are a group of *Aspergillus* spp. usually characterized by biseriate conidial heads, in shades of yellow-green to brown colonies and dark green sclerotia s(Samson & Varga, 2009). The most common species of this section are *A. flavus, A. parasiticus* and *A. nomius*. *Aspergillus flavus* and *A. parasiticus* are the most toxigenic and economically important in spoilage of food and production of aflatoxins (Ezekiel et al., 2014; Koenning & Payne, 2018; Samson & Varga, 2009). *Aspergillus flavus* mainly produces aflatoxin B1 and B2 while *A. parasiticus* produces all the four aflatoxins (CAST, 2003). When grown on maize kernels, *A. flavus* has typical yellow green appearance (Koenning & Payne, 2018).

Consumption of aflatoxin contaminated food stuffs contaminates blood with the toxin, causing diseases called aflatoxicoses (Williams et al., 2005). Chronic exposure to aflatoxin may result in growth impairment in animals and children, liver cancer and hepatic failure (Williams et al., 2005; Wu & Khlangwiset, 2010). Pregnant mothers whose blood is contaminated with aflatoxin deliver anemic infants with significantly low mean birth weight (De Vries et al., 1989; Ismail et al., 2014; Smith et al., 2017). In addition to its effects on human health and nutrition, aflatoxin contamination of maize has household and national economic and food security implications (Ismail et al., 2014).

Economic implications of aflatoxin contamination of maize include yield losses, reduced crop value due to mycotoxin contamination and reduced animal productivity and human health costs.

### 2.4.2 Fumonisins

Fumonisins are a group of mycotoxins produced by *Fusarium*, mainly produced by *F. verticillioides,* and to a lesser extent by *F. proliferatum* (Leslie & Summerell, 2006; Olga, 2009). *Fusarium verticillioides* is characterized by white to salmon color on maize kernels (Koenning & Payne, 2018; Leslie & Summerell, 2006). The fungi associated with fumonisins cause *Fusarium* ear rot in maize, whose infection is increased by physical damage to kernels by insect feeding. Fumonisins exist in over 28 different forms designated as A, B, C and P-series (JECFA, 2018; Schmale & Munkvold, 2009). Fumonisin B1 is considered the most common and economically important followed by B2 and B3 (Schmale & Munkvold, 2009).

Consumption of maize contaminated with fumonisins has been implicated as a possible cause of various clinical symptoms including apoptosis of the liver and kidneys, pulmonary edema, esophageal cancer, neural tube birth defects and toxification of the nervous system (IARC, 1972; Koenning & Payne, 2018; Olga, 2009). Production of fumonisin is optimum during drought period in well aerated environment, at temperatures > 15°C, pH of 2.5 – 5.0 and in the presence of limited (Leslie & Summerell, 2006). Drying the grains below 14% moisture content stops fumonisin production in storage, but fumonisins produced before harvest and drying will remain intact ( Ono et al., 2002).

### 2.4.3 Deoxynivalenol

Deoxynivalenol **(**DON**)** belongs to a category of mycotoxins called trichothecenes type "B" group, and is also called vomitoxin due to its high emetic effect after consumption (Gutleb et al., 2002; Kushiro, 2008). Deoxynivalenol is more important in wheat than in maize and is mainly produced by *F. graminearum* Booth (Leslie & Summerell, 2006). However, *F. culmorum*, and *F. pseudograminearum* have been reported to produce the toxin too (Koenning & Payne, 2018; Wagacha et al., 2010). In addition to vomiting, intake of deoxynivalenol disrupts protein function by inhibiting protein synthase enzyme (Sobrova et al., 2010). Chronic exposure to DON results in decreased weight gain, feed refusal, anorexia, decreased nutritional efficiency and altered immune function (Koenning & Payne, 2018; Sobrova et al., 2010). The toxin also decreases hematopoiesis, damages the nervous, gastrointestinal and cardiovascular systems. On maize kernels, *F. graminearum* appears pinkish to reddish in color. The amount of DON produced in grain is usually positively correlated to the fungal biomass in the grains and production is favored by temperatures below 24°C (Beattie et al., 1998; Wagacha et al., 2016). Human beings can be exposed to the toxin directly through ingestion of contaminated cereals or indirectly through ingestion of products from animals fed on contaminated cereals (Leslie & Summerell, 2006).

### 2.4.4 Zearalenone

Zearalenone (ZEA) is a mycotoxin found in food and feed, mainly produced by *F. graminearum* and mostly co-occur with deoxynivalenol (Coker, 1997; Zinedine et al., 2007). Zearalenone mainly occur in food and feed in temperate regions but also occur in tropical regions in low concentrations. The maximum tolerable daily intake of ZEA was established as 0.5 µg/kg of body weight (Zinedine et al., 2007). Exposure to zearalenone contaminated maize causes reproductive disorders in livestock and hyper estrogenic syndrome in humans. Contamination of maize with zearalenone

can occur in the field or during storage at temperature below 24°C (Queiroz et al., 2012). Human exposure to zearalenone can be through consumption of contaminated cereals like maize or consumption of products from animals that were fed on contaminated maize or silage (Queiroz et al., 2012).

### 2.4.5 Other mycotoxins contaminating maize

Ochratoxins and ergot alkaloids are less common mycotoxins of maize (CAST, 2003). Ochratoxins are mainly produced by *Penicillium verrucosum* and *Aspergillus ochraceous*, and less frequently by *Aspergillus* section *Circumdati* (Frisvad et al., 2004; Palencia et al., 2014; Scudamore & Patel, 2000). Even low concentrations of ochratoxins cause endemic kidney disease in animals and suppresses the immune system in humans (Petzinger & Weidenbach, 2002). European regulation set maximum ochratoxin limit in cereals as 5 µg/kg (Petzinger & Weidenbach, 2002). Ergot alkaloids are mainly produced by *Claviceps* spp., plant pathogens that elaborate their toxins in fungal masses known as sclerotia. Ergotism was reported to have killed thousands of people in Europe in the last 1000 years (Bragg et al., 2017; UNDP/FAO, 1989). The main impact of ergots is reduced grain yield because ergot resting structures replace maize ovaries (Tenberge, 1999). Ergots produce a metabolite called ergot alkaloids, which cause deadly ergotism in animals and humans when consumed in contaminated grains.

## 2.5 Methods for mycotoxin analysis

Mycotoxin analysis could either qualitative or quantitative. In either case, the method ought to be simple, rapid, robust, accurate, and selective to enable simultaneous determination of different mycotoxins (Jones, 2009). Commonly used methods of mycotoxin analysis are solid phase

Enzyme Linked Immuno Sorbent Assay (ELISA), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Jones, 2009; Tittlemier et al., 2019). The method for analysis selected depends on the nature of the samples, the number of samples, how quickly the results are needed, and the amount of details required from the analyses (Shephard, 2009).

### 2.5.1 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is one of the most popular quantitative methods of mycotoxin analysis foods and feeds because it is superior and reliable. The method uses immunoaffinity clean-up to separate mycotoxins (Krska et al., 2008). HPLC can detect and quantify multi mycotoxins simultaneously including aflatoxins, deoxynivalenol, zearalenone and fumonisins (Jones, 2009). Detection step in HPLC is either by UV or fluorescence. However, HPLC with fluorescent detection is more common because it uses readily available short and high-resolution columns and has highly sensitive detectors. HPLC technique is sophisticated and require a highly trained personnel to operate (McMaster, 2007; Sciencing, 2020). Also, more than one compounds with similar structure and polarities can be eluted together while some very strongly bound chemical compounds may fail to leave the beads during elution. The technique is also very expensive to develop compared to other techniques like solid-phase ELISA.

### 2.5.2 Enzyme-Linked Immuno Sorbent Assay

Solid phase ELISA are antibody-antigen reaction methods that have been developed for estimating aflatoxin, zearalenone, fumonisin, ochratoxin and T-2 toxin in a variety of commodities (Krska et al., 2008). The methods are simple, rapid and commercially available (Krska et al., 2005). The

antigen in the sample directly or indirectly compete with the antibodies coated microtiter plates (Pittet, 2005). The technique is cheap to acquire and undertake compared most of automated techniques. Enzyme-Linked Immuno-sorbent Assay is preferred for screening purposes because it allows simultaneous analysis of many samples and require no technical training. In addition, ELISA requires limited use of organic solvent and inexpensive equipment. However, ELISA has narrow detection range and there is possibility of false positive/negative, matrix interference and cross-reactivity with related mycotoxins (Krska et al., 2008; Shephard, 2009). Most ELISA techniques are limited to certain raw material, and therefore, one must get different kits for each raw material.

### 2.5.3 Thin Layer Chromatography

Thin layer chromatography (TLC) is a qualitative method that is used for screening of samples for contamination with mycotoxins (Krska et al., 2008). This technique involves applying the sample extract as a spot or band on the origin of layer on the plate and identification separation zones by comparing with those of standards (Sherma, 2006; Tuzimski & Sherma, 2019). However, the method is not easy trichothecenes are non-emitter or weak emitters of fluorescence therefore sensitive to detect with this technique. This technique is widely used for detection of aflatoxins, fumonisins and citrinin. TLC has advantages of being easy to do, analysis of multiple samples, usage of little solvent per sample and high accuracy (Sherma, 2006). However, TLC is limited due to lack of fully automated systems and limited capability of resolution. Also, TLC does not quantify the mycotoxin.

### 2.5.4 Liquid chromatography with tandem mass spectrometry

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods analyzes for several mycotoxins in multiple samples simultaneously (Krska et al., 2008; Tittlemier et al., 2019). These methods are mainly used for analysis of *Fusarium* mycotoxins including deoxynivalenol, T-2, HT-2, zearalenone, fumonisins and their derivatives. Extraction and clean-up depend on the LC-MS/MS method chosen. LC-MS/MS is highly selective, and sensitive (Taylor, 2005). Like HPLC, however, there is a possibility of coelution of compounds with similar polarities. This technique is limited by the need of a highly trained personnel to operate.

### 2.5.5 Gas chromatography

Gas chromatography (GC) is a technique used for analysis of samples for type A trichothecenes because they are not analyzable by HPLC (Pittet, 2005). The technique can with high sensitivity detect and quantify for multiple mycotoxins simultaneously. Moreover, GC can be coupled with mass spectrometry(GC_MS) and be used for simultaneous detection of trichothecenes A and trichothecenes B (Biselli et al., 2004). This technique is however, limited to volatile samples and is not suitable for thermal labile samples. In addition, GC is less efficient in terms of time and uses complex derivatization procedure (Zhang et al., 2018).

### 2.5.6 High-performance Thin Layer Chromatography

High-performance Thin Layer Chromatography (HPTLC) is an automated form of TLC that has efficient adsorption and separation in a short time (Shaise, 2011). Sample preparation and analysis by HPTLC is simple. Compared to TLC, HPTLC can simultaneously analyze more samples and standards with better accuracy and precision within a shorter time. However, sample preparation

does not include clean-up and therefore, there are chances of presence of impurities. The technique is also very expensive to acquire.

### 2.6 Strategies to reduce fungal and mycotoxin contamination in maize

Mycotoxin contamination of maize can be managed pre-harvest, during harvest and post-harvest (Kabak et al., 2006; Wu & Khlangwiset, 2010). Kabak et al. (2006) reported that most mycotoxin contamination of maize starts in the field and continues during storage. Thus, pre-harvest control of mycotoxin contamination is critical to safe maize from contamination with phytopathogenic fungi like *Fusarium* and associated mycotoxins in storage.

Pre-harvest management strategies include breeding for resistant maize varieties to reduce the potential of mycotoxin management (Kabak et al., 2006). This, however, has not being achieved because there is lack of resistant genotypes. More so, there is no legislation on genetically modified food in Africa. Additionally, cultural practices such as crop rotation, proper tillage, irrigation, appropriate application of proper soil nutrition and use of fertilizers (Munkvold, 2003; Strosnider et al., 2006; Wagacha & Muthomi, 2008). Crop rotation reduce mycotoxin contamination by breaking the chain of production of fungal inocula. To achieve this, rotation crop should be a non-cereal. Proper and timely use of fertilizers reduce crop stress, especially during seed development, thus, reducing the chances of opportunistic infection by fungi. This because nutrient deficiencies, especially nitrogen, has been reported to make maize more susceptible to infection fungi and subsequent contamination by associated mycotoxins (Abbas et al., 2009). Irrigation reduce plant stress but should not be done in order to avoid facilitation of development, spread and infection of ears with fungal inocula. Pre-harvest practices reduce mycotoxin contamination of maize by

altering the conditions under which the crop is grown so that insect pest infestation and fungal infection are avoided.

Pre-harvest biological control of mycotoxins has been done by use of non-toxigenic strains of fungi to competitively displace the mycotoxigenic strains. For instance, use of non-toxigenic *A. flavus* isolates (aflasafe) has been widely tested under field conditions for control of aflatoxin-producing *A. flavus* in different countries in Africa ( Abbas et al., 2009; Mohamed, 2016). Biological control of insects through strategies such as use of Bt maize also results in reduced occurrence of mycotoxins as a result of reduced fungal infection courts ( Wu, , & Casman, 2004). Use of insecticides and fungicides to control insects and fungal infection while maize grow would also control mycotoxin contamination of the grains (Magan et al., 2003).

Harvesting management strategies such as drying the grains to safe moisture level < 13% and removal of unwanted and rotten grains reduce fungal infection and mycotoxin contamination (Kabak et al., 2006). Maize that has symptoms of ear rots have been reported to have significantly higher levels of mycotoxins than asymptomatic maize (Ono et al., 2006; Owuor et al., 2018). Removal of maize stalks from the field after harvest would reduce primary inocula of the fungus in subsequent seasons since mycotoxigenic *Fusarium* spp. survive in cereal crop residues across seasons. The crop residues act as a source of inoculum for the next cropping season (CAST, 2003; Njeru et al., 2016). Moisture affects the growth of mycotoxigenic fungi and mycotoxin production. Therefore, while in storage maize should be kept at adequate aeration and moisture content in the storage structures should be monitored periodically. Natural and chemical agents such as phosphine have been used to control storage insects and mold infection reduce mycotoxin contamination.

Pre-harvest, harvest and post-harvest cultural practices described above have little impact on the levels of mycotoxins in maize at harvest. Most of the other strategies described above are expensive and impractical for small scale mixed farmers who depend on maize farming for food and as source of income. This creates the need to evaluate more robust, cheap and sustainable strategies. The main short coming of aflasafe technology is the need development of molecular techniques for tracking fungal population of *A. flavus* during growth, which is expensive and requires trained technician.

## 2.7 Push-pull technology in maize farming

### 2.7.1 Principles of push-pull technology

Push-pull is a farming technology that was developed by scientists at *icipe* in Kenya and Rothamsted Research in the UK, in collaboration with other research institutions in East Africa, primarily for control of stemborer and striga weed (Frank et al., 2008; Khan et al., 2000). The technology combines behavior modifying stimuli to manipulate the spread and abundance of insect and their natural enemies. The stimuli used for behavioral modification in push-pull includes visual and semio-chemical cues that work by non-toxic mechanisms (Cook, Khan, & Pickett, 2007). Push-pull uses three components: the cereal (maize/sorghum), non-food legume intercrops as "push" crops and grass border crops as "pull" crop (Cook et al., 2007; Frank et al., 2008).

The strategy for "push-pull" involves the release of repellant volatiles from the push crop and attractive volatiles from the pull plants. Insects use these chemicals to determine their host and non-host plants. The crop being protected (maize/sorghum) and the trap plant must have some volatiles in common. Plants that have been identified as effective "pull" plants include Sudan grass (*Sorghum vulgare* Sudanese), napier grass (*Pennisetum purpureum*) and Brachiaria grass while plants identified as effective "push" plants include molasses grass (*Melinis minutiflora*) and

Desmodium *(Desmodium uncinatum* and *D. intortum*) (Frank et al., 2008). The conventional push-pull intercrops maize with *D. uncinatum* and Napier grass is planted around as the border crop, while the climate smart push-pull intercrop maize with *D. intortum* and Brachiaria grass is planted as the border crop (Midega et al., 2010).

### 2.7.2 Mechanisms of action of push-pull technology in management of insects and striga

The technology integrates insect, striga weed and declining soil fertility management in maize and sorghum farming in Africa (Khan et al., 2018; Midega et al., 2018). Molasses grass attracts stemborers by production of five attractant compounds; (*E*)-ß-ocimene, α-terpinolene, ß-caryophyllene, humulene, and (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene. In addition to being attractive volatiles for stemborers, ocimene and nonatrience in some circumstances would be repellant to ovipositing stemborers ( Khan et al., 2000). Desmodium produces (E)-ß-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene, together with large quantities of other sesquiterpenes. These are the same chemicals produced by damaged plants as self-defense mechanism (Khan et al., 2010; Midega et al., 2015; Midega et al., 2018).

Napier/Brachiaria grass attracts gravid stemborers by producing large amounts of nonanal, naphthalene, 4-allyl anisole, eugenol and linalool. The amount of these chemicals increase in the first hour of nightfall, the period when gravid stemborer moths look for host plants for oviposition, thus the preferential oviposition on napier grass (Khan et al., 2010). Napier grass also produces gummy substance that restricts larval development. Therefore, only approximately less than 20% of emerged larvae survive to adulthood (Frank et al., 2008). Use of Sudan grass as the pull crop not only attracts the stemborers but also attracts natural enemies of the stemborers. This makes Sudan grass a less preferred ‘pull’ crop. Desmodium controls striga by increasing soil fertility

through nitrogen fixation, soil shading and production of two sets of allelopathic chemicals from the roots that induce suicidal germination of striga seeds ( Khan et al., 2000).

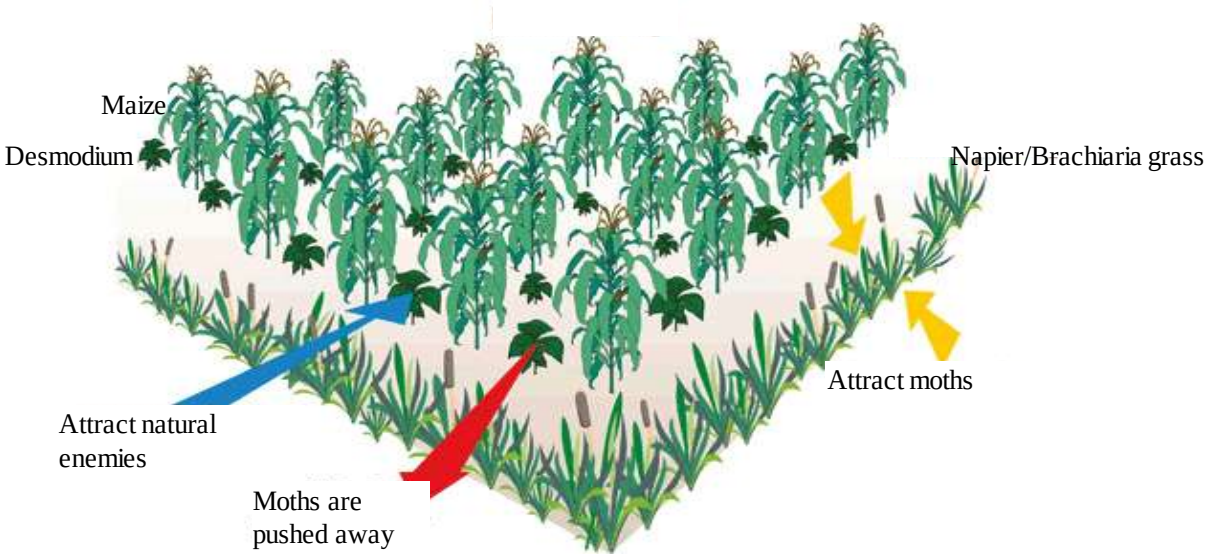


Figure 2. 2: Diagrammatic presentation of push-pull technology for management of stemborers and fall armyworm in maize

Source: (Fredalette, 2017)

### 2.7.3 Other benefits of the push-pull technology

Control of stem borers and striga weed in maize and sorghum by push-pull cropping system has several advantages over conventional strategies including reduction in cost of production and increased sustainability (icipe, 2019). Both desmodium and napier/Brachiaria grass are nutritious fodder for livestock thereby allowing crop-livestock integration (Khan et al., 2008). Desmodium has high dry matter (20-26%) and protein content (15-20%), which result in improved milk production (The Organic Farmer, 2019). Desmodium grows extensively, both during the rainy and the dry seasons thus acting as live mulch and extending striga weed control to when the host crop is not in season ( Khan et al., 2011). The cropping system is beneficial to resource constrained small holder farmers because it fits well with traditional mixed farming systems in Africa, uses

locally available resources and is therefore, easy to adopt without investing much time and resources (icipe, 2019).

The strategy is highly compatible with other strategies for insect pest control such as use of chemicals and cultural practices like crop rotation. Push-pull reduce the amount of insecticides required and subsequently the opportunity for pests to become resistant (Cook et al., 2007). Together with the grass, desmodium also conserves soil moisture, prevents soil erosion, enhances arthropod abundance and diversity and protects maize from strong winds (Khan et al., 2008). The roots of desmodium fix nitrogen, avail phosphorus to the cereal crop and improves soil organic matter (Khan et al., 2008; Midega et al., 2013). The result of the striga and insect control through the diverse mechanisms described, coupled with the ecosystem services, is increased grain yield (Khan et al., 2008). Push-pull farmers also earn money from selling desmodium and milk.

### 2.8 Effects of root exudates and soil nutrition on fungal populations in soil

Different plants produce secondary chemical compounds from different tissues, which are categorized based on their biosynthetic pathway (Bourgaud et al., 2001). Plants growing in agricultural soils are in contact with pathogenic and beneficial fungal community (Broeckling et al., 2008). Both the beneficial and pathogenic fungi in soils are diverse and the diversity is positively or negatively influenced by the crops grown, through their root exudate profiles (Wang et al., 2017). Root exudates of different plants contain different groups of chemical compounds, and exudates of certain plants stimulate or inhibit growth of fungal spores. Therefore, the fungal biomass in soil changes when non-resident plant species and genotypes are introduced. The diversity and fungal biomass in the soil could also be influenced by developmental stages of the resident plants (Hooper et al., 2015; Karagiannidis et al., 1997; Mougel et al., 2006). Beneficial

microbial community in the soil, rhizosphere and endosphere, facilitates the shuttling of nutrients and information in and out with the soil matrix (Lundberg et al., 2012).

Over time, cultivated soils become increasingly depleted of nutrients, most importantly nitrogen and phosphorus (Pasuquin et al., 2014; Tamene et al., 2016). Replenishment of essential nutrients in agricultural soils can be done by application of mineral fertilizers, organic manure or companion cropping of cereals with legumes ( Jama et al., 2000; Kifuko et al., 2007; Ndung'u et al., 2006). Use of mineral fertilizers in maize farming in Sub-Saharan Africa is limited by many constraints including but not limited to limited availability, high cost of buying and inadequate application in farms (Kerr et al., 2007; Vanlauwe & Giller, 2006). Organic manure is made from decomposed highly nutritious plants such as *Tithonia diversifolia* which is high in nitrogen, potassium and phosphorus on dry matter basis and farm yard manure. However, processing and application of organic manure is labor intensive. The quality of nutrients available in soils amended with organic manure at different times is different thus limiting the expected benefits (Gachengo et al., 2004).

Soil pH, soil types, soil nitrogen, phosphorus, and carbon content, soil porosity, temperature and relative humidity among others, are important environmental factors that may influence microbial biomass in the soil (Zhao et al., 2016). Availability of adequate nutrients in the soil is an important aspect for optimum maize growth and production (Pasuquin et al., 2014; Tamene et al., 2016). Previous studies reported nitrogen and phosphorus deficiency as important constraints to maize production in western Kenya (Weisskopf et al., 2009). The diversity and abundance of fungal communities in the soil also shifts with changes in cropping systems. (Debenport et al., 2015) reported that some bacterial and fungal communities in millet fields were higher in shrub intercropping, while others were higher in millet monocrop. They also reported that a difference in the species of the same fungal genera present in millet monocrop and millet-shrub intercrop.

# CHAPTER THREE

## SOCIO-ECONOMIC AND AGRONOMIC FACTORS ASSOCIATED WITH EAR ROTS AND MYCOTOXIN CONTAMINATION OF MAIZE UNDER PUSH-PULL AND NON-PUSH PULL PRODUCTION SYSTEMS IN WESTERN KENYA

### 3.1 Abstract

Maize farmers in western Kenya practice diverse cropping systems, and many of them have adopted the push-pull technology for management of stemborers and striga weed. A household survey that covered 255 respondents, 116 push-pull and 139 non-push-pull, was conducted to collect data on socio-economic and agronomic factors that influence farmers' knowledge and occurrence of ear rots and contamination of maize with mycotoxins. Maize ear samples were collected from the standing crop from each farmer's field and analysed for ear rot pathogens, aflatoxin and fumonisin. Twenty six percent of respondents were knowledgeable about aflatoxin while 50% were knowledgeable about ear rots in maize. *Aspergillus flavus* and *Fusarium verticillioides* were isolated in significantly ($P < 0.05$) lower frequencies in samples from push-pull farms. Push-pull reduced contamination of maize samples with aflatoxin levels above 10 µg/kg by 4% and fumonisin levels above 1000 µg/kg by 46%. Socio-economic and agronomic factors of farmers did not differ between cropping systems. Farmers from different counties differed with their knowledge of ear rots and aflatoxin ($P < 0.05$). Farmers practicing push-pull had less knowledge of ear rots ($P < 0.05$), while farmers with better education were significantly ($P < 0.05$) more aware of ear rots. Furthermore, the elderly (45-60 years) were more knowledgeable of aflatoxin than younger respondents. Significantly higher ($P < 0.05$) aflatoxin and fumonisin levels were detected in samples from DAP fertilizer treated fields. Furthermore, levels of aflatoxin were positively associated with knowledge of stemborer damage of maize. These results indicate that contamination of maize with the two mycotoxins was influenced both by pre-harvest farming practices and adaptation of push-pull cropping system. These results imply that creating awareness is key to mitigation of ear rots and mycotoxin contamination of maize.

**Key words:** Aflatoxin, ear rots, fumonisin, mycotoxins, push-pull, *Zea mays*

## 3.2 Introduction

Nearly all Kenyan agricultural households grow maize, 70% of them are small holder (Keya & Rubaihayo, 2013; Kibet, 2011). Western Kenya is one of the country's major maize production and consumption regions. However, production and consumption of maize in this region is constrained by numerous factors, including low soil fertility, insect pests, weeds, diseases and mycotoxins (Rajcan & Swanton, 2001; Schmale & Munkvold, 2009; Vanlauwe et al., 2008). One of the major diseases of maize is ear rots, whose visual characteristics are colored molds on and between grains (Bigirwa et al., 2007). some of the fungi that cause ear rots including *Fusarium*, *Aspergillus*, and *Penicillium* contaminate infected maize with mycotoxin (Dragich & Nelson, 2014).

Most common mycotoxins associated with maize are aflatoxins, fumonisins, deoxynivalenol and ochratoxins (Haschek & Voss, 2013; Kimanya, 2015). Aflatoxin is produced by *A. flavus* and *A. parasiticus* while fumonisin is mainly produced by *F. verticillioides*, and to a lesser extent by *F. proliferatum* (Leslie & Summerell, 2006; Mutegi et al., 2018; Samson & Varga, 2009). In spite of the presence of several reports on occurrence of aflatoxin and fumonisin in maize in western Kenya (Mutiga et al., 2015), there has been little success on alleviating the problem. (Owuor et al., 2018) reported reduced occurrence of ear rots and associated mycotoxins in maize grown under the push-pull cropping system. However, whether cropping systems and farming practices have mechanisms that can control mycotoxins has not been studied. Farming practices such as continuous cultivation, tillage practice, handling of crop residues, time of planting and harvesting and drying efficiency after harvest have been reported to influence mycotoxin accumulation in maize(Bruns, 2003; Mbuge et al., 2016; Mutiga et al., 2014).

Push-pull cropping system, is a companion cropping system in which maize is intercropped with insect repellent crops ('push') and planting attractive trap plants ('pull') around this intercrop (Khan et al., 2011). The cropping system effectively control lepidopteran pests, such as stemborers and fall armyworm, resulting in improved crop yields (Midega et al., 2010, 2018). The foliage of the intercrop emits repellant chemicals that 'push' away the insects while the border crop produces chemicals attract them. The roots of *Desmodium* spp. suppress growth of the parasitic striga weed and enhance soil health through a number of mechanisms including biological nitrogen fixation, increase in organic matter content and conservation of soil moisture (Khan et al., 2000). The objective of this study was to determine the socio-economic and agricultural practices of push-pull and non-push-pull maize farmers in western Kenya, including their awareness on maize ear rots and mycotoxins, and the influence of these factors on production of aflatoxin and fumonisin in maize.

## 3.3 Materials and Methods

### 3.3.1 Description of the study sites

This study was conducted in Kisumu, Vihiga, Siaya, Kakamega and Migori counties of western Kenya. These five counties are representative of regions where the push-pull cropping system has been widely adopted by smallholder farmers for management of stemborer and striga weed in maize fields. In these counties, farmers depend on maize for both food and source of family income. The five counties have characteristic two rainy seasons: the long rains farming season which run from March to August and the short rain farming season from October to January. Table 3.1 shows the agro-ecological features of the study sites while the sampling sites and points are shown in Figure 3.1. One hundred and sixteen respondents practicing push-pull cropping system and 139 who did not were arbitrarily selected from a listed provided by International Centre of

Insect Physiology (icipe) field-based staff in consultation with local government extension officers based on presence of a standing crop at the time the survey was conducted.

Table 3. 1**:** Agro-ecological characteristics of the sub-counties in five counties where household survey was conducted, and maize samples were collected

| County | Sub-counties | AEZ | Altitude (m) | Rainfall [a] | Temperature [b] |
|---|---|---|---|---|---|
| Siaya | Ugunja | LM 1, 2 | 1200 - 1500 | 1450 - 1900 | 20.9 - 22.3 |
| Kakamega | Khwisero | UM 1, LM1 | 1300 - 1900 | 1650 - >2000 | 18.5 - 22.2 |
| Kisumu | Kisumu west | LM 2, 3 | 1140 - 1500 | 1050 - 1600 | 20.9 - 22.7 |
| Migori | Rongo, Awendo | LM 1, 2 | 1300 - 1550 | 1300 -1800 | 20.4 - 21.7 |
| Vihiga | Emuhaya, Luanda | UM 1, LM1 | 1300 - 1900 | 1650 - >2000 | 20.1 - 22.2 |

AEZ – agro-ecological zones, LM – lower midland, UM – upper midland, [a] – average annual rainfall (mm), [b] – average annual temperature (°C)
Source: (Jaetzold et al., 2009)

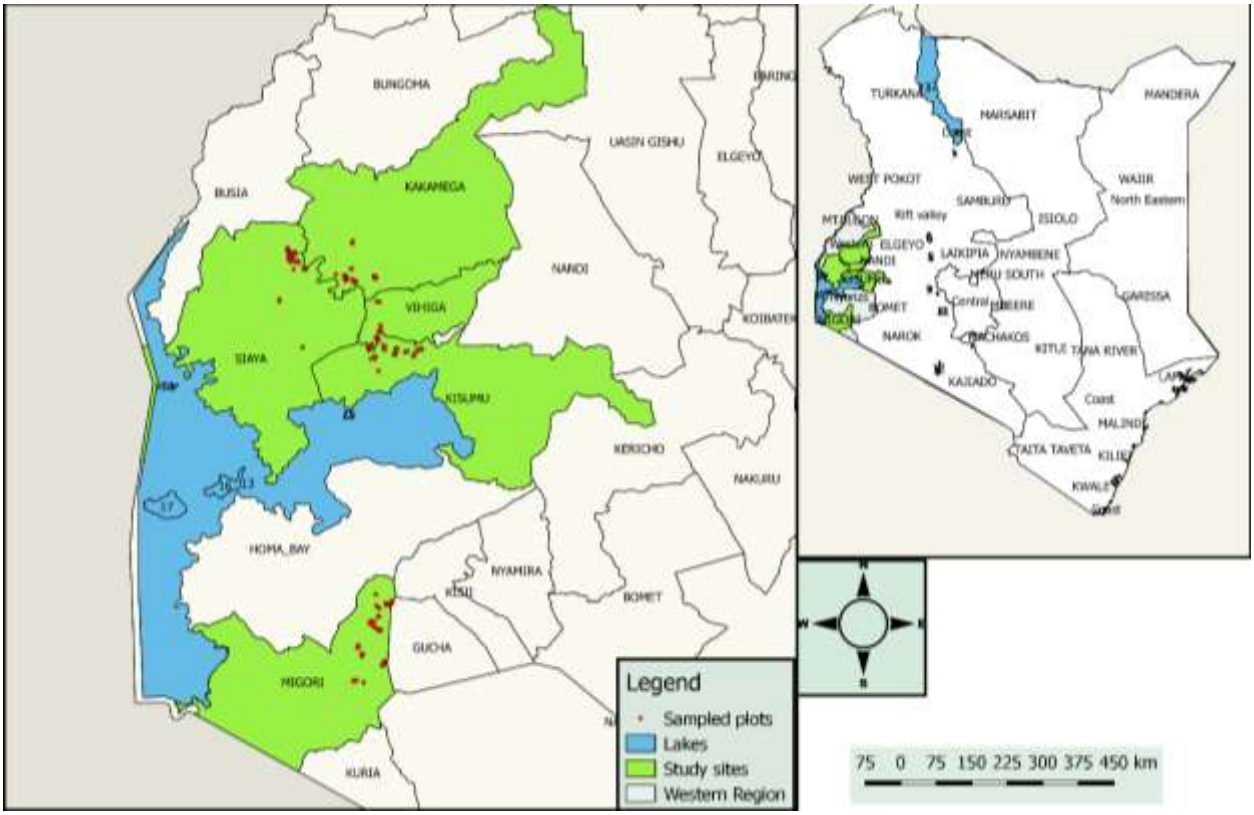

Figure 3. 1: Map of western Kenya showing the study sites and sampling points

### 3.3.2 Collection of socio-economic and agronomic data and maize sampling

A farm survey was conducted between January and February 2017 using a semi-structured questionnaire (Appendix 1). Socio-economic and agronomic data of maize farmers were collected using face to face interviews. Socio and economic data recorded included sex, age, highest level of education, size of land under maize production, knowledge of aflatoxin and fumonisin, maize farming experience, membership of welfare group(s), average annual family income and source of funds for farming activities. For the agronomic practices data collected included type of maize varieties, crop rotation program adopted, sources of seeds, practice of intercropping, cultivation method, soil amendment, handling of stovers after harvest, and awareness about ear rots of maize. The enumerators asked the questions in local languages of the respondents. A maize sample of 10 to 20 ears was sampled from ready to harvest farms of interviewed farmers. The samples were transported to the laboratory where they were sun-dried, after which they were shelled manually, finely ground (Bunn-O-Matic Corporation Coffee Mill, G3-000) and stored at 4°C until analyzed for ear rot pathogens, aflatoxin and fumonisin.

### 3.3.3 Isolation and identification of *Aspergillus* spp. and *Fusarium* spp. in the maize samples

One-gram maize flour sub-sample was mixed with 9 ml sterile distilled water, vortexed for 30 seconds and serially diluted to $10^{-2}$. One hundred microliter aliquots were spread on half strength potato dextrose agar (PDA – 17g, $KH_2PO_4$ – 1g, $KNO_3$ – 1g; $MgSO_4$ – 0.5g, agar - 10g) amended with 50 mg of antibiotics tetracycline, streptomycin and chloramphenicol and 50 mg of antifungal (PCNB). Pentachloronitrobenzene was added before autoclaving while the antibiotics were added after the media was cooled to 45°C in a water bath. Each sample was replicated thrice Three replicates were kept per sample and the inoculated plates were incubated at 25°C for three days,

after which the number of colonies of each all types of fungi in each plate was counted. The number of colony forming units per gram (CFU/g) of ground maize was determined as follows:

$$\text{CFU/g} = \frac{\text{No. of colonies}}{\text{Volume plated (ml)} \times \text{Dilution factor}}$$

The frequency of different fungal genera was calculated as:

$$\text{Frequency (\%)} = \frac{\text{Number of isolates of a genus in a sample}}{\text{Total number of isolates of all the genera in a sample}} \times 100$$

Characteristic colonies of *Aspergillus* and *Fusarium* were transferred separately with sterile toothpicks to PDA and incubated at 25°C for 7-14 days. Colonies of *Fusarium* spp. were also transferred to Synthetic Nutrient Agar (SNA: (Nirenberg, 1981), $KH_2PO_4$ 1.0g, $KNO_3$ 1.0g, $MgSO_4$ 0.5g, KCl 0.5g, Glucose 0.2g, Agar 20g) and incubated for 14-21 days at near UV light to enhance sporulation (Su, Qi, & Cai, 2012). *Aspergillus* spp. colonies were sub-cultured on Czapex Dox agar and incubated for 5 – 7 days at 25°C. Fungal genera were identified using the identification manual by (Humber, 1997). Colonies of *Fusarium* spp. sub-cultured on SNA were used for microscopic identification at ×400 magnification while those transferred to PDA were used for cultural characterization. *Fusarium* spp. were identified using manuals by (Nelson, 1983) and (Leslie & Summerell, 2006) while *Aspergillus* spp. were identified using the manual by (Klich, 2007a). Features used for identification of *Fusarium* spp. included growth pattern, color of aerial mycelia, reverse colony color, macroconidia presence and morphology, microconidia presence and morphology, type of conidiophore, type of phialides and presence or absence of chlamydospores. *Aspergillus* spp. were identified based on colony color, colony diameter, reverse color, sclerotia formation, seriation, size and shape of vesicles and conidia color, size and surface texture at ×400 magnification.

### 3.3.4 Determination of aflatoxin and fumonisin levels in maize samples

Two sub-samples of 20 g each were extracted with 100 ml of methanol (70%) for aflatoxin and 40 ml of 90% methanol for fumonisin analysis. Extraction was done by shaking the mixtures of sample and methanol in sealed containers for two minutes for aflatoxin and one minute for fumonisin. The residue was allowed to settle, and the supernatant was filtered through Whatman No.1 filter paper. Extracts for fumonisin analysis were diluted (1:20 v: v) with distilled water. Presence and quantity of aflatoxin and fumonisin in the samples was determined by direct competitive Enzyme Linked Immuno-Sorbent Assay (ELISA) following instructions by the manufacturer (Helica Biosystems Inc.). The ELISA kit included conjugate solutions, standards, 96 antibody coated microliter wells, substrate/chromogen, stop solution, dilution wells and PBS-buffer.

The aflatoxin test kit had limit of detection ranging between 1 and 20 µg/kg, while the corresponding limits for fumonisin kit were 100 and 6000 µg/kg, respectively. A standard curve for each kit was plotted and intrapolated to determine the levels of toxin in each sample. Samples that had toxin levels beyond the upper limit of the kits were diluted with distilled water and the toxin analysis repeated, putting into consideration the additional dilution factor.

### 3.3.5 Data analyses

Data from the survey was statistically analyzed using SPSS version 22 to determine percentages, frequencies, means and standard errors. Data on populations of *Fusarium* and *Aspergillus* spp. were analyzed by linear mixed models fitted by REML and means were separated by LSD in R Studio version 3.5.1 (RStudio Team, 2015). In the model, cropping system and county were set as fixed effects while farmer identity was set as random effects.. Aflatoxin and fumonisin data were categorized into: (i) below limit of detection of the kits, (ii) levels below the regulatory threshold

set by Kenya Bureau of Standards (KEBS) for aflatoxin and European Commission (EC), which Kenya adopts for fumonisin, and (iii) levels above the regulatory threshold. Spearman correlation was performed in SPSS to establish the relationship among *A. flavus, F. verticillioides* and their respective toxins. The association among farmers' awareness of ear rots, aflatoxin, fumonisin and socio-economic characteristics was tested by binary logistic regression while logistic regression tested the association between farming practices with aflatoxin and fumonisin levels in maize.

## 3.4 Results

### 3.4.1 Socio and economic features of small holder push-pull and non-push-pull farmers in the five study counties in western Kenya

All the respondents were small holder farmers whose average annual income were below 55,000 Kenyan shillings and depended on maize for food and income to finance farming activities and other needs (Table 3.2). More than half of the respondents were female, aged 31 to 60 years. However, non-push-pull respondents had more female (70%) than push-pull respondents (57%). A significantly ($P < 0.05$) lower percentage of push-pull respondents was less than 30 years. Most (> 80%) respondents were members of one or more welfare group(s). Majority of the respondents had some level of literacy, as indicated by the large (> 60%) number of respondents with at least primary school education (Table 3.2). Nevertheless, significantly higher number of non-push-pull respondents lacked formal education ($P < 0.05$). Approximately 27% of the respondents were knowledgeable on aflatoxin, but only one respondent was knowledgeable on fumonisin. Over 50% of respondents had knowledge on ear rots in maize, though, the ear rots occurred in low incidence. None of the respondents had any knowledge on management practices of ear rots, and the two mycotoxins. More than 50% had been farming maize for at least 10 years.

Table 3. 2: Socio and economic features of small holder push-pull and non-push-pull maize farmers in the five study counties in western Kenya

| Features | | Proportion of farmers (%) | | |
|---|---|---|---|---|
| | | Push-pull | Non-push-pull | P value |
| Age (years) | 18-30 | 4 | 11 | 0.133 |
| | 31-45 | 33 | 37 | |
| | 46-60 | 38 | 33 | |
| | Over 60 | 25 | 19 | |
| Level of formal education | None | 2 | 5 | 0.205 |
| | Not completed primary | 19 | 24 | |
| | Primary | 28 | 32 | |
| | Secondary | 37 | 32 | |
| | Tertiary | 14 | 7 | |
| Members of welfare group(s) | | 79 | 94 | 0.004 |
| Farming experience (years) | < 10 | 41 | 30 | 0.078 |
| | 10-20 | 28 | 41 | |
| | > 20 | 31 | 29 | |
| Sources of income | Surplus farm produce | 71 | 66 | 0.213 |
| | Casual labour | 7 | 12 | 0.117 |
| | Small scale business | 11 | 9 | 0.367 |
| | Welfare groups | 15 | 11 | 0.226 |
| Annual income (kes) | 20,000-35,000 | 38 | 41 | 0.557 |
| | 36,000-55,000 | 18 | 19 | |
| | 56,000-75,000 | 15 | 11 | |
| | 76,000-100,000 | 13 | 18 | |
| | Above 100,000 | 16 | 11 | |
| Knowledge on aflatoxin? (yes) | | 39 | 30 | 0.042 |
| Size of land owned (acres) | | 2.5 | 2.0 | 0.053 |

kes – Kenyan shillings, 100 kes = 1 USD

### 3.4.2 Agronomic practices in maize production by push-pull and non-push-pull farmers in five counties in western Kenya

Land preparation was the most (99%) common pre-season practices by the farmers. Additionally, some of them applied farmyard and compost manure (< 10%) and some others (≈ 15%) planted short duration crops including sweet potatoes and vegetables. Land preparation was mostly done by hand-hoe digging, by significantly ($P < 0.001$) more push-pull respondents (Table 3.3). Majority of respondents did not practice crop rotation because they lacked enough land. However, for those that did, groundnuts, sweet potatoes, cassava and millet were the key crops grown.

Significantly ($P < 0.05$) more non-push-pull respondents intercropped maize with other food crops, mainly beans.

Most push-pull respondents planted certified seeds bought from agro-shops or provided by welfare projects. On the other hand, more of the non-push-pull farmers kept seeds from the previous crop for planting (Table 3.3). Farmers from both cropping systems preferred local than hybrid maize varieties. The hybrid maize varieties planted by farmers in order of decreasing popularity were Pioneer, DK8031, WH505, WH513, WH517, WH511, WH515, H516, DH04, H813, H113, Simba 61, G30, IR, prestige and Tarco. Significantly ($P < 0.05$) high number of respondents applied compost and farmyard manure, but, more push-pull (44%) than non-push-pull (22%) respondents used compost manure.

Maize was mainly harvested manually by removing the husk from the ear followed by sun-drying then manually shelling or by cutting whole maize plants followed by drying, husk removal and shelling (Table 3.3.). After harvest, stovers of maize were mainly harvested and used as feed for livestock or left in the field where they were ploughed-in during tillage. Significantly ($P < 0.05$) more of non-push-pull (42%) as opposed to push-pull (33%) respondents ploughed-in maize stovers. Maize stovers were also directly grazed on by cattle, burnt in the field or used as firewood. Over 80% of the respondents stored the shelled maize in polythene sacks on a raised surface above the floor of the house, while less than 10% of the respondents stored the maize in polythene bags on the house floor. The rest of the respondents either stored shelled grains in traditional granaries and drums or used husks to hang ears in the house.

More than 50% of the farmers reported that they encountered slightly rotten maize, with over 90% removing them during shelling. Most respondents mainly fed the rotten maize to livestock such as chicken, cattle or pigs (Table 3.3), while some sold the unwanted maize to local brewers or mixed

it in small ratios with clean maize before taking to posho mill. Maize grain yield was significantly ($P < 0.05$) higher in the push-pull system, as indicated by the respondents.

Table 3. 3: Percentage of push-pull and non-push-pull farmers in five counties in western Kenya who practiced different agronomic practices

| Practice | | Push-pull | Non-push-pull | P value |
|---|---|---|---|---|
| Tillage method | Oxen ploughing | 29 | 41 | 0.023 |
| | Hand hoe digging | 86 | 69 | 0.001 |
| Crop rotation | | 25 | 30 | 0.271 |
| Intercropping food crops | | 60 | 85 | <0.001 |
| | Intercropping with beans | 53 | 71 | 0.002 |
| | Intercropping with groundnuts | 22 | 30 | 0.106 |
| | Intercropping with other crops | 5 | 13 | 0.019 |
| Soil amendments | DAP | 40 | 46 | 0.264 |
| | CAN | 29 | 25 | 0.635 |
| | Compost manure | 44 | 22 | 0.001 |
| | Farmyard manure | 64 | 75 | 0.031 |
| | Others | 7 | 4 | 0.212 |
| Sources of seeds | Own | 42 | 50 | 0.487 |
| | Certified | 51 | 44 | |
| | other | 7 | 6 | |
| Maize variety | Local | 53 | 65 | <0.001 |
| | Hybrid | 40 | 29 | <0.001 |
| Harvesting method | De-husking in the field | 49 | 51 | 0.260 |
| | Cut stovers with ears | 43 | 40 | |
| | Other | 12 | 9 | |
| Use of maize stovers | Harvest for cattle feed | 27 | 27 | 0.522 |
| | Direct grazing of cattle | 34 | 32 | 0.417 |
| | Ploughing in | 33 | 42 | 0.087 |
| | Others | 25 | 11 | 0.004 |
| Sorting maize | | 94 | 99 | 0.257 |
| Reasons for sorting | Avoid eating rotten maize | 65 | 68 | 0.312 |
| | Keep the best for seeds | 8 | 11 | 0.311 |
| | Avoid cross contamination | 9 | 9 | 0.541 |
| | Others | 16 | 12 | 0.272 |
| Use of rotten maize | Feed livestock | 70 | 64 | 0.161 |
| | Sell to local brewers | 7 | 12 | 0.179 |
| | Make compost manure | 2 | 6 | 0.078 |
| | Dispose | 17 | 17 | 0.563 |

DAP – diammonium phosphate, CAN – calcium ammonium nitrate

### 3.4.3 Population of *Aspergillus* and *Fusarium* spp. in maize samples

Only 18% of the total population of fungi recovered from maize samples was from push-pull farms. Five main fungal genera: *Fusarium*, *Aspergillus*, *Penicillium*, *Verticillium* and *Acremonium* spp. were frequently isolated, while 10 others were isolated in low frequencies of less than 0.01%

(Figure 3.2A), with the total population of the fungi isolated being significantly lower in maize samples from push-pull relative to those from non-push-pull farms (Table 3.4). The average population of *Fusarium* and *Aspergillus* spp. was significantly ($P < 0.001$) lower in push-pull maize samples (Table 3.4). Over 80% of *Fusarium* spp. isolates recovered from maize samples from the two systems was *F. verticillioides* and it was the most frequently recovered fungus from both push-pull and non-push-pull maize samples.

*Aspergillus flavus* was isolated in low frequencies in both cropping systems (Figure 3.2B). The average populations of *A. flavus* and *F. verticillioides* were significantly ($P < 0.001$) reduced in maize from push-pull fields, but the populations did not differ among counties (Table 3.5, 3.6). *Aspergillus fumigatus*, *A. niger*, *A. parasiticus*, *A. ostianus* and *A. ochraceous* were isolated from the maize samples in very low frequencies. The population of *F. proliferatum*, though very low, was more reduced in maize samples from push-pull cropping system ($P < 0.001$).

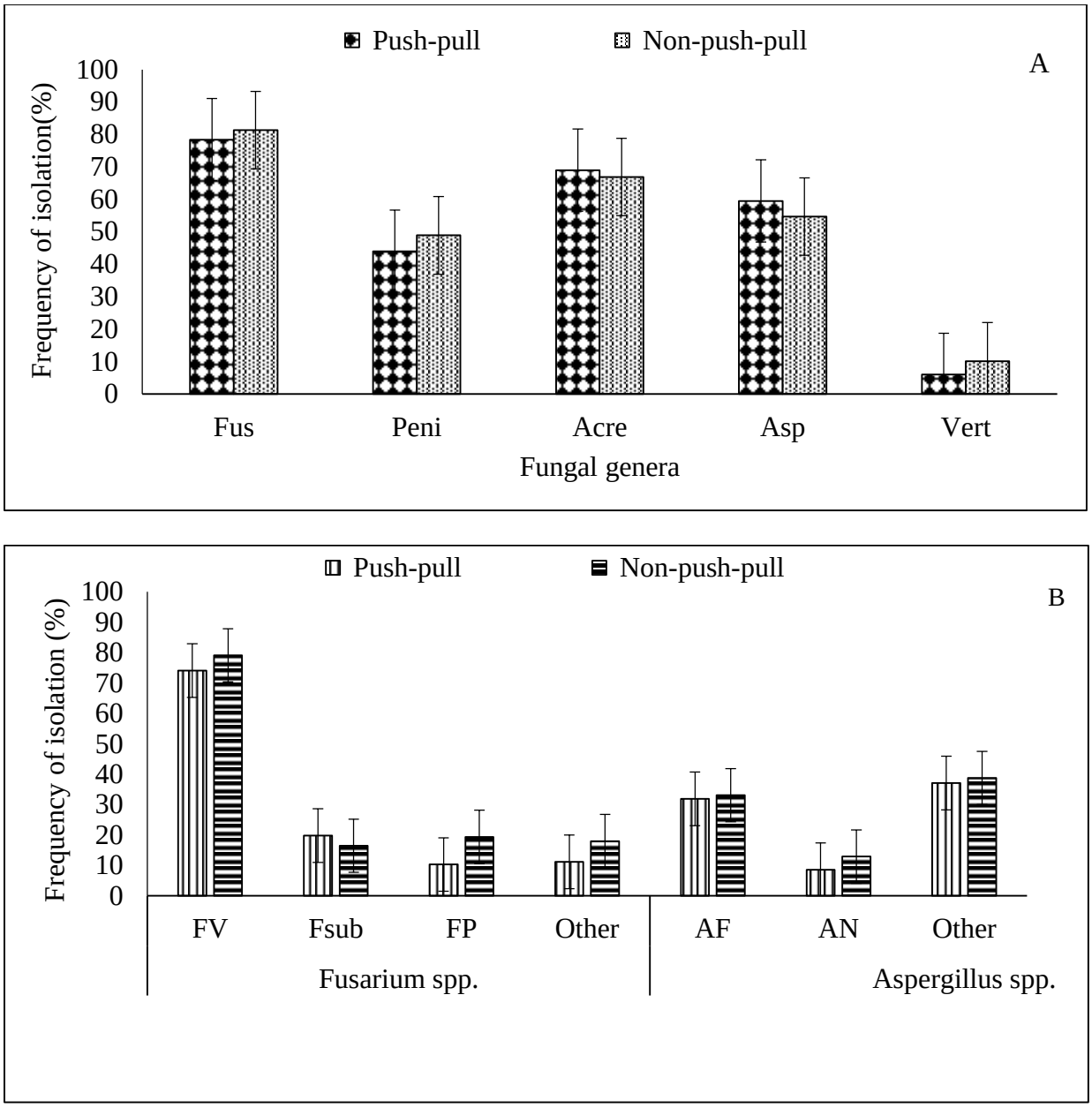


Figure 3. 2: Frequency (%) of isolation of (A) major fungal genera, (B) *Fusarium* and *Aspergillus* spp. in maize sampled from push-pull and non-push-pull farms in western Kenya

Asp – *Aspergillus*, Fus – *Fusarium*, Acre – *Acremonium*, Vert – *Verticillium*, Peni – *Penicillium*, FV – *Fusarium verticillioides*, FP – *F. proliferatum*, Fsub – *F. subglutinans*, AF – *Aspergillus flavus*, AN – *A. niger* (Error bars represent the standard error of the mean)

Table 3. 4: Population (CFU/g) of different fungal genera in maize from push-pull and non-push-pull farms from five counties in western Kenya

| Fungal genera | Cropping system | Kakamega | Kisumu | Migori | Siaya | Vihiga | Mean |
|---|---|---|---|---|---|---|---|
| *Aspergillus* | PPT | 75±31 | 126±51 | 621±337 | 58±17 | 49±28 | 236±99 |
| | NPPT | 165±41 | 2,781±1,295 | 148±59 | 2,594±978 | 6±2 | 1,241±266 |
| | Mean | 130±28d | 1748±797a | 388±173c | 1,334±501b | 27±14e | 785±206 |
| | P value | 0.146 | <0.001 | 0.033 | <0.001 | 0.059 | <0.001 |
| *Fusarium* | PPT | 246±62 | 4,535.4±2,085 | 1,496±431 | 1,126±387 | 2,026±763 | 1,837±424 |
| | NPPT | 2,202±1,087 | 3,112±757 | 22,283±10,876 | 53,020±20,052 | 5,107±2,126. | 17,582±4818 |
| | Mean | 1,453±674d | 3,665±931c | 11,734±5,398b | 27,230±10,260a | 3,613±1,161c | 10,427±2,649 |
| | P value | <0.001 | 0.405 | <0.001 | <0.001 | 0.070 | <0.001 |
| Other fungi | PPT | 5,003±2,570 | 6,307.5±2,659 | 1,513±260 | 1,838±541 | 463±149 | 2,850±646 |
| | NPPT | 3,128±989 | 1,145.4±274 | 4,246±1,471 | 2,948±935 | 1,148±394 | 2,647±453 |
| | Mean | 3,846±1,155a | 3,152±1,061b | 2,859±741c | 2,396±542d | 816±217e | 2,740±384 |
| | P value | 0.265 | <0.001 | 0.001 | 0.179 | 0.048 | 0.660 |
| Total population | PPT | 5,325±2,616 | 10,654.4±4,425 | 3,644±619 | 3,023±667 | 2,516±756 | 4,869±939 |
| | NPPT | 5,502±1,544 | 7,246.5±1,638 | 26,658±11,169 | 58,563±20,750 | 6,225±2,213 | 21,511±4,994 |
| | Mean | 5,434±1,377d | 8,571±1,987c | 14,979±5,556b | 30,961±10,635a | 4,427±1,206e | 13,949±2,773 |
| | P value | 0.927 | 0.160 | <0.001 | <0.001 | 0.012 | <0.001 |

Data are means ± standard error of the mean
PPT – push-pull technology, NPPT – non-push-pull technology

Table 3. 5: Population (CFU/g) of different *Aspergillus* spp. in maize from push-pull and non-push-pull farms from five counties in western Kenya

| *Aspergillus* spp. | | Kakamega | Kisumu | Migori | Siaya | Vihiga | Mean |
|---|---|---|---|---|---|---|---|
| *A. flavus* | PPT | 2.7±1.9 | 19.2±7.7 | 12.8±4.4 | 24.9±13.6 | 7.3±4.7 | 14.5±3.8 |
| | NPPT | 101.1±31.0 | 176.4±88.8 | 21.7±9.0 | 1052.4±448.0 | 1.5±0.5 | 276.4±93.0 |
| | Mean | 63.4±19.5c | 115.3±54.6b | 16.2±5.0d | 541.8±228.3a | 4.2±2.3e | 157.4±51.0 |
| | P value | <0.001 | 0.011 | 0.533 | <0.001 | n/s | <0.001 |
| *A. niger* | PPT | 0.9±0.5 | 4.9±3.5 | 0.1±0.1 | 5.5±3.8 | 2.1±2.1 | 2.6±1.1 |
| | NPPT | 3.3±1.5 | 322.1±224.9 | 10.2±10.1 | 1,513.0±892.4 | 2.4±2.0 | 381.0±185.9 |
| | Mean | 2.4±1.0a | 198.8±137.8a | 5.1±5.0a | 763.8±451.4a | 2.2±1.4 | 208.1±101.6 |
| | P value | n/s | 0.006 | 1.000 | <0.001 | 0.369 | 0.948 |
| Aspergilli | PPT | 71.6±31.3 | 101.8±45.3 | 31.2±12.0 | 28.2±11.0 | 40.0±24.7 | 50.7±11.0 |
| | NPPT | 61.0±22.6 | 2,282.6±1108.6 | 116.7±58.6 | 28.7±11.8 | 2.7±2.0 | 585.6±265.4 |
| | Mean | 65.0±18.3b | 1,434.6±681.5a | 73.3±30.0b | 28.5±8.0 b | 20.8±12.1b | 342.5±145.1 |
| | P value | 0.823 | 0.003 | 0.144 | 0.982 | n/s | 0.867 |

Data are means ± standard error of the mean

PPT – push-pull technology, NPPT – non-push-pull technology

Table 3. 6: Population (CFU/g) of different *Fusarium* spp. in maize from farms of push-pull and non-push-pull respondents in the five study counties

| *Fusarium* spp. | CS | Kakamega | Kisumu | Migori | Siaya | Vihiga | Mean |
|---|---|---|---|---|---|---|---|
| *F. verticillioides* | PPT | 239.3±62.3 | 3577.9±1455.3 | 1383.6±432.5 | 1,000.7±383.9 | 2,025.2±763.7 | 1600.9±325.9 |
| | NPPT | 2199.0±1087.2 | 3106.3±757.1 | 22082.3±10880.0 | 53,020.0±20,052.5 | 5,104.9±2,127.0 | 17532.2±4818.6 |
| | Mean | 1,448.4±674.6 | 3,289.7±728.5 | 11,578.5±5,399.3 | 27,168.0±10,261.4 | 3,611.6±1,161.4 | 10.292.6 |
| | P value | <0.001 | 0.766 | <0.001 | <0.001 | 0.080 | <0.001 |
| *F. proliferatum* | PPT | 0.0±0.0 | 4.8±3.5 | 1.0±1.0 | 1.2±1.2 | 0.0±0.0 | 1.4±0.8 |
| | NPPT | 2.4±2.3 | 4.0±2.5 | 43.5±23.4 | 0.0±0.0 | 2.5±1.7 | 12.1±5.6 |
| | Mean | 1.5±1.4 | 4.3±2.0 | 21.9±11.6 | 0.6±0.6 | 1.3±0.9 | 7.2±3.1 |
| | P value | 1.000 | 1.000 | <0.001 | 1.000 | 1.000 | <0.001 |
| *F. subglutinans* | PPT | 0.0±0.0 | 317.8±317.5 | 88.1±37.2 | 124.6±76.6 | 0.0±0.0 | 112.4±60.9 |
| | NPPT | 1.1±1.1 | 1.0±1.0 | 140.4±78.4 | 0.0±0.0 | 0.0±0.0 | 33.7±18.7 |
| | Mean | 0.7±0.7 | 124.2±123.4 | 113.8±42.9 | 61.9±38.2 | 0.0±0.0 | 69.4±29.5 |
| | P value | 1.000 | 1.000 | <0.001 | 0.895 | n/s | 0.255 |
| Fusaria | PPT | 7.6±4.8 | 634.9±445.3 | 23.5±14.8 | 0.0±0.0 | 1.0±1.0 | 122.8±81.0 |
| | NPPT | 0.0±0.0 | 1.3±1.0 | 17.2±9.1 | 0.4±0.3 | 0.0±1.0 | 4.5±2.2 |
| | Mean | 2.9±1.9 | 247.7±174.0 | 20.4±8.7 | 0.2±0.1 | 0.5±0.5 | 58.2±36.9 |
| | P value | 1.000 | 1.000 | <0.001 | 1.000 | 1.000 | 0.010 |

Data are means ± standard error of the mean

PPT – push-pull technology, NPPT – non-push-pull technology

### 3.4.4 Concentration of aflatoxin and fumonisin in maize sampled from push-pull and non-push-pull farms in five counties in western Kenya

Approximately 8% and 12% of the maize sampled during the survey were contaminated with aflatoxin (Table 3.7). Contamination of maize with aflatoxin was significantly ($P < 0.05$) associated with the study county ($P = 0.023$). Individual counties had only below 10% of maize from push-pull containing aflatoxin, except Siaya which had 12%. However, the proportion was on slightly higher for non-push-pull samples ($P = 0.414$), ranging from 6% - 25% in Vihiga and Siaya, respectively. All the maize samples from push-pull were contaminated with aflatoxin levels below 10 µg/kg, which is KEBS' regulatory threshold while an average of 4.3% of samples from farms not under push-pull had concentrations above the regulatory threshold. Proportion of samples contaminated with aflatoxin was significantly ($X^2 = 9.611$, $P = 0.049$) higher in samples from Siaya and Kakamega than in Migori, Kisumu and Vihiga.

The percentage of samples with fumonisin contamination was, however, higher in all the counties and cropping systems (Table 3.7). Overall, 5.1% from push-pull and 9.4% of samples from non-push-pull farms had fumonisin concentrations above 1000 µg/kg, EC regulatory threshold ($P = 0.002$). The percentage of samples from push-pull that were contaminated with fumonisin ranged between 5.6% and 23.8%, while the corresponding proportions from non-push-pull samples were 11.3% and 37.5%. A significantly ($P < 0.05$) larger number of samples from non-push-pull were contaminated with fumonisin concentrations higher than 1000 µg/kg across the counties. The number of samples contaminated with fumonisin did not vary significantly ($X^2 = 5.460$, $P = 0.243$) among the five counties.

Ninety-two percent of samples from non-push-pull farms contaminated with aflatoxin concentrations above KEBS' limit was from Siaya and Kakamega counties, while 50% of samples contaminated with fumonisin above EC threshold were from Migori and the other 50% were from

Siaya and Kakamega counties. Samples from Migori and Vihiga counties displayed higher difference between cropping systems in terms of proportions of samples with fumonisin levels above 1000 µg/kg. Only 2.8% of total samples were co-currently contaminated with both mycotoxins, with 0.9% and 4.3% of push-pull and non-push-pull maize samples, respectively, contaminated with both mycotoxins.

Table 3. 7: Proportion (%) of maize samples contaminated with aflatoxin and fumonisin levels below limit of detection of the kits and above the KEBS and EC regulatory threshold

| County | Cropping system | N | Proportion of samples (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Aflatoxin (µg/kg) | | | Fumonisin (µg/kg) | | |
| | | | < 1 | < 10 | > 10 | < 100 | < 1000 | > 1000 |
| Kakamega | Push-pull | 18 | 94.4 | 5.6 | 0 | 94.4 | 5.6 | 0 |
| | Non-push-pull | 29 | 79.3 | 10.4 | 10.3 | 89.7 | 3.4 | 6.9 |
| Kisumu | Push-pull | 21 | 95.2 | 4.8 | 0 | 76.2 | 19.0 | 4.8 |
| | Non-push-pull | 34 | 97.1 | 2.9 | 0 | 73.5 | 23.6 | 2.9 |
| Migori | Push-pull | 34 | 91.2 | 8.8 | 0 | 79.4 | 14.7 | 5.9 |
| | Non-push-pull | 32 | 96.9 | 3.1 | 0 | 81.3 | 0.0 | 18.7 |
| Siaya | Push-pull | 27 | 88.9 | 11.1 | 0 | 88.9 | 7.4 | 3.7 |
| | Non-push-pull | 28 | 75 | 17.9 | 7.1 | 71.4 | 14.3 | 14.3 |
| Vihiga | Push-pull | 16 | 93.8 | 6.2 | 0 | 87.5 | 12.5 | 0 |
| | Non-push-pull | 16 | 93.7 | 0.0 | 6.3 | 62.5 | 25.0 | 12.5 |
| P value (cropping systems) | | | | | 0.029 | | | 0.328 |

N – sample size, 1 - lower limit of detection for aflatoxin, 100 µg/kg - lower limit of detection for fumonisin, 10 µg/kg – KEBS regulatory threshold, 1000 µg/kg – EC regulatory threshold

### 3.4.5 Associations among socio-economic characteristics of farmers, knowledge on ear rots, knowledge of aflatoxin, farming activities, *F. verticillioides*, *A. flavus*, aflatoxin and fumonisin contamination in maize

Older farmers, particularly farmers aged 46 to 60 years, were significantly ($P < 0.05$) more (48%) aware of aflatoxin regardless of the cropping system adopted (Table 3.8). Respondents from Kisumu were the most knowledgeable on aflatoxin compared to respondents from the other counties. The percentage of push-pull respondents who were aware of maize ear rots was 4% lower than that of non-push-pull respondents. Regression analysis showed that this was 0.34 times

significantly ($P < 0.05$) lower than non-push-pull (Table 3.8). Respondents who completed primary school education were 5 times more knowledgeable on maize ear rots than those without formal education, with respondents from Kisumu being more knowledgeable than respondents from the other counties. Knowledge on ear rots and aflatoxin were positively correlated with each other ($P < 0.05$) ($r = 0.338^{**}$).

Maize from farms whose soil was amended with DAP fertilizer at planting had aflatoxin levels increased 3.9 times above 10 µg/kg ($P < 0.05$) (Table 3.9). Aflatoxin levels were also significantly ($P < 0.05$) higher in maize damaged by stemborer in the field (2.0 times) and in maize grown together with other food crops such as sorghum and cassava (0.3 times). Other agronomic practices that showed association with high aflatoxin levels included use of compost manure, hand hoe tillage, ploughing in harvested maize stovers, grazing of cattle on maize remains directly in the field and intercropping maize with beans. Number of samples contaminated with levels of fumonisin greater than 1000 µg/kg significantly increased (0.3 times) in maize from soils amended with DAP at planting ($P < 0.05$) (Table 3.8). Majority of the other agronomic practices tested showed insignificant influence on fumonisin levels.

The population of *A. flavus* and aflatoxin levels in maize were positively and significantly correlated, irrespective of the cropping system ($r=0.134^{*}$). Similarly, an increase in population of *F. verticillioides* significantly ($P < 0.05$) increased with the levels of fumonisin ($r=0.248^{**}$). There was, however, no correlation in the occurrence and levels of the two mycotoxins.

Table 3. 8: Association among socio-economic traits of farmers, awareness of aflatoxin and maize ear rots of push-pull and non-push-pull farmers in the five study counties

| Socio-economic trait | Awareness of aflatoxin | | | Awareness of ear rots | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | P value | Odds ratio | 95% CI | P value |
| Age group (years) | | | 0.004 ** | | | 0.195 |
| 18-30 | 0.47 | 0.03-6.45 | 0.574 | 0.38 | 0.06-2.39 | 0.304 |
| 31-45 | 2.37 | 0.65-8.71 | 0.192 | 1.33 | 0.38-4.65 | 0.653 |
| 45-60 | 7.52 | 2.14-6.43 | 0.002 ** | 2.16 | 0.67-6.97 | 0.196 |
| above 60 | 0[a] | 0a | | 0[a] | 0a | |
| Highest education | | | 0.589 | | | 0.134 |
| No formal education | 0.67 | 0.05-8.67 | 0.76 | 1.38 | 0.10-17.71 | 0.804 |
| Not completed primary | 0.24 | 0.04-1.53 | 0.132 | 6.8 | 1.19-38.56 | 0.03 * |
| Completed primary | 0.37 | 0.07-2.10 | 0.263 | 5.32 | 1.00-28.18 | 0.05 * |
| Secondary | 0.51 | 0.11-2.49 | 0.408 | 2.59 | 0.55-12.22 | 0.23 |
| Tertiary | 0[a] | 0a | | 0[a] | 0a | |
| Maize farming experience (years) | | | 0.579 | | | 0.214 |
| Less than 10 | 1.07 | 0.35-3.23 | 0.912 | 2.37 | 0.74-7.55 | 0.145 |
| 20-Oct | 1.67 | 0.59-4.74 | 0.337 | 0.92 | 0.32-2.57 | 0.873 |
| Over 20 | 0[a] | 0a | | 0[a] | 0a | |
| Push-pull | 1.95 | 0.81-4.70 | 0.137 | 0.34 | 0.13-0.82 | 0.017 * |
| Non-push-pull | 0[a] | 0a | | 0[a] | 0a | |
| County | | | 0 *** | | | 0 *** |
| Siaya | 0.23 | 0.07-0.77 | 0.016 * | 0.2 | 0.04-0.99 | 0.049 * |
| Kisumu | 1.1 | 0.33-3.61 | 0.878 | 0.49 | 0.09-2.61 | 0.4 |
| Kakamega | 0 | 0 | 0.997 | 0 | 0 | 0.996 |

[a] – Parameter used as reference

* Significant at 0.05%, ** significant at 0.001%, *** significant at 0.0001%

Table 3. 9: Relationship among aflatoxin, fumonisin and agronomic practices of farmers in the five study counties

| Agronomic practice | Aflatoxin | | | Fumonisin | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | P value | Odds ratio | 95% CI | P value |
| Application of DAP during planting (1=Yes 0=No) | 3.88 | 1.22-12.38 | 0.022* | 0.28 | 0.09-0.90 | 0.032* |
| Application of FYM during planting (1=Yes 0=No) | 1.07 | 0.38-3.05 | 0.9 | 0.91 | 0.31-2.63 | 0.86 |
| Application of compost manure (1=Yes 0=No) | 0.61 | 0.24 -1.52 | 0.287 | 0.52 | 0.21-1.29 | 0.158 |
| Hand hoe digging cultivation (1=Yes 0=No) | 1.12 | 0.43-2.95 | 0.814 | 1.33 | 0.53-3.37 | 0.541 |
| Oxen ploughing cultivation (1=Yes 0=No) | 0.9 | 0.36-2.23 | 0.817 | 0.88 | 0.37-2.11 | 0.776 |
| Keep seeds from previous crop (1=Yes 0=No) | 2.01 | 0.35-11.57 | 0.433 | 1.36 | 0.28-6.51 | 0.704 |
| Plant certified seeds (1=Yes 0=No) | 1.39 | 0.23-8.37 | 0.717 | 2.18 | 0.43-10.94 | 0.345 |
| Maize variety planted (1=Local 0=Hybrid) | 1.14 | 0.54-2.39 | 0.733 | 1.55 | 0.75-3.19 | 0.239 |
| Crop rotation (1=Yes 0=No) | 0.79 | 0.42-1.49 | 0.471 | 0.64 | 0.34-1.19 | 0.156 |
| Intercrop maize with other food crops (1=Yes 0=No) | 0.26 | 0.08-0.89 | 0.032* | 0.72 | 0.20-2.53 | 0.603 |
| Intercrop maize with beans (1=Yes 0=No) | 2.64 | 0.91-7.67 | 0.074 | 2.81 | 0.91-8.68 | 0.072 |
| Intercrop maize with groundnuts (1=Yes 0=No) | 0.74 | 0.33-1.63 | 0.452 | 1.46 | 0.67-3.17 | 0.339 |
| Harvest for maize stovers for hay (1=Yes 0=No) | 0.87 | 0.39-1.94 | 0.736 | 1.03 | 0.47-2.27 | 0.943 |
| Directly graze livestock on maize stovers (1=Yes 0=No) | 0.67 | 0.33-1.39 | 0.283 | 1.41 | 0.69-2.87 | 0.347 |
| Ploughing in maize stovers in the soil (1=Yes 0=No) | 0.74 | 0.39-1.41 | 0.362 | 1.53 | 0.80-2.90 | 0.196 |
| Stemborers are the main insect (1=Yes 0=No) | 1.99 | 1.03-3.87 | 0.041* | 1.05 | 0.55-2.01 | 0.873 |
| Cropping system (1=Push-pull 0=Non-push-pull) | 1.25 | 0.64-2.44 | 0.514 | 0.91 | 0.48-1.70 | 0.755 |

* Significant at 0.05%; Aflatoxin category > 10 µg/kg category was set as reference, fumonisin category > 1000 µg/kg category was set as reference

### 3.5 Discussion

The results of the survey showed no difference in social, economic and agronomic practices between push-pull and non-push-pull farmers, except that push-pull farmers did not plough-in maize stovers. This implied that any differences in fungal species and mycotoxins recorded in this study are mainly due to the differences in the cropping systems. The push-pull cropping system involved intercropping maize with the fodder legume desmodium and planting napier/Brachiaria grass at the border of the intercrop while the non-pull-pull cropping systems were mainly maize monocrop, maize+beans intercrop and maize+groundnuts intercrop cropping systems. This also implied the effects of farming practices on aflatoxin and fumonisin levels reported in the study were regardless of the cropping system practiced.

Women were indicated as the most respondents, implying they were the ones managing farming activities in their families. This concurs with reports of previous studies in western Kenya (Midega et al., 2011). FAO report showed that women are an important resource in agriculture and rural economy worldwide (Sofa & Doss, 2011). The largest proportion of smallholder maize farmers in the study sites were aged 31 to 60 years. Majority of the respondents were resource constrained, as indicated by the low average annual income recorded. This could explain why most respondents sold surplus food produce to acquire farm inputs, because they did not have off-farm income. These results also indicated that though literate, many respondents lacked awareness on aflatoxin, fumonisin and maize ear rots. This lack of knowledge poses a great risk to the process of producing and use of safe maize for food and feed.

The most common agricultural activities practiced by majority of respondents in the region included no crop rotation, hand hoe tillage, keeping seeds for planting from previous crop and feeding livestock on rotten/unwanted maize. Previous studies have reported that these practices

are incompatible with integrated approaches for managing maize ear rots, aflatoxin and fumonisin contamination because they allows maize residues to survive for long on the soil surface, which allow survival of toxigenic fungal pathogens until the next crop (Govaerts et al., 2008; Njeru et al., 2016; Nyangi, 2016). For example, directly grazing cattle on stovers moves fungi infected soil and stovers across farms. This makes the method of disposing harvested stovers an important agronomic practice in pre-harvest mitigation of mycotoxin contamination in maize. This is because the practice of burying, destroying or/and removing maize stovers most likely reduce accumulation of saprophytic fungi in the farm (Edwards, 2004; Parsons & Munkvold, 2012) and (Ndemera et al., 2018) reported that the amount of fumonisin B1 in maize is directly affected by the quality of planted seeds, especially with respect to *F. verticillioides* and other systemic fungi.

Both organic and inorganic soil amendments were applied by most respondents at different stages of maize growth, especially at planting. Soil amendments are meant to increase crop productivity. However, if not applied in the recommended amount, timely and correctly, application may enhance mycotoxin contamination of maize and other crops (Arino et al., 2009; Blandino et al., 2008; Hassegawa et al., 2008). This study supported this finding, with significant association between aflatoxin and fumonisin levels and application of DAP fertilizer at planting. Fertilizers influence mycotoxin contamination of maize by altering the rate of decomposition of crop residues, induction of physiological stress on the crop and/or changing the structure of the plant canopy by reducing crop vigor. High levels of phosphorus can harm plants by decreasing the amount of oxygen available for the plant while deficiency of phosphorus make the plant look weak, characterized by thin stems. The effects make the crop susceptible to infection by opportunistic

fungi, for example *A. flavus*, which contaminates maize grains with aflatoxin (Dolezal et al., 2014).

Most farmers hand-sorted maize before shelling in order to remove the rotten ears. Rotten maize has been reported to be highly likely to be contaminated with high concentrations of mycotoxins (Owuor et al., 2018), and therefore sorting lowers the levels of mycotoxins. This, however, depends on availability of more clean than rotten maize to mask the effect of rotting (Afolabi et al., 2006). Unfortunately, some of the rotten maize in the current study ended up in food and feed, because some of the respondents either used the unwanted maize to feed livestock or sold it to brewers of local alcohol. Animal products such as liver, eggs, milk and kidneys obtained from livestock fed on rotten maize exposes consumers to mycotoxicosis (Fink-Gremmels, 2008; Jovaiš Iene ˙ et al., 2016). Therefore, agronomic practices and technologies that reduce the occurrence of ear rots would indirectly lower incidences of human and animal mycotoxicosis through reduction of mycotoxin contamination of maize (Alakonya et al., 2009).

The most predominant fungal species isolated from maize sampled from both cropping systems was *F. verticillioides* but it was significantly lower under push-pull cropping system. Previous studies also reported *F. verticillioides* as the pre-dominant fungus in maize (Alakonya et al., 2009; Kedera et al., 1999). High population of *F. verticillioides* in maize from non-push-pull plots is an indicator of high risk of exposure to fumonisin and other *Fusarium* mycotoxins such as deoxynivalenol and zearalenone while the maize is in storage. The population of *F. verticillioides* recovered from maize sampled from push-pull is also of concern. The low population was responsible for the detected fumonisin in the samples, 5.1% of which was above the EC threshold.

This represents a serious threat if the grain is stored under conducive conditions for fungal population multiplication, particularly with reference to moisture and temperature.

The population and frequency of isolation of *A. flavus* from maize samples was low in both cropping systems but significantly reduced in push-pull farms. The low population could, however, increase and produce more aflatmilloxin after harvest, if the maize is not properly handled and stored. *Aspergillus flavus* is an opportunistic fungus that infects food crops both before and after harvest (Klich, 2007a). The population levels observed in this study could have resulted from multiplicity of factors, including environmental conditions during the study period and agronomic practices employed by the farmers. For example, the farmers applied di-ammonium phosphate (DAP) fertilizer at planting, that has been reported to reduce the population and infection of plants with *A. flavus* (Dereje A, 2014). Occurrence of other fungal species such as *A. parasiticus, F. proliferatum, A. niger, A. ostianus, A. ochraceous* and *Penicillium* spp., though in lower frequencies, implied possible risks of co-occurrence of different mycotoxins, under favorable conditions of temperature and moisture.

According to the findings of this study, fumonisins appeared to be more common mycotoxins than aflatoxins in maize in the study region. This was indicated by the high number of samples contaminated with fumonisin compared to the number contaminated with aflatoxin. Nonetheless, samples contaminated with the two mycotoxins above the KEBS and EC regulatory threshold was reduced by the push-pull cropping system. This accord with the findings of a previous study that reported levels of aflatoxin and fumonisins in maize in the same trend in Bahati District in Tanzania ((Nyangi, 2016). A lower proportion of samples contaminated with the two toxins above the KEBS and EC threshold was, however, lower in the current study than those previously

reported ((Kamala et al., 2015; Mutiga et al., 2015; Nyangi, 2016; Sirma, 2016). This discrepancy could be due to differences in times of sampling, because previous studies targeted stored maize while in this study maize was collected from standing crop. Stored maize that had mycotoxigenic fungi at harvest could have had more mycotoxins produced in storage, in the event of improper storage conditions of moisture and temperature (Chulze, 2010). Additionally, temperature and precipitation conditions of a cropping season also influence mycotoxins (Tirado et al., 2010; Viegas et al., 2016). Climate also, indirectly influences mycotoxin contamination of maize through its influence on human behavior of handling crops in the field. Siaya and Kakamega are warm and that may be why more samples from these sites were contaminated with aflatoxin. Production of aflatoxin is optimal at temperature between 25 to 30°C.

Samples from Vihiga, Siaya and Migori counties had both high population of *F. verticillioides* and high contamination with fumonisin levels above 1000 µg/kg. This suggests that these three sites have similar and conducive environmental conditions of temperature, moisture and relative humidity for *F. verticillioides* proliferation and infection of maize and other cereals, as well as fumonisin production under field conditions. Despite the co-occurrence of the two mycotoxins in the maize samples, there was no correlation in their occurrence as well and between the populations of their producing fungi, *A. flavus* and *F. verticillioides*, respectively. This implies that the presence of aflatoxin and *A. flavus* does not influence the presence of fumonisin and *F. verticillioides*, and vice versa (Mutiga et al., 2015). The correlation between *A. flavus* and aflatoxin and *F. verticillioides* and fumonisin indicates that population of the fungi could be used as an indicator of presence and risk of future exposure to the associated mycotoxins.

The knowledge of aflatoxin was significantly higher among older respondents as opposed to younger ones. It could be that older farmers may have had many channels of learning about it, including national and local news channels during previous aflatoxin outbreaks such as the 2004-2005 incident in lower eastern Kenya (Lewis et al., 2005). There was no correlation between knowledge of aflatoxin and the level of education. Farmers' knowledge on ear rots in maize was the only listed factor that was significantly associated with the cropping system adopted, with push-pull farmers being less knowledgeable of it. This could be because maize under push-pull cropping system did not rot as much as maize grown under other cropping systems. The low mean population of fungi in push-pull maize samples was an indicator of low fungal infection and consequently ear rots of maize. Moreover, level of education below secondary school was associated with lower knowledge on rotting disease of maize as compared to secondary school and tertiary education. This is an implication of the importance of literacy in management of occurrence of ear rots in maize.

Despite the established association among socio-economic factors, agronomic factors, aflatoxin and fumonisin concentrations in maize, maize under push-pull cropping system had reduced levels of both mycotoxins. *Desmodium* foliage emit semio-chemicals that repel gravid lepidopteran moths. The moths are at the same time attracted by the border crop, where they lay eggs. The border grass on the other hand although preferred for egg laying by the moths does not support significant survival and development of the pest larvae. There is also high abundance and activity of the pest's natural enemies, further contributing to lower pest levels and crop damage under the push-pull system. *Desmodium* roots also produces a wide range of allelopathic chemicals, that stop the development of striga weed while others are responsible for improved soil nutrition (Khan et

al., 2000; Midega et al., 2010). Through the above described mechanisms of striga and insect pests management, the cropping system result in increased maize grain yield (Khan et al., 2003). It is also possible that some of the functionality mechanisms of push-pull could be contributing to the observations in the current study, the lower populations of *A. flavus* and *F. verticillioides* as well as the lower contamination of maize under push-pull cropping system with aflatoxin and fumonisin.

### 3.6 Conclusions and recommendations

Exposure to aflatoxin was significantly higher in Siaya and Kakamega than in Vihiga, Kisumu and Migori, while exposure to fumonisin did not differ among counties. Contamination of maize with fumonisin and the fungi that produce them was significantly reduced under push-pull cropping system. This is an implication that push-pull cropping system possess mechanisms that suppress the populations of toxigenic fungi and mycotoxin contamination in maize. It could also be that the reduced tendency to plough-in harvested maize stovers by push-pull farmers reduced survival of fungal inocula on the soil. Further studies are recommended to elucidate the mechanisms involved in reduction of ear rots, ear rot fungi and mycotoxin contamination in maize grown under push-pull. This information will be the key to implementation of push-pull cropping system as a mycotoxin management strategy.

Contamination of maize samples with aflatoxin and fumonisin in the study region was correlated with the socio-economic and agronomic practices of the farmers. Therefore, farmers can be trained to manipulate such practices for management of mycotoxins and ear rots. This creates the need for agricultural agencies to invest in mycotoxin awareness and management trainings. Surveillance

studies are also recommended to reduce occurrence of acute toxicosis caused by these mycotoxins and in development of robust integrated management tools for mycotoxins.

# CHAPTER FOUR

## EFFECT OF STEMBORER AND FALL ARMYWORM MANAGEMENT UNDER PUSH-PULL CROPPING SYSTEM ON OCCURRENCE OF MAIZE EAR ROTS AND MYCOTOXINS

### 4.1 Abstract

Push-pull is a conservation agriculture technology that was developed for farmers in Africa integrating of management of cereal stemborers and Striga. The technology has also been observed to effectively control fall armyworm. This study evaluated the impact of stemborer and fall armyworm damage management by the push-pull cropping system on incidence of maize ear rots and pre-harvest contamination with aflatoxin and fumonisin mycotoxins. The study was conducted between March 2017 and August 2018 during three maize cropping seasons under push-pull and non-push-pull. Incidence of stemborer and fall armyworm damage was determined as percentage of damaged plants while incidence of ear rots was determined as percentage of ears with ear rot symptoms. Maize sampled at harvest was analyzed for aflatoxin and fumonisin producing fungi, aflatoxin and fumonisin levels. Incidence of stemborer and fall armyworm damage of maize was significantly ($P = 0.001$) reduced by over 50% under push-pull cropping system compared to non-push-pull. This resulted in a significant ($P < 0.001$) reduction of the presence of *F. verticillioides* (60%) and that of *A. flavus* (86%), which was reflected by a reduced incidence of ear rots (50%) ($P = 0.001$). The level of fumonisin in the maize from push-pull farms was significantly ($P = 0.048$) reduced by 39% but there was no significant effect on aflatoxin. Incidence of *Fusarium* ear rot significantly correlated with the incidence of stemborer and FAW ear damage, while population of *F. verticillioides* had significant correlation with the incidence of *Fusarium* ear rot. Accordingly, fumonisin levels had significant positive correlation with the population of *F. verticillioides* and incidence of *Fusarium* ear rot. The study showed that push-pull cropping system is an effective strategy for managing maize ear rots and fumonisins.

**Key words:** aflatoxin, ear rots, fall armyworm, fumonisin, push-pull, stemborer, *Zea mays*

## 4.2 Introduction

Complex interaction of climate factors, insect infestation and pre- and post-harvest handling of maize have been associated with fungal infection of maize (Cotty & Jaime-Garcia, 2007; Fountain et al., 2014; Miller, 2008). Climate change causes erratic rainfall or drought, which in turn influence temperature, precipitation and relative humidity which are the most important ecological factors that influence mycotoxin contamination in grains (Fountain et al., 2014; Miller, 2008). Infection of maize ears by *F. verticillioides* at any growth stage of maize can cause *Fusarium* ear rot, either symptomatic or asymptomatic (Bush et al., 2003; Kedera et al., 1999). *Fusarium* ear rot has been reported to be associated with contamination of maize with fumonisins (Bigirwa et al., 2007; Mukanga et al., 2010; Leslie & Summerell, 2006). A study conducted in China suggested a relationship between exposure to fumonisin and human esophageal and liver cancer (Sun et al., 2007). Burger et al. (2017) reported that cancer has no direct association with fumonisin but promotes liver cancer by interrupting lipid metabolism.

Symptoms caused by *A. flavus*, are not always severe in the field but the fungus can still be present at levels that can contaminate maize with aflatoxins (Maina et al., 2016; Mukanga et al., 2010). However, infections caused by *A. flavus* are mostly invisible and therefore shows no visible spore masses on the surface of the kernels (Schoeman, 2012). In fact, visually healthy maize can be highly contaminated by the fungus and aflatoxin. Aflatoxins are human and animal hepatoxic mycotoxins mainly produced by *A. flavus* in maize (Lewis et al., 2005; Tola & Kebede, 2016; Warburton & Williams, 2014). Chronic exposure to aflatoxin exacerbates the epidemics of many diseases such as malaria, tuberculosis and AIDs through suppression of the immune system and interference with nutrition (Williams et al., 2005). Aflatoxins contribute a great deal to the disease

burden in sub-Saharan African countries with repeated exposure (Schmaile III & Munkvold, 2009).

Previous studies reported a significant increase in both symptomatic and asymptomatic infection of maize by mycotoxin-producing fungi with increased infestation of the maize by insects like stemborer (Dowd, 2003; Mehl et al., 2018; Sobek & Munkvold, 1999). These reports indicated that insects can contribute to infection of maize with ear rot fungi both by acting as a vector for ear rot fungal inoculum and exposing kernels to infection by inocula from the environment dispersed by wind and rainfall. A recent study reported that push-pull cropping system reduces the incidence of ear rots and associated mycotoxins in harvested maize (Owuor et al., 2018). These findings created the need to evaluate the mechanisms and effectiveness by which push-pull cropping system controls occurrence of ear rots and mycotoxins.

Push-pull is a companion cropping system that entails intercropping cereals, mainly maize and sorghum, with insect repellent *Desmodium* and planting around this intercrop napier or Brachiaria grass (Hassanali et al., 2008; Khan et al., 2000; Midega et al., 2018). The desmodium emits repugnant volatiles that 'push' away the stemborer and fall armyworm that are pulled by the napier or Brachiaria trap plants that emit attractive volatiles (Khan et al., 2011; Khan et al., 2018; Midega et al., 2018). Insects feeding on maize ears may act as vectors for ear rot fungi to infect the maize (Sobek & Munkvold, 1999). The tunnels and holes made by the insects acts as infection court for fungal inocula, some of which contaminate the developing kernels with mycotoxins. In this study, the impact and effectiveness of reduction of stemborer and fall armyworm damage the under push-pull cropping system on incidence of ear rots and levels of aflatoxin and fumonisin in maize was determined.

## 4.3 Materials and Methods

### 4.3.1 Description of the study site

The study was conducted for three maize cropping seasons between March 2017 and August 2018 in Siaya, Vihiga and Migori that are agro-ecologically different counties in western Kenya. The sampling sites and points are shown in Figure 4.1. The actual agro-ecological zones and sub-counties where sampling was done are showed in Section 3.3.1, Table 3.1. One hundred and twenty fields were sampled each season; 20 push-pull and 20 maize non-push-pull per site were randomly selected from the previous list of farmers interviewed during the farm survey (Section 3.3.1). The same farms were maintained in each county across the three cropping seasons and same maize variety was planted in both push-pull and maize monocrop plots in a season. The farms were approximately 0.04 to 0.10 ha in size, and a push-pull and a non-push-pull were either side by side or up to 100 m apart.

### 4.3.2 Determination of stemborer and fall armyworm damage in maize

Damage of maize with stemborers and fall armyworm was assessed at milk growth stage (R1-R3) (Pioneer, 2019b). The incidence of foliage and ear damage was determined as the number of plants showing damage out of 100 arbitrarily selected maize plants in each farm while the extent of damage was assessed on a scale of 0 – 4, (0=no damage, 1=slight damage, 2=moderate damage, 3=serious damage, 4=dead heart). Stemborer damage on leaves was characterized by pin holes and window-panning marks on the leaves (Overholt, Maes, & Goebel, 2001), while damage on the stem was characterized by the number of entry and exit holes and tunnel length caused by stemborer larvae feeding. Ears damaged by stemborer had sawdust-like feaces. Fall armyworm damage on leaves was characterized by extensively skeletonized leaves and windowed whorls with

loads of frass produced by feeding larvae while ear damage was characterized by frass-filled holes (Goergen et al., 2016). The number of stemborer and fall armyworm larvae recovered from damaged ears was also recorded.

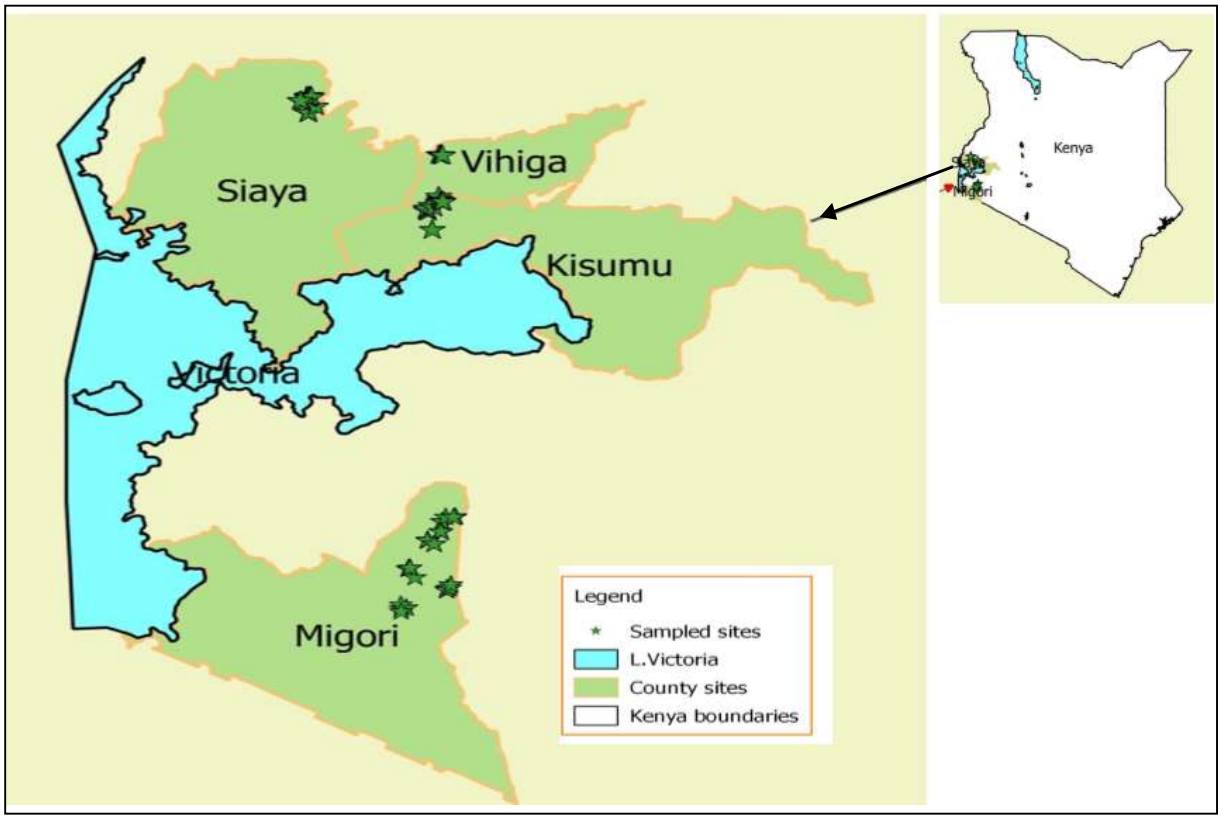


Figure 4. 1: Map of western Kenya showing the study sites and sampling points

### 4.3.3 Determination of ear rots infection in maize

At harvest (R6 growth stage) (Pioneer, 2019b), 100 maize ears were arbitrarily handpicked from each farm, dehusked and assessed for symptoms and extent of ear infection with maize ear rots. The incidence of common types of ear rots was determined as the number in infected ears out of the 100 randomly harvested ears while the severity was determined on a scale of 1 – 5, where 1 = < 25% and 5 = 100 ( icipe, 2013). The type of ear rot was identified based on causal fungi as illustrated in a compendium of maize diseases (CIMMYT, 2004). There were no visual symptoms

of *Aspergillus* ear rots, which are usually characterized by greenish-yellow moldiness of the maize ear. *Fusarium* ear rot infected ears showed whitish-pinkish to violet moldy kernels scattered among healthy-looking kernels (Pioneer, 2019a). *Diplodia* ear rot was characterized by white moldiness over and between maize kernels, starting from the bottom of the ear which left the kernels lightweight and greyish brown. *Gibberella* ear rot was observed as white to pink mold covering the tip to the upper half of the maize ear while *Penicillium* ear rot developed as blue green to green mold at the tips of damaged maize ears. Figure 4.2 shows stemborer and fall armyworm damaged ears and different types of ear rots encountered. Ten to 20 maize ears were randomly sampled from the 100, put into Khaki bags, and transported to the laboratory for fungal isolation, aflatoxin and fumonisin analyses. The maize samples were dried under the sun, followed by manual shelling and finely ground (Bunn-O-Matic Corporation Coffee Mill, G3-000) before being stored in Khaki bags at 4°C until analyses.

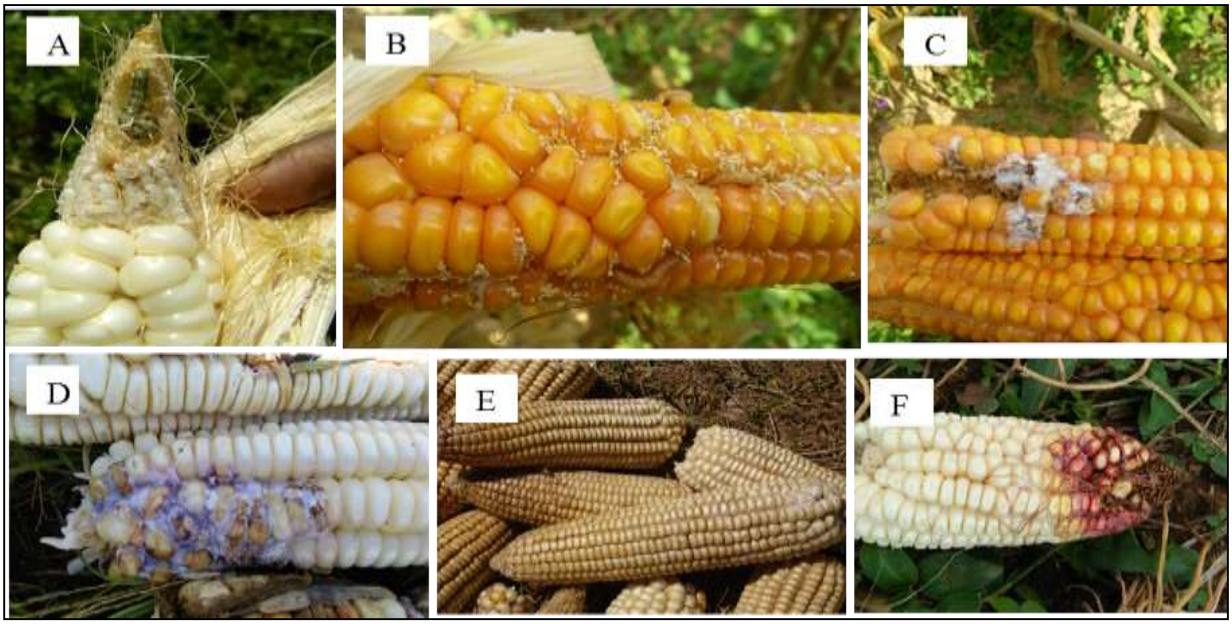


Figure 4. 2: Maize ears showing (A) Fall armyworm damage, (B) stemborer damage, (C, D) *Fusarium* ear rot, (E) *Diplodia* ear rot, and (F) *Giberella* ear rot in maize in western Kenya

### 4.3.4 Isolation and identification of *Aspergillus* and *Fusarium* spp. from maize

One-gram maize flour sub-sample was mixed with 9 ml of sterile distilled water, vortexed for 30 seconds and serially diluted to $10^{-2}$. One hundred microliter aliquots were spread on half strength potato dextrose agar (PDA – 17g, $KH_2PO_4$ – 1g, $KNO_3$ – 1g; $MgSO_4$ – 0.5g, agar - 10g) amended with 50 mg of each tetracycline, streptomycin chloramphenicol and pentachloronitrobenzene (PCNB). Pentachloronitrobenzene was added before autoclaving while the antibiotics were added after autoclaving and media was cooled to 45°C. Each sample was replicated three times and incubated at 25°C for three days. The number of colonies of each fungus was counted in each plate.

The number of colony forming units per gram (CFU/g) of ground maize and the frequency of isolation of different fungal genera were calculated as described in section 3.3.3. Characteristic colonies of each fungus were sub-cultured on full strength PDA and incubated for 7-14 days at 25°C. Colonies of *Fusarium* spp. were also sub-cultured on SNA and incubated for 14-21 days at near UV light to enhance sporulation (Nirenberg, 1981; Su et al., 2012). Fungal genera, *Aspergillus* and *Fusarium* spp. were identified as described in section 3.3.3.

### 4.3.5 Aflatoxin and fumonisin analyses in maize

Two sub-samples of 20 g of homogenized ground maize were extracted with 100 ml of 70% methanol for aflatoxin and 40 ml of 90% methanol for fumonisin analysis as described in section 3.3.4. Aflatoxin and fumonisin levels were detected and quantified using ELISA. After carefully following the instructions, the micro-wells were immediately read by a microplate reader at 450 nm. A standard curve was drawn to intrapolate the optical densities of the samples to determine the levels of respective mycotoxin in the test samples. Maize samples contaminated with

mycotoxin concentrations higher than the upper limit of the kits were serially diluted and the mycotoxin levels re-quantified, with consideration of the additional dilution factor in the interpretation of the results.

### 4.3.6 Data analyses

Data on incidence of stemborer, fall armyworm and maize ear rots was analyzed using linear mixed models fitted by REML in R studio software and means were separated by Fisher's LSD test. Data for each season was analyzed separately and then combined to compare the differences among the seasons. Cropping system (push-pull vs monocrop), county, season and their interactions were used as fixed factors while farmer identity was set as random effects in the model. Means of foliage and ear damage by stemborer and fall armyworm were compared using paired T test. Cross tabulation procedure of SPSS version 22 (IBM Corp, 2013, New York, USA) was used to categorize aflatoxin and fumonisin data into three categories: (i) samples below limit of detection of the kits, (ii) samples contaminated with toxin levels below the regulatory threshold and (iii) samples with toxin levels above the regulatory threshold set by Kenya Bureau of Standards (KEBS) for aflatoxin and European Commission (EC), which Kenya adopts, for fumonisin. Ordinal logistic regression and chi-square tests were performed to establish the association between different levels of aflatoxin and fumonisin with cropping system, county and season. Non-parametric correlations and linear regression were performed in SPSS to establish the relationship among insect damage, ear rots, ear rot fungi and their respective mycotoxins.

## 4.4 Results

### 4.4.1 Incidence of stemborer and fall armyworm damage of maize

The incidence of both stemborer and fall armyworm damage of the maize was significantly lower ($P < 0.05$) in the ears than in the foliage (Figure 4.3). Damage of both foliage and ears was significantly ($P < 0.05$) reduced in maize grown under push-pull cropping system than in non-push-pull. Incidence of foliage damage by stemborer was significantly ($P < 0.05$) lower than the incidence by fall armyworm damage. Foliage damage by stemborer was significantly ($P < 0.05$) low in maize grown under push-pull maize system across the three cropping seasons while ear damage was only significantly low ($P < 0.05$) during the 2017 short rain cropping season (Table 4.1). Both foliage and ear damage by fall armyworm were significantly ($P < 0.05$) lower under push-pull across the three cropping seasons. The lowest damage of both foliage and ear by stemborer was during 2018 long rain cropping season with incidence of up to 1%.

The number of fall armyworm larvae recovered from damaged ears was significantly ($P < 0.05$) higher than the number of stemborer larvae. However, the number of the larvae of the two insects was significantly ($P < 0.05$) lower in maize ears under push-pull cropping system (Table 4.2). The number of both stemborer and fall armyworm larvae recovered varied significantly ($P < 0.05$) across the seasons. The lowest number of stemborer larvae was recovered during the 2018 long rain cropping season while the lowest number of fall armyworm larvae was recovered during the 2017 short rain cropping season. The number of stemborer larvae were not significantly different between the push-pull and non-push-pull during individual cropping seasons, but the number of fall armyworm larvae were significantly ($P < 0.05$) lower under push-pull cropping system during all the three cropping seasons.

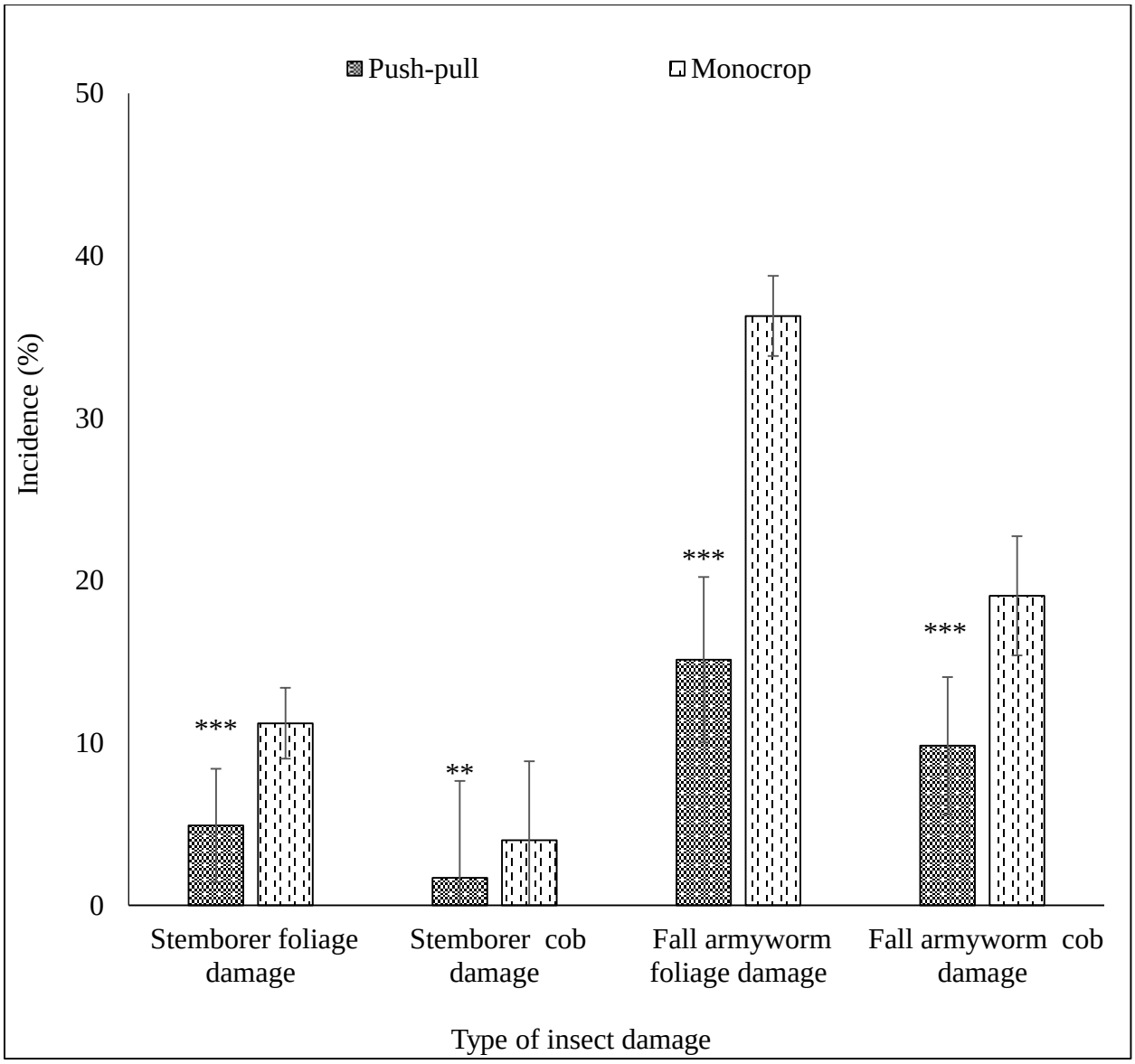


Figure 4. 3: Percent incidence of stemborers and fall armyworm damage on foliage and ears of maize grown under push-pull and non-push-pull farms during three cropping seasons in western Kenya (Error bars represent the standard error of the mean)

Table 4. 1: Percent incidence of stemborer and fall armyworm damage on foliage and ears of maize grown during three cropping seasons in three counties of western Kenya

| | Type of damage | County | Long rain 2017[a] | | | P | Short rain 2017[a] | | | P | Long rain 2018[a] | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PPT | NPPT | Mean | value | PPT | NPPT | Mean | value | PPT | NPPT | Mean | value |
| Stemborer | Foliage | Siaya | 4.25 ± 0.9 | 10.0 ± 2.1 | 7.1c | 0.012 | 4.2 ± 0.7 | 6.1 ± 1.3 | 4.3b | 0.178 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1a | 1.000 |
| | | Vihiga | 9.9 ± 2.2 | 24.7 ± 3.0 | 17.3b | 0.001 | 6.4 ± 1.5 | 17.5 ± 2.4 | 9.8a | <0.001 | 0.0 ± 0.0 | 0.7 ± 0.3 | 0.4a | 0.995 |
| | | Migori | 15.6 ± 2.1 | 24.5 ± 2.3 | 20a | 0.008 | 3.4 ± 0.7 | 6.1 ± 1.3 | 10.0a | <0.001 | 0.1 ± 0.1 | 0.4 ± 0.2 | 0.2a | 0.086 |
| | | Mean | 9.9 ± 1.2 | 19.7 ± 1.7 | | | 4.7 ± 0.6 | 13.2 ± 1.2 | | | 0.1 ± 0.0 | 0.4 ± 0.1 | | |
| | | P (CS) | <0.001 | | | | <0.001 | | | | 0.003 | | | |
| | Ear | Siaya | 3.6 ± 0.9 | 2.6 ± 0.7 | 3.1a | 0.409 | 1.5 ± 0.6 | 1.4 ± 0.6 | 1.7b | 0.866 | 0.3 ± 0.2 | 0.6 ± 0.2 | 0.4a | 0.191 |
| | | Vihiga | 1.7 ± 0.6 | 4.8 ±1.0 | 3.2a | 0.020 | 3.2 ± 0.9 | 19.5 ± 6.6 | 5.4a | 0.004 | 1.5 ± 0.6 | 1.7 ± 0.4 | 1.8a | 0.832 |
| | | Migori | 2.3 ± 0.6 | 3.9 ± 0.7 | 3.1a | 0.110 | 0.6 ± 0.3 | 1.4 ± 0.5 | 1.5b | 0.460 | 0.7 ± 0.2 | 1.2 ± 0.3 | 0.9a | 0.228 |
| | | Mean | 2.5 ± 0.4 | 3.7 ± 0.5 | | | 1.7 ± 0.4 | 7.0 ± 2.5 | | | 0.8 ± 0.2 | 1.2 ± 0.2 | | |
| | | P (CS) | 0.084 | | | | 0.004 | | | | 0.275 | | | |
| Fall armyworm | Foliage | Siaya | 17.1 ± 2.5 | 36.3 ± 4.8 | 26.7b | <0.001 | 10.7 ± 1.1 | 12.8 ± 1.5 | 18.6c | 0.328 | 8.9 ± 1.0 | 25.6 ± 3.6 | 18.6c | <0.001 |
| | | Vihiga | 11.2 ± 2.6 | 17.1 ± 3.6 | 14.1c | 0.187 | 16.0 ± 3.1 | 37.6 ± 6.8 | 20.8b | <0.001 | 10.3 ± 1.9 | 32.9 ± 2.9 | 20.8b | <0.001 |
| | | Migori | 22.6 ± 2.8 | 54.5 ± 2.7 | 38.5a | <0.001 | 19.6 ± 4.5 | 12.8 ± 1.5 | 37.4a | <0.001 | 19.3 ± 1.4 | 51.4 ± 2.6 | 37.4a | <0.001 |
| | | Mean | 16.9 ± 1.6 | 36.0 ± 2.9 | | | 15.4 ± 1.9 | 35.9 ± 3.5 | | | 13.0 ± 1.1 | 37.0 ± 2.2 | | |
| | | P (CS) | <0.001 | | | | <0.001 | | | | <0.001 | | | |
| | Ear | Siaya | 9.6 ± 1.3 | 23.4 ± 3.7 | 16.5b | <0.001 | 6.4 ± 1.1 | 4.2 ± 0.7 | 14.0a | 0.070 | 15.4 ± 1.6 | 26.3 ± 3.0 | 17.3c | 0.004 |
| | | Vihiga | 3.0 ± 0.7 | 7.9 ± 1.3 | 5.4c | 0.001 | 1.5 ± 0.5 | 2.3 ± 0.9 | 8.6c | 0.567 | 14.2 ± 2.8 | 22.9 ± 3.9 | 21.6b | 0.089 |
| | | Migori | 10.5 ± 1.5 | 25.7 ± 3.6 | 18.1a | <0.001 | 4.3 ± 1.2 | 4.2 ± 0.7 | 20.8a | <0.001 | 24.2 ± 1.5 | 32.5 ± 2.9 | 35.3a | 0.008 |
| | | Mean | 7.7 ± 0.8 | 19.0 ± 2.0 | | | 4.1 ± 0.6 | 11.3 ± 2.3 | | | 18.0 ± 1.3 | 27.2 ± 1.9 | | |
| | | P (CS) | <0.001 | | | | <0.001 | | | | <0.001 | | | |

PPT – push-pull technology, NPPT – non-push-pull technology, CS – cropping system, [a] – mean ± standard error of the mean

Table 4. 2: Number of stemborer and fall armyworm larvae recovered per farm from damaged maize ears grown during three cropping seasons in three counties of western Kenya

| Season | County | Number of stemborer larvae | | | Number of fall armyworm larvae | | |
|---|---|---|---|---|---|---|---|
| | | Push-pull[a] | Non-push-pull[a] | P value | Push-pull[a] | Non-push-pull[a] | P value |
| Mean | | 0.7±0.1 | 1.1±0.2 | | 4.5±0.4 | 10.7±1.2 | |
| LR2017 | Siaya | 1.4±0.4 | 0.8±0.3 | 0.265 | 9.7±1.6 | 14.0±2.8 | 0.199 |
| | Migori | 1.3±0.4 | 3.5±1.2 | 0.020 | 3.9±1.2 | 14.3±3.8 | 0.001 |
| | Vihiga | 0.6±0.3 | 1.9±0.5 | 0.036 | 1.8±0.7 | 5.3±1.4 | 0.026 |
| | Mean | 1.1±0.2 | 2.0±0.5 | | 5.1±0.8 | 11.2±1.7 | |
| | P value | 0.039 | | | 0.001 | | |
| SR2017 | Siaya | 1.7±0.6 | 1.5±0.5 | 0.927 | 0.8±0.6 | 0.7±0.3 | 0.919 |
| | Migori | 0.4±0.2 | 1.5±0.5 | 0.833 | 3.4±1.0 | 30.6±3.6 | <0.001 |
| | Vihiga | 0.2±0.1 | 0.8±0.3 | 0.044 | 0.6±0.3 | 2.1±0.8 | 0.092 |
| | Mean | 0.7±0.2 | 0.8±0.2 | | 1.6±0.4 | 11.1±2.8 | |
| | P value | 0.724 | | | <0.001 | | |
| LR2018 | Siaya | 0.1±0.1 | 0.2±0.1 | 0.246 | 4.7±1.0 | 9.2±1.3 | 0.012 |
| | Migori | 0.5±0.1 | 0.7±0.2 | 0.883 | 9.4±1.0 | 10.0±1.2 | <0.001 |
| | Vihiga | 0.3±0.2 | 0.7±0.3 | 0.883 | 6.1±2.0 | 10.1±2.4 | <0.001 |
| | Mean | 0.3±0.1 | 0.5±0.1 | | 6.8±0.8 | 9.8±1.0 | |
| | P value | 0.089 | | | 0.030 | | |
| P (CS overall) | | 0.017 | | | <0.001 | | |
| P (seasons) | | <0.001 | | | <0.001 | | |
| P (insects) | | <0.001 | | | | | |

LR – long rain cropping season, SR – short rain cropping season, [a] - mean ± standard error of the mean, CS – cropping system, insect – stemborer and fall armyworm

### 4.4.2 Incidence and severity of common types of maize ear rots

The most commonly recorded types of maize ear rots in order of decreasing incidence were *Fusarium, Penicillium* and *Diplodia* (Figure 4.4A). *Giberella* ear rot was also recorded but in low incidence of less than 1%, which was not significantly different between cropping systems. Prevalence of *Fusarium* ear rots was highest during the 2018 long rain cropping season ($P < 0.001$) (Figure 4.4B). The incidence of *Fusarium* ear rot and total ear rots were significantly ($P < 0.05$) lower in maize grown under push-pull cropping system than maize grown as a non-push-pull during the 2017 short rain and 2018 long rain cropping seasons (Table 4.3). Occurrence of *Fusarium* ear rot varied significantly ($P = 0.007$) among seasons and had a significant ($P = 0.001$) county, and variation among counties was significantly influenced by season. The severity of *Fusarium* ear rot was low (< 50% in over 80% of farms) and did not vary significantly between the push-pull and non-push-pull and across the season, except for 2017 short rain cropping season when 7.8% of the maize grown under non-push-pull had moderate infection (Table 4.4). The maize ears with moderate *Fusarium* ear rot severity were from Vihiga and Migori counties.

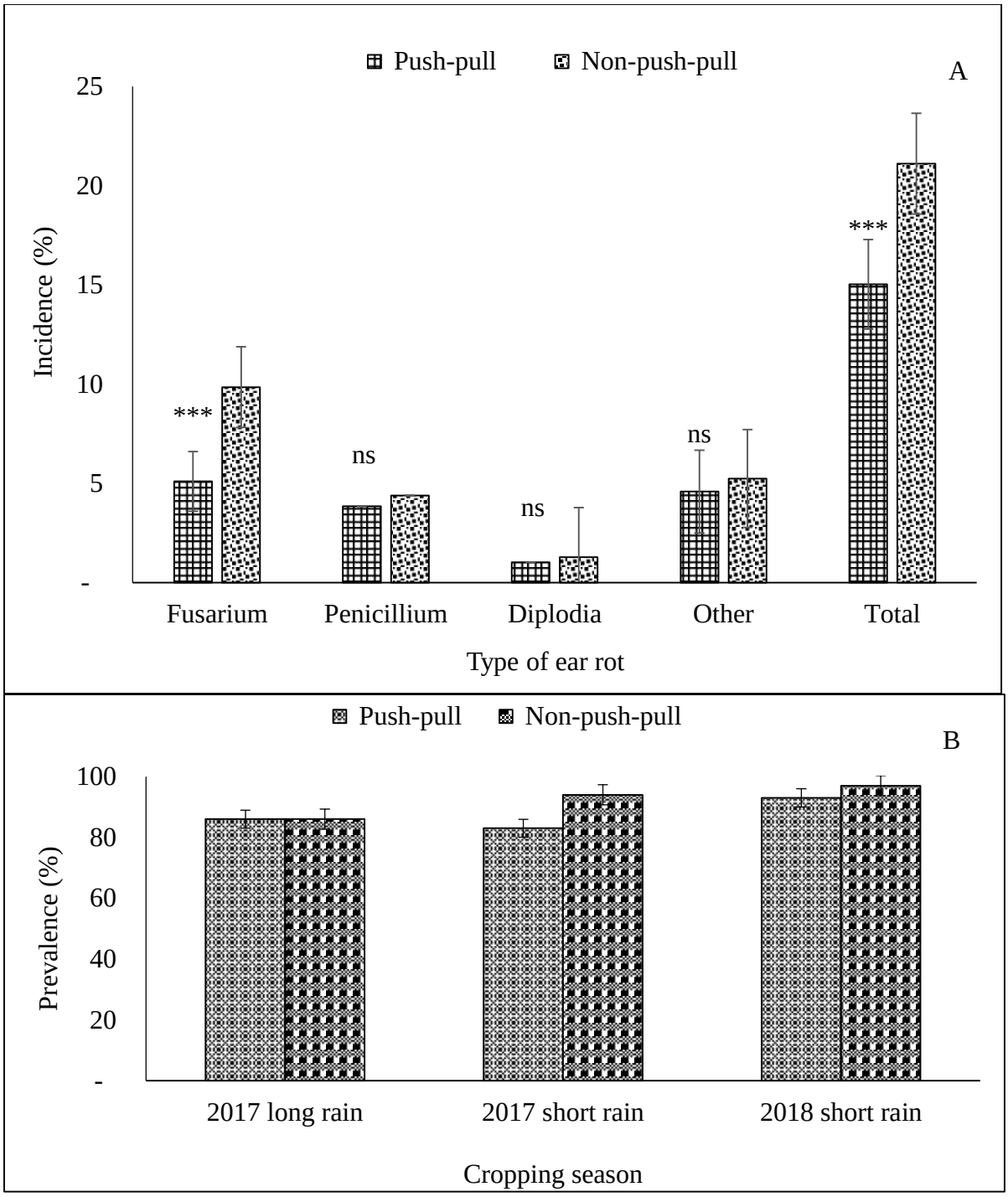


Figure 4. 4: Percentage (A) incidence of different types of ear rots and (B) prevalence of *Fusarium* ear rot in maize grown during three cropping seasons in western Kenyan ns – not significant (Error bars represent the standard error of the mean)

Table 4. 3: Percent incidence of different types of ear rots in maize grown under push-pull and non-push-pull for three cropping seasons in three counties of western Kenya

| Type of ear rot | County | Long rain 2017[a] | | | Short rain 2017[a] | | | Long rain 2018[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Push-pull | Non-push-pull | P value | Push-pull | Non-push-pull | P value | Push-pull | Non-push-pull | P value |
| *Fusarium* | Siaya | 9.0 ± 1.2 | 12.2 ± 3.0 | 0.001 | 2.1 ± 0.3 | 5.8 ± 1.1 | <0.001 | 4.0 ± 0.6 | 12.8 ± 2.2 | 0.065 |
| | Vihiga | 8.8 ± 1.7 | 10.9 ± 2.4 | 0.552 | 5.2 ± 1.2 | 9.7 ± 1.7 | 0.026 | 5.4 ± 0.9 | 10.3 ± 1.8 | <0.001 |
| | Migori | 3.7 ± 1.5 | 8.8 ± 2.0 | 0.017 | 4.4 ± 1.2 | 9.8 ± 2.1 | 0.039 | 3.3 ± 0.8 | 8.3 ± 1.2 | <0.001 |
| | Mean | 7.2 ± 0.9 | 10.5 ± 1.4 | | 3.9 ± 0.6 | 8.4 ± 1.0 | | 4.3 ± 0.5 | 10.5 ± 1.0 | |
| | P (CS) | 0.759 | | | <0.001 | | | <0.001 | | |
| *Penicillium* | Siaya | 8.4 ± 2.1 | 4.6 ± 2.0 | 0.685 | 1.2 ± 0.6 | 4.3 ± 2.2 | 0.470 | 5.2 ± 1.9 | 4.3 ± 1.1 | 0.910 |
| | Vihiga | 3.6 ± 1.1 | 5.8 ± 1.8 | 0.870 | 0.8 ± 0.3 | 1.6 ± 0.8 | 0.0369 | 6.4 ± 1.5 | 6.6 ± 1.6 | 0.325 |
| | Migori | 5.9 ± 1.7 | 7.4 ± 2.0 | 0.584 | 1.0 ± 0.4 | 0.8± 0.5 | 0.594 | 2.4 ± 0.7 | 3.4 ± 0.9 | 0.440 |
| | Mean | 5.9 ± 1.0 | 5.9 ± 1.1 | | 1.0 ± 0.3 | 2.3 ± 0.9 | | 4.6 ± 0.4 | 4.7 ± 0.7 | |
| | P (CS) | 0.507 | | | 0.379 | | | 0.954 | | |
| *Diplodia* | Siaya | 0.1 ± 0.3 | 0.3 ± 0.2 | 0.943 | 1.9 ± 0.4 | 1.6 ± 0.6 | 0.736 | 0.9 ± 0.4 | 0.3 ± 0.1 | 0.550 |
| | Vihiga | 0.2 ± 0.1 | 0.5 ± 0.2 | 0.035 | 0.8 ± 0.5 | 0.6 ± 0.2 | 0.724 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.493 |
| | Migori | 1.2 ± 0.4 | 2.5 ± 0.8 | 0.160 | 2.3 ± 0.6 | 3.9 ± 1.1 | 0.178 | 1.4 ± 0.7 | 1.7 ± 0.4 | 0.751 |
| | Mean | 0.6 ± 0.2 | 1.1 ± 0.3 | | 1.6 ± 0.3 | 2.1 ± 0.5 | | 0.8 ± 0.3 | 0.8 ± 0.2 | |
| | P (CS) | 0.131 | | | 0.540 | | | 0.872 | | |
| Other | Siaya | 5.2 ± 1.4 | 7.5 ± 2.0 | 0.121 | 4.1 ± 0.6 | 4.2 ± 1.1 | 0.875 | 2.6 ± 0.5 | 6.5 ± 1.3 | 0.766 |
| | Vihiga | 6.9 ± 1.4 | 6.8 ± 1.2 | 0.344 | 1.7 ± 0.4 | 3.1 ± 0.8 | 0.109 | 6.0 ± 1.0 | 2.7 ± 0.7 | 0.780 |
| | Migori | 8.1 ± 1.4 | 10.0 ± 2.2 | 0.539 | 2.9 ± 0.6 | 2.6 ± 0.8 | 0.766 | 3.9 ± 0.8 | 2.8 ± 0.5 | 0.258 |
| | Mean | 6.7 ± 0.8 | 8.2 ± 1.1 | | 2.9 ± 0.3 | 3.3 ± 0.6 | | 4.2 ± 0.5 | 4.0 ± 0.6 | |
| | P (CS) | 0.958 | | | 0.448 | | | 0.809 | | |
| Total | Siaya | 23.1 ± 2.8 | 24.6 ± 5.2 | 0.012 | 11.2 ± 1.3 | 16.3 ± 2.6 | 0.013 | 12.7 ± 2.2 | 24.1 ± 3.5 | 0.246 |
| | Vihiga | 19.9 ± 2.6 | 24.3 ± 3.9 | 0.236 | 8.5 ± 1.4 | 15.3 ± 2.0 | 0.010 | 18.2 ± 1.9 | 19.9 ± 2.0 | 0.133 |
| | Migori | 19.2 ± 3.4 | 29.1 ± 3.4 | 0.031 | 11.1 ± 1.5 | 17.9 ± 2.5 | 0.012 | 11.4 ± 1.6 | 16.7 ± 1.6 | 0.030 |
| | Mean | 20.7 ± 1.7 | 26.0 ± 2.5 | | 10.2 ± 0.8 | 16.5 ± 1.4 | | 14.2 ± 1.1 | 20.2 ± 1.5 | |
| | P (CS) | 0.621 | | | <0.001 | | | 0.001 | | |

**CS –** cropping system, a – mean ± standard error of the mean

Table 4. 4: Percentage of maize under different severity range of *Fusarium* ear rot in maize grown during three cropping seasons in western Kenya

| Severity | Push-pull | | | Non-push-pull | | |
|---|---|---|---|---|---|---|
| | LR2017 | SR2017 | LR2018 | LR2017 | SR2017 | LR2018 |
| < 50% | 86.4 | 82.8 | 97.6 | 96.1 | 86.3 | 95.2 |
| 50 - 75% | 0.0 | 0.0 | 0.0 | 0.0 | 7.8 | 0.0 |
| 76 – 100% | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

LR – long rain cropping season, SR – short rain cropping season

### 4.4.3 Population of *Aspergillus* and *Fusarium* spp. in maize

*Fusarium* spp. was the most frequently isolated fungal genus across the seasons and counties, in maize samples from both cropping systems. *Aspergillus*, *Penicillium*, *Acremonium* and *Verticillium* spp. were also isolated in varying frequencies and colony forming units. In addition, there were approximately five fungal genera that were unidentified and therefore recorded as 'other'. The 'other' fungi were mainly isolated in low frequencies (< 0.01%) and their populations were low (< 1% CFU/g maize). The overall population of fungi was significantly ($P < 0.05$) lower in maize grown under push-pull cropping system than in maize grown as non-push-pull in all the three cropping seasons (Table 4.5). The population of *Fusarium* spp. was significantly ($P < 0.05$) lower in push-pull maize during the 2017 short rains and 2018 long rain cropping seasons while the population of *Aspergillus* spp. was only significantly lower under push-pull cropping system during the 2017 long rain cropping season. However, the overall populations of both *Fusarium* and *Aspergillus* spp. were significantly lower in maize under push-pull cropping system. Mean population of *Fusarium* and *Aspergillus* spp. were highest in maize grown during the 2017 short rain season and lowest in maize grown during the 2018 long cropping season.

The most frequent *Fusarium* and *Aspergillus* spp. were *F. verticillioides* and *A. flavus*, respectively (Table 4.6). *Fusarium proliferatum*, *F. subglutinans*, *A. parasiticus*, *A. niger*, *A. ostianus*, *A.*

*fumigatus*, *A. tamarii* and *A. ochraceous* were isolated in low frequencies. The population of *F. verticillioides* was significantly ($P < 0.05$) lower in maize grown under push-pull cropping system compared to maize grown as a non-push-pull during the 2017 short rain and 2018 long rain (Table 4.6). On the contrary, the population of *A. flavus* was only significantly lower ($P < 0.05$) in maize grown under push-pull overall but not during individual cropping seasons. The population of *F. verticillioides* and *A. flavus* were highest during the 2017 short rain cropping season.

Table 4. 5: Population (Colony forming units/gram) of *Aspergillus* and *Fusarium* spp. in maize grown during three cropping seasons in three counties of western Kenya

| Season | County | Overall | | | *Fusarium* spp. | | | *Aspergillus* spp. | | | Other | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PPT | NPPT | P value | PPT | NPPT | P value | PPT | NPPT | P value | PPT | NPPT | P value |
| LR2017 | Migori | 56,792 | 38,852 | 0.562 | 29,636 | 34,786 | 0.853 | 60 | 24 | <0.001 | 150 | 18 | 0.055 |
| | Siaya | 28,903 | 133,830 | 0.011 | 22,037 | 37,216 | 0.455 | 275 | 2,689 | 0.134 | 35 | 2,597 | 0.002 |
| | Vihiga | 53,588 | 190,803 | 0.017 | 31,839 | 69,246 | 0.212 | 65 | 7,529 | 0.001 | 758 | 58 | 0.033 |
| | Mean | 46,126 | 121,162 | | 27,737 | 47,081 | | 136 | 3,414 | | 310 | 891 | |
| | P value | 0.006 | | | 0.211 | | | 0.001 | | | 0.188 | | |
| SR2017 | Migori | 58,753 | 152,688 | 0.021 | 24,536 | 113,077 | 0.018 | 2,958 | 660 | 0.292 | 2,327 | 1,543 | <0.001 |
| | Siaya | 76,601 | 154,346 | 0.116 | 34,684 | 81,357 | 0.094 | 3,947 | 2,840 | 0.857 | 1,232 | 1,026 | 0.888 |
| | Vihiga | 87,326 | 247,665 | 0.024 | 73,931 | 212,228 | 0.048 | 1,522 | 10,544 | 0.274 | 85 | 2,281 | 1.000 |
| | Mean | 74,187 | 184,871 | | 44,548 | 136,111 | | 2,790 | 4,643 | | 1,214 | 1,626 | |
| | P value | <0.001 | | | <0.001 | | | 0.602 | | | 0.715 | | |
| LR2018 | Migori | 10,598 | 17,103 | 0.170 | 3,461 | 7,385 | <0.001 | 33 | 243 | 0.123 | 155 | 107 | 1.000 |
| | Siaya | 12,037 | 65,588 | <0.001 | 7,250 | 33,798 | 0.013 | 600 | 6,507 | 0.050 | 152 | 282 | 0.995 |
| | Vihiga | 27,268 | 49,245 | 0.207 | 23,446 | 43,800 | 0.297 | 234 | 7 | 1.000 | 1240 | 265 | 0.252 |
| | Mean | 16,793 | 43,233 | | 11,529 | 28,139 | | 278 | 2,106 | | 528 | 216 | |
| | P value | <0.001 | | | 0.004 | | | 0.259 | | | 0.941 | | |
| Mean | | 45,864 | 116,085 | | 28,033 | 69,801 | | 1,078 | 3,381 | | 687 | 906 | |
| P value | | <0.001 | | | <0.001 | | | 0.06 | | | 0.565 | | |

PPT – push-pull technology, NPPT – non-push-pull technology, LR – long rain, SR – short rain, other – unidentified fungal genera

Table 4. 6: Population (Colony forming units/gram) of *Aspergillus* and *Fusarium* spp. in maize grown under push-pull and non-push-pull during three cropping seasons in three counties of western Kenya

| Season | County | *Fusarium* spp. | | | | | | *Aspergillus* spp. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *F. verticillioides* | | | Other *Fusarium* sp. | | | *A. flavus* | | | Other *Aspergillus* sp. | | |
| | | PPT | NPPT | P value | PPT | NPPT | P value | PPT | NPPT | P value | PPT | NPPT | P value |
| LR2017 | Migori | 23,603 | 34,782 | 0.650 | 567 | 0 | 1.000 | 26 | 0 | 0.010 | 0 | 0 | 0.996 |
| | Siaya | 21,996 | 33,677 | 0.552 | 42 | 2 | 0.900 | 275 | 349 | <0.001 | 0 | 2,338 | <0.001 |
| | Vihiga | 30,739 | 68,564 | 0.226 | 0 | 12 | 1.000 | 26 | 354 | <0.001 | 39 | 0 | 1.000 |
| | Mean | 25,387 | 45,675 | | 200 | 5 | | 112 | 234 | | 13 | 780 | |
| | P value | 0.174 | | | n/a | | | 0.525 | | | n/a | | |
| SR2017 | Migori | 23,697 | 109,695 | 0.017 | 8 | 347 | <0.001 | 168 | 35 | <0.001 | 1,527 | 620 | <0.001 |
| | Siaya | 36,597 | 81,224 | 0.115 | 298 | 93 | 0.996 | 105 | 2,711 | <0.001 | 53 | 130 | <0.001 |
| | Vihiga | 73,745 | 212,228 | 0.048 | 10 | 0 | 1.000 | 1,502 | 10,000 | 0.001 | 20 | 544 | <0.001 |
| | Mean | 44,816 | 134,882 | | 102 | 151 | | 600 | 4,202 | | 541 | 440 | |
| | P value | <0.001 | | | n/a | | | 0.25 | | | 0.873 | | |
| LR2018 | Migori | 3350 | 6,175 | 0.251 | 35 | 45 | ns | 18. | 160 | 0.900 | 15 | 83 | <0.001 |
| | Siaya | 7,232 | 33,259 | 0.022 | 0 | 19 | 1.000 | 109 | 5,444 | 1.000 | 186 | 80 | 1.000 |
| | Vihiga | 23,378 | 42,297 | 0.347 | 52 | 3 | ns | 233 | 0 | 1.000 | 0 | 7 | 1.000 |
| | Mean | 11,461 | 27,036 | | 3 | 22 | | 121 | 1,7449 | | 63 | 56 | |
| | P value | 0.004 | | | n/a | | | 0.237 | | | 0.873 | | |
| Grand mean | | 27,322 | 68,554 | | 111 | 59 | | 280 | 2,027 | | 208 | 429 | |
| P value | | <0.001 | | | 0.545 | | | 0.026 | | | 0.434 | | |

LR – long rain, SR – short rain, n/a – positive data set not enough for P value to be calculated

### 4.4.4 Levels of aflatoxin and fumonisin in maize

Aflatoxin contamination of maize varied significantly among the seasons ($P < 0.05$) (Table 4.7). Maize grown during the 2018 long rain cropping season had the highest proportion of maize samples contaminated with aflatoxin, as compared to the other two seasons. Overall, the number of push-pull samples contaminated with levels above the Kenya Bureau of Standards (KEBS) of 10 µg/kg regulatory threshold was slightly lower than the proportion of maize grown under non-push-pull. Overall and during the 2017 short rain cropping season, the percentage of maize samples contaminated with different levels of aflatoxin varied among the counties ($P < 0.001$) but not between the cropping systems,. However, there was significantly ($P < 0.05$) lower percentage of push-pull samples from Siaya County contaminated with aflatoxin levels above 10 µg/kg. The 2017 short rain cropping season had the lowest number of samples contaminated with aflatoxin but had the highest differences in proportion of samples contaminated with aflatoxin levels above 10 µg/kg.

The number of samples contaminated with fumonisin was significantly higher than the number contaminated with aflatoxin ($P < 0.05$) (Table 4.8). Overall, the proportion of samples contaminated with fumonisin was significantly ($P = 0.048$) reduced under the push-pull cropping system. The proportion of sample contaminated with fumonisin levels above the European Commission (EC) regulatory threshold of 1000 µg/kg was significantly ($P < 0.001$) reduced in maize grown under the push-pull cropping system as opposed to maize grown in non-push-pull system. There was significantly ($P = 0.005$) lower proportion of maize grown under push-pull cropping system contaminated with different levels of fumonisin during the 2018 long rain cropping season as compared to 2017 long and short rain cropping seasons. During the 2017 long rain cropping season, the levels of fumonisin significantly ($P = 0.017$) varied among counties. There was significantly ($P = 0.044$) lower proportion of push-pull samples from Vihiga County

contaminated with high (> 1000 µg/kg) levels of fumonisin, followed by Migori and lastly Siaya counties.

Table 4. 7: Percentage of samples contaminated with aflatoxin levels (µg/kg) under different categories in maize grown during three cropping seasons in three counties of western Kenya

| Season | County | Push-pull | | | Non-push-pull | | | $X^2$ | P value (CS) |
|---|---|---|---|---|---|---|---|---|---|
| | | < 1 | < 10[a] | > 10 | < 1 | < 10 | > 10 | | |
| LR2017 | Migori | 42.1 | 52.6 | 5.3 | 40.0 | 55.0 | 5.0 | 0.137 | 0.934 |
| | Siaya | 50.0 | 50.0 | 0.0 | 60.0 | 30.0 | 5.0 | | |
| | Vihiga | 52.6 | 36.8 | 5.3 | 55.0 | 35.0 | 0.0 | | |
| | Mean | 48.3 | 46.6 | 3.4 | 51.7 | 40.0 | 3.3 | | |
| SR2017 | Migori | 95.0 | 0.0 | 5.0 | 95.0 | 0.0 | 5.0 | 0.967 | 0.617 |
| | Siaya | 73.7 | 15.8 | 5.3 | 66.7 | 11.1 | 11.1 | | |
| | Vihiga | 60.0 | 35.0 | 0.0 | 47.4 | 47.4 | 5.3 | | |
| | Mean | 76.3 | 16.9 | 3.4 | 70.2 | 19.3 | 7.0 | | |
| LR2018 | Migori | 35.0 | 60.0 | 0.0 | 35.0 | 65.0 | 0.0 | 1.434 | 0.488 |
| | Siaya | 27.8 | 66.7 | 5.6 | 44.4 | 44.4 | 11.1 | | |
| | Vihiga | 20.0 | 75.0 | 5.0 | 35.0 | 65.0 | 0.0 | | |
| | Mean | 27.6 | 67.2 | 3.4 | 37.9 | 58.6 | 3.4 | | |
| Grand mean | | 50.9 | 43.4 | 3.4 | 53.1 | 39.4 | 5.6 | | |

LR – long rain, SR – short rain, 1 µg/kg – lower limit of detection of kit, [a] – Kenya Bureau of Standards regulatory threshold, CS – cropping systems

Table 4. 8: Percentage of samples contaminated with fumonisin levels (µg/kg) under different categories in maize grown during three cropping seasons in three counties of western Kenya

| Season | County | Push-pull | | | Non-push-pull | | | $X^2$ | P value (CS) |
|---|---|---|---|---|---|---|---|---|---|
| | | <100 | <1000[b] | > 1000 | <100 | <1000 | > 1000 | | |
| LR2017 | Migori | 47.4 | 21.1 | 31.6 | 45.0 | 35.0 | 20.0 | 3.110 | 0.211 |
| | Siaya | 15.0 | 45.0 | 40.0 | 15.0 | 20.0 | 65.0 | | |
| | Vihiga | 15.8 | 52.6 | 31.6 | 25.0 | 20.0 | 55.0 | | |
| | Mean | 25.9 | 39.7 | 34.5 | 28.3 | 25.0 | 46.7 | | |
| SR2017 | Migori | 20.0 | 25.0 | 55.0 | 15.0 | 25.0 | 60.0 | 2.070 | 0.355 |
| | Siaya | 10.5 | 57.9 | 31.6 | 33.3 | 33.3 | 33.3 | | |
| | Vihiga | 40.0 | 25.0 | 35.0 | 21.1 | 15.8 | 63.2 | | |
| | Mean | 23.7 | 35.6 | 40.7 | 22.8 | 24.6 | 52.6 | | |
| LR2018 | Migori | 55.0 | 40.0 | 5.0 | 35.0 | 35.0 | 30.0 | 11.756 | 0.003 |
| | Siaya | 61.1 | 16.7 | 22.2 | 11.1 | 50.0 | 38.9 | | |
| | Vihiga | 35.0 | 45.0 | 20.0 | 20.0 | 35.0 | 45.0 | | |
| | Mean | 50.0 | 34.5 | 15.5 | 22.4 | 39.7 | 37.9 | | |
| Grand mean | | 33.1 | 36.6 | 30.3 | 24.6 | 29.7 | 45.7 | | |

LR – long rain, SR – short rain, 100.0 µg/kg - lower limit of detection of kit, [b] – European Commission regulatory threshold, CS – cropping systems

### 4.4.5 Correlations among insect damage, ear rot, ear rot fungi and mycotoxin levels in maize

Though ear damage by both stemborer and fall armyworm was significantly lower than foliage damage, incidence of ear damage significantly ($P < 0.05$) increased with increased foliage damage (Table 4.9). Accordingly, ear damage by both insects caused significant increase in the incidence of *Fusarium* and total ear rots ($P < 0.05$). The correlation coefficients were, however, weak. Similarly, reduced incidence of *Fusarium* ear rot caused a corresponding stronger significant ($P < 0.05$) reduction in *F. verticillioides* population. The levels of fumonisin had strong positive correlation with the population of *F. verticillioides* while the levels of aflatoxin had weak positive and significant correlation with *A. flavus*, and in both cases the correlations were significant ($P < 0.05$).

Table 4. 9: Correlation among insect damage, ear rot and mycotoxin levels in maize

| Variables | Stemborer foliage damage | Stemborer ear damage | *Fusarium* ear rot | *F. verticillioides* | Fumonisisn | FAW foliage damage | FAW ear damage | *A. flavus* |
|---|---|---|---|---|---|---|---|---|
| Stemborer ear damage | 0.193 ** | - | | | | | | |
| *Fusarium* ear rot | 0.097 | 0.167* | - | | | | | |
| *F. verticillioides* | 0.112 * | -0.010 | 0.388** | - | | | | |
| Fumonisin | 0.142 ** | 0.056 | 0.429** | 0.672 ** | - | | | |
| FAW foliage damage | 0.232 ** | -0.003 | 0.204** | -0.029 | 0.044 | - | | |
| FAW ear damage | -0.235 ** | 0.043 | 0.107** | -0.143 ** | -0.075 | 0.376 ** | - | |
| *A. flavus* | 0.021 | -0.080 | -0.124* | -0.062 | -0.007 | -0.036 | -0.067 | - |
| Aflatoxin | -0.052 | 0.004 | 0.023 | -0.001 | -0.020 | 0.019 | 0.161** | 0.159** |

FAW – fall armyworm, ** - significant at $p < 0.01$ (two tailed), * - significant at $p < 0.05$ (two tailed)

## 4.5 Discussion

Insect pests, ear rots and mycotoxins are major constraints to maize production and utilization, but feasible management strategies to combat them are limited for resource constrained smallholder farmers such as those in western Kenya. The current study demonstrated that the 'push-pull' cropping system can contribute to effective reductions in crop damage and mycotoxin contamination in maize. There was significant reduction in infestation of maize by stemborer and fall armyworm under push-pull cropping system. These findings concur with previous findings that reported reduced incidence of damage by the two insects under push-pull cropping system (Khan et al., 2010; Midega et al., 2018). The cropping system was initially developed as a control strategy for cereal stemborer and striga weed in Africa (Midega et al., 2010). The incidence of damage of maize by both stemborer and fall armyworm, however, significantly varied across seasons and study counties. This is an indication that the extent of damage by the insects depend on multiple factors such as cropping season and geographical region (Manu et al., 2019; Sarmento et al., 2002). Different cropping seasons have different climatic characteristics of rainfall, temperature, humidity, and sky cloudiness that influence the incidence of disease, insect and weed and consequently quantity and quality of grain yield.

To the best of our knowledge, there is no previous study on incidence of stemborer and fall armyworm simultaneously and the possible consequence on maize quality in the same study in Kenya. Fall armyworm caused significantly more damage than stemborer, indicating that the extent of insect damage also greatly depends on the species. Fall armyworm was very aggressive feeders and caused extended skeletonization of leaves and windowed whorls (Goergen et al., 2016) while stemborer infestation caused window-panning marks on the leaves (Overholt et al., 2001). Both insects, however, bore holes in growing maize ears.

Control of stemborer and fall armyworm moths through the use of insecticides is very difficult because they are both nocturnal and therefore difficult to target for spraying (Kfir et al., 2002). The larvae of both insects also bore holes into the host plants, making it difficult to target by use of insecticides. On the other hand, push-pull cropping system produce chemicals that modify the behavior of insects and that of their natural enemies (Cook et al., 2007).

*Fusarium* ear rot was the most prevalent type of maize ear rot across the seasons and with the disease incidence being significantly reduced in push-pull cropping system, which concur with findings by (Owuor et al., 2018). The current findings from maize monocrops, however, disagree with observations of previous studies that reported *Fusarium* ear rot as the third most common type of maize ear rots and in the region (Bigirwa et al., 2007; Opande et al., 2017). In the current study, the incidence of *Fusarium* ear rot significantly varied across seasons, indicating that the climatic conditions of temperature and moisture in a cropping season are key determinants of presence and extent of infection of maize by ear rot fungi, since dominant fungi are greatly influenced by climate (Vacher et al., 2008). The reduction of *Fusarium* ear rot under push-pull could be caused by reduced entry of inocula as a result of reduced insect damage. Another explanation is possible release of antifungal compounds into the soil by desmodium roots.

Ear rots fungi mainly gain entry into maize kernels through wounds caused by insect infestation or systemically from the soil through the stalk (Munkvold et al., 1997). Therefore, maize ears under push-pull cropping system, from which low damage by stemborer and fall armyworm larvae was recorded, has significantly low ear rots infection. Insect feeding on maize ears act as vectors of ear rot fungi (Sobek & Munkvold, 1999). Maize ear rot fungi also cause infection by entering through the silk as the kernels develop (Thompson et al., 2018). *Fusarium verticillioides*, *A. flavus* and *F. graminearum* are the most common silk-entering fungal pathogens of maize (Thompson et

al., 2018). This could possibly why even maize sampled from push-pull farms was contaminated with *A. flavus* and *F. verticillioides*, which are aflatoxin and fumonisin producers, respectively, even though there was significantly lower damage to maize by both stemborer and fall armyworm larvae.

*Fusarium verticillioides* was isolated in the highest prevalence and frequency in maize samples overall and the population was significantly lower under push-pull cropping system. As with incidence of *Fusarium* ear rot, the population of *F. verticillioides* also varied across the cropping seasons. This is because population of fungal species is dependent on climate, temperature, and moisture during its growth (Manu et al., 2019; Medina et al., 2017; Vacher et al., 2008). Leslie and Summerrell (2006) reported that food substrate contaminated with high population of *F. verticillioides* is unlikely to be contaminated by *A. flavus*. Previous studies have also reported *F. verticillioides* as the most prevalent fungus isolated in maize (Bush et al., 2003; Degraeve et al., 2015). Correlation analysis confirmed *F. verticillioides* as the cause of maize *Fusarium* ear rot across the three cropping seasons. Mukanga et al. (2010) and Duan et al. (2016) also reported that *F. verticillioides* was correlated with *Fusarium* ear rot in maize. Isolation of *F. verticillioides* in maize at harvest is an indication of risk of contamination of maize with fumonisin during storage.

The lower population of *A. flavus* was lower than the population of *F. verticillioides*. This could possibly explain the higher number of samples contaminated with fumonisin compared to the number contaminated with aflatoxin, as also reported in a previous study (Mutiga et al., 2015). Aflatoxin and fumonisin co-occurred in a small proportion of the maize samples. This concurs with previous studies by Guo et al. (2017) and Mutiga et al. (2015). Although *A. flavus* was confirmed by correlation analyses as the main producer of aflatoxin in the maize samples, there was no physical damage by the fungus on maize kernels. This concurs with findings of a previous

study which reported that *Aspergillus* ear rot mainly causes little damage to maize kernels, which cannot be visually observed as discoloration as is the case for most other ear rot fungi (Schoeman, 2012). The low population of *A. flavus* could be due to the presence of high population of *F. verticillioides*. A previous study reported that presence of *F. verticillioides* in maize samples lowered the likelihood of being infected by *Aspergillus* spp. and another *Fusarium* spp. (Leslie and Summerell, 2006). *Fusarium verticillioides* and *A. flavus* infection in maize can also be asymptomatic (Bigirwa et al., 2006; Owuor et al., 2018; Schoeman, 2012). Therefore *A. flavus* was isolated in maize samples even though there was no single case of *Aspergillus* ear rot recorded. Presence of *A. flavus* in very low colony forming units per gram of maize sample could also possibly explain the absence of visual symptoms of *Aspergillus* ear rots.

This study implied that push-pull cropping system significantly reduced the occurrence of maize ear rots, particularly *Fusarium* ear rot, which was consequently resulted in low population of *F. verticillioides,* which is the main producer of fumonisin. This was strongly supported by the positive significant correlation among incidence of *Fusarium* ear rot, population of *F. verticillioides* and levels of fumonisin. The association between *Fusarium* ear rots, *F. verticillioides* and fumonisin was consistent across the three cropping seasons. The mechanism of reduction of *Fusarium* ear rot infection and production of fumonisin in maize under push-pull cropping system was through reduction in maize damage by stemborer and fall armyworm. This implication was supported by the reduced incidence of stemborer and fall armyworm infestation, and the significant association between stemborer and fall armyworm damage with the incidence of *Fusarium* ear rot across the three cropping seasons. These findings concur with previous studies that reported association between insect pest damage and the level of mycotoxin contamination of

cereals by reducing the ear rot pathogen inoculum that would infect the maize through wounded ears (Mays, 2015; Sobek & Munkvold, 1999).

The functionality of push-pull cropping system in control of stemborer and fall armyworm damage of maize recorded in this study concurs with reports of previous studies (Khan et al., 2010; Midega et al., 2015, 2018). `Push-pull` cropping system was primarily developed as a tool for control of stemborers that attack maize in Africa. Control of stemborer and fall armyworm by push-pull cropping system is mediated by repellent chemicals emitted by the desmodium, the 'push' crop (Khan et al., 2010; Midega et al., 2018). The repelled insects are pulled by chemicals produced by the 'pull' crop, planted at the border of the plot. The 'pull' crop is not suitable for development of the larvae, hence few larvae survive in the end (Frank et al., 2008). Additional mechanism of control of stemborer and fall armyworm under push-pull cropping system is through attracting natural enemies of the insects (Midega et al., 2006).

In addition, a previous study reported that crop production practices that increase grain yield help control mycotoxin contamination in food crops (Bruns, 2003). Striga control and improved soil nutrition under push-pull increase crop vigor and canopy. This would result in reduced infection of developing kernels by fungal inocula from the atmosphere. This could be hypothesized as another mechanism by which push-pull cropping system controlled the occurrence of maize ear rots. The cropping system has been proved to increase maize grain yield as compared to non-push-pull (Midega et al., 2018). The push-pull cropping system increase grain yield through conservation of moisture, increasing soil organic matter content and availing nutrients like nitrogen and phosphorus which increase crop vigor (Khan et al., 2011; Khan & Pickett, 2004), thus reducing crop stress which increase infection by fungal pathogens (Bruns, 2003).

### 4.6 Conclusions and recommendations

Push-pull cropping system reduced the incidence of maize ear rots and associated mycotoxins through reduced stemborer and fall armyworm damage. It is, therefore, recommended that the push-pull cropping system be integrated into existing methods of mycotoxin control. The cropping system is easy and cheap to maintain and encourages livestock keeping by resource constrained smallholder farmers because the components: desmodium, napier and Brachiaria grass and maize stalks, are an important source of quality fodder. Reduction in insect damage was, however, not the only mechanism by which push-pull reduced the incidence of ear rots, as shown by the weak correlation between ear damage and *Fusarium* ear rot. It is, therefore, recommended that further studies be carried out on potential mechanisms of mycotoxin control under push-pull cropping system. Proper maintenance of the 'push' and 'pull' crops is recommended in order to optimize the benefits of the system. It is also important to coat maize seeds for planting with fungicides to reduce systemic infection of the maize under push-pull with ear rot fungi like *F. verticillioides*. Wounding of maize ears by birds should also be controlled, so that the impact of the push-pull on ear rots and mycotoxins is increased. These would reduce infection courts of maize by ear rot fungi and contamination with aflatoxin and fumonisin levels above tolerable levels.

# CHAPTER FIVE

## RELATIONSHIP BETWEEN SOIL NUTRITION AND POPULATION OF MYCOTOXIGENIC FUNGI IN SOILS UNDER PUSH-PULL CROPPING SYSTEM


### 5.1 Abstract

The levels and sources of soil nutrients influence the diversity and abundance of soil microorganisms. Desmodium component of the push-pull technology is a legume that fixes nitrogen and avail phosphorus. The objective of this study was to establish the correlation between soil nutrition and populations of mycotoxigenic fungi in soils under the push-pull cropping system. Soils were sampled at planting, at silking and at harvest from 60 push-pull farms and 60 neighboring non-push-pull farms for three cropping seasons in three counties western Kenya. The soils were analyzed for pH, organic carbon, total nitrogen, available phosphorus and populations of *Aspergillus* and *Fusarium* spp. There was no detectable improvement in soil nutrition under the push-pull cropping systems during the three sampling regimes. The populations of *Aspergillus* and *Fusarium* spp. did not vary in soils from push-pull and non-push-pull across the sampling regimes. *Aspergillus* section *Fumigati* was the most isolated fungus with a frequency of 6 – 24%, while *A. flavus* had an isolation frequency of up to 2%. *Fusarium oxysporum* and *F. verticillioides* were isolated in frequencies of up to 4% and < 1%, respectively. The populations of *A. flavus* and *A. niger* were significantly ($P < 0.05$) increased with increase in soil nutrients. However, the correlation was independent of the cropping system. From the findings of this study, soil nutrients and fungal population were not correlated with the cropping system and sampling time, and were not correlated between themselves.



**Key words:** *Aspergillus*, *Fusarium*, mycotoxin management, push-pull, soil fertility

## 5.2 Introduction

In companion cropping of maize with legumes, the legumes replenish soil nitrogen through biological nitrogen fixation (Matusso et al., 2014). This nitrogen maybe available to the associated crop in the current cropping season or as residual nitrogen for the succeeding cereal crops in subsequent seasons (He et al., 2003). The potential transfer of nitrogen by legumes is varied among different legume species, depending on root tissue composition and legume population density (Louarn et al., 2015). Intercropping maize with desmodium under push-pull technology, has been proven to be a more viable option for replenishing soil fertility (Kifuko-Koech, 2013). Desmodium is a fodder legume that is used as intercrop in the push-pull technology of maize farming and is adaptable to resource poor areas of rain-fed cereal production (Frank et al., 2008; Khan et al., 2002, 2010).

'Push-pull' technology is the farming system that involves intercropping maize with a fodder legume commonly known as desmodium, and planting a grass like napier/Brachiaria at the border of the intercrop (Cook et al., 2007; Khan et al., 2002). Desmodium pushes gravid stemborer and fall armyworm, which are simultaneously attracted by the border grass which act as the 'pull' (Khan et al., 2000; Midega et al., 2018). The desmodium controls the parasitic striga weed by producing allelochemicals that induce germination of their seeds, while at the same time inhibiting attachment of germinated seedlings onto the maize (Khan et al., 2000). The roots of desmodium fix nitrogen, avail phosphorus to the cereal crop and improves soil organic matter (Khan et al., 2008; Midega et al., 2013). The result of the striga and insect control and nutrient fixation is increased grain yield (Khan et al., 2008).

Studies have shown that the levels and sources of soil nutrients influence the diversity and abundance of soil microorganisms (Augusto et al., 2018; Kanwal et al., 2017; Medina et al., 2008).

Continuous use of mineral fertilizers has recently been reported to negatively affect availability of organic carbon and total nitrogen, which result in increased populations of pathogenic fungi (Augusto et al., 2018; Kanwal et al., 2017; Waithaka, et al. 2007). Density of fungi is also influenced by crop composition (Horn, 2003).

Agricultural soils in western Kenya have been reported to face continuous nutrient depletion, with nitrogen and phosphorus being the most limiting (Makokha et al., 1999; Marenya & Barrett, 2009; Vanlauwe et al., 2008; Tittonell, & Mukalama, 2006; Weisskopf et al., 2009). However, farmers apply both organic and inorganic inputs to manage soil fertility (Odendo et al., O2009). The objective of the current study was to establish the relationship between soil nutrients with populations of mycotoxigenic fungi in the soils under push-pull cropping system.

## 5.3 Materials and Methods

### 5.3.1 Soil sampling

Soil samples were collected from the farms selected in Section 4.3.1. for three cropping seasons. Top soil samples were collected at planting, at silking (R1-R2 growth stage) and at harvest (R6 growth stage) (Pioneer, 2019a), from the top 15 cm by driving a soil auger at 10 points per plot, achieved by traversing two diagonal lines across the field. Samples from the 10 sampling points were homogenized to make one composite sample of 0.5 – 1.0 kg per farm. While sampling soil, the rhizosphere of desmodium and maize plants were strictly avoided. After oven drying at 40°C for seven days, the soil samples were finely ground and sub-divided into two sub-samples: one for fungal isolation and the other one for determination of selected soil fertility parameters.

### 5.3.2 Determination of levels of nutrients in soil

The soil samples were sent to Kenya Agricultural and Livestock Research Organization (KALRO), Kabete, for chemical analysis. Soil pH was determined using electrochemical method where a pH meter was inserted into soil - water suspension (1:1 w/v). Total nitrogen was determined by Kjeldahl method (Page, Miller, & Kenney, 1982). The soil samples were first digested with concentrated sulphuric acid ($H_2S0_4$) at a temperature of 350°C, after which total N was measured by titration after distillation.

The percentage of organic carbon was determined by calorimetric method (Anderson & Ingram, 1993). The samples were oxidized and the digests were thoroughly mixed and allowed to stand overnight, after which carbon was measured at 600 nm on a spectrophotometer. Sub-samples for testing available phosphorus, sodium, calcium, potassium and magnesium were extracted by Mehlich Double Acid method (Mehlich et al., 1962), where Na, Ca and K were quantified with a flame photometer while Mg and Mn were determined spectrophotometrically.

Exchangeable acidity was determined for soil samples with a pH of 5.5 and below by adding 12.5 ml of 1 M KCl into 5 g of dry soil sub-sample in a 50 ml container and stirring the contents with a clean glass rod and allowing them to stand for 30 minutes, before filtering through a funnel. The filtrate was leached five times with 12.5 ml aliquots of 1 M KCl. Titration was done with 0.1 M NaOH after addition of phenolphthalein indicator. The burette reading of the volume (ml) of NaOH used was recorded and the titration readings corrected for a blank of titration of 75 ml KCl solution. Sub-samples for testing for available trace elements (Fe, & Cu) were extracted (1:10 w/v) with 0.1 M HCl and elements determined with atomic absorption spectrophotometer (AAS).

### 5.3.3 Isolation and identification of *Aspergillus* and *Fusarium* spp. from soil

Isolation of fungal pathogens was done on half strength PDA as described in section 3.3.3. Samples with high incidence of *Mucor* and *Rhizopus* were re-isolated on Dicloran Rose Bengal Chloramphenicol agar (DRBC) (Oxoid Microbiology Products: Glucose – 10g, Peptone – 5g, $MgSO_4.7H_2O$ – 0.5g, $KH_2PO_4$ – 1.0g, agar – 15g, Dicloran – 2mg, Rose Bengal – 25mg, chloramphenicol – 0.1g, pH- 5.6). After incubation, the number of colonies of each fungus was counted in each plate. The number of fungal colony forming units per gram (CFU/g) of soil and the frequency of isolation of different fungal genera were calculated as described in section 3.3.3.

Characteristic colonies of each fungus were transferred separately with sterile toothpicks to PDA and incubated at 25°C for 7-14 days. Colonies of *Fusarium* spp. were also sub-cultured on SNA (Nirenberg, 1981) and incubated for 14-21 days at near UV light to enhance sporulation. Colonies of *Aspergillus* spp. were transferred to Czapex Dox agar and incubated for 5 – 7 days at 25°C. Fungal genera, *Aspergillus* and *Fusarium* spp. was identified as described in section 3.3.3.

### 5.3.4 Data analyses

Data on soil nutrients and populations of *Aspergillus* and *Fusarium* spp. were analyzed by linear mixed models fitted by REML in R studio software. Crosstab procedure of SPSS version 22 was used to calculate frequencies of isolation of fungal species and proportions of samples with adequate levels of nutrients. Chi-square test was performed to establish association among adequate levels of total nitrogen, organic carbon, available P, cropping system, county and sampling times. Correlation analysis was performed in SPSS to establish the association among soil nutrients and populations of *Aspergillus* and *Fusarium* spp. in the soil.

## 5.4 Results

### 5.4.1 Levels of soil nutrients in soils

Overall, the levels of organic carbon, total nitrogen, available phosphorus, pH and ECEC were not significantly ($P > 0.05$) different in soil samples collected from push-pull and non-push-pull cropping systems during the three sampling regimes across the seasons (Table 5.1). However, pH levels were significantly higher in soil samples from push-pull farms at planting. The proportion of samples with adequate amounts of total nitrogen and available phosphorus did not differ significantly between push-pull and non-push-pull, but the proportion of samples with adequate amount total of nitrogen and available phosphorus were significantly higher in soils under push-pull cropping system ($P < 0.05$) (Figure 5.1). Presence of adequate organic carbon was only significantly ($P < 0.001$) associated with sampling time and was highest at silking and harvest (R6). Adequacy of total nitrogen in soil was significantly ($P = 0.013$) different among counties. Adequacy of levels of available phosphorus was different between push-pull and non-push-pull ($P = 0.001$), among sampling times ($P < 0.001$) and counties ($P < 0.001$) (Figure 5.1). All the soil samples analyzed had adequate levels of the micronutrients copper and iron.

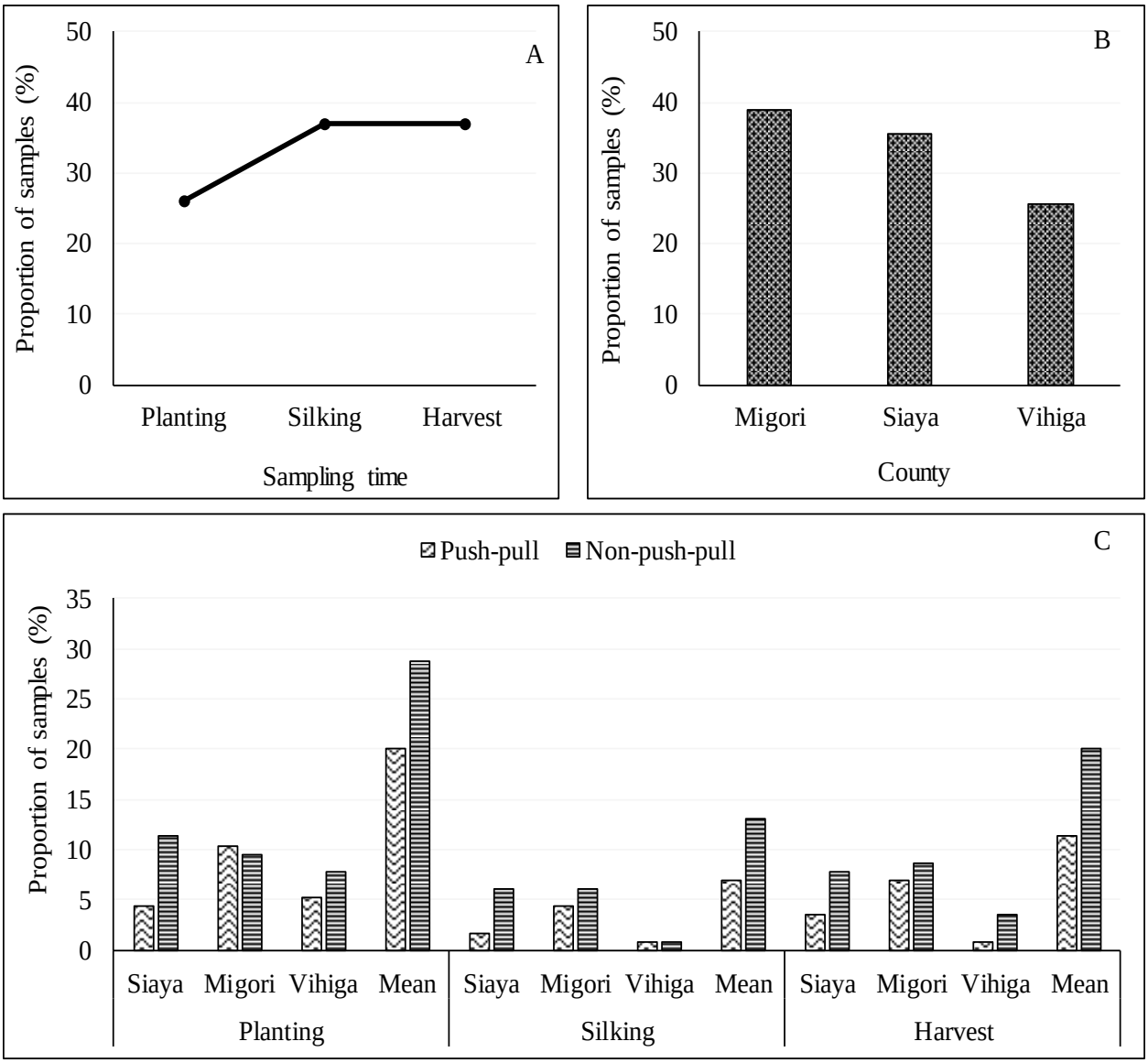


Figure 5. 1: Proportion (%) of soil samples with adequate levels of organic carbon (A), total nitrogen (B) and available phosphorus (C)

Table 5. 1: Levels of nutrients in soils sampled at three sampling regimes for three cropping seasons in three counties in western Kenya

| Soil nutrient | Sampling time | LR2017 | | | | SR2017 | | | | LR2018 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Siaya | Migori | Vihiga | Mean | Siaya | Migori | Vihiga | Mean | Siaya | Migori | Vihiga | Mean |
| Organic carbon (g/kg) | Planting | 13.4 | 16.4 | 15.9 | 15.2 | 13.6 | 14.8 | 13.7 | 14.0 | 12.5 | 12.5 | 12.4 | 12.5 |
| | Silking | 12.7 | 14.8 | 13.8 | 13.8 | 12.8 | 14.9 | 14.6 | 14.1 | 13.8 | 11.2 | 13.9 | 12.9 |
| | Harvest | 12.8 | 13.4 | 15.2 | 13.8 | 10.7 | 10.7 | 12.2 | 11.2 | 13.1 | 13.4 | 15.6 | 14.0 |
| | P value | | | | 0.039 | | | | <0.001 | | | | 0.005 |
| Total nitrogen (g/kg) | Planting | 1.6 | 10.1 | 1.4 | 4.2 | 1.4 | 1.4 | 1.4 | 1.4 | 1.3 | 1.3 | 1.2 | 1.3 |
| | Silking | 1.3 | 1.4 | 1.3 | 1.4 | 1.3 | 1.5 | 1.4 | 1.4 | 1.3 | 1.2 | 1.4 | 1.3 |
| | Harvest | 1.3 | 1.4 | 1.4 | 1.4 | 1.2 | 1.1 | 1.3 | 1.2 | 1.3 | 1.3 | 1.5 | 1.4 |
| | P value | | | | 0.374 | | | | <0.001 | | | | 0.015 |
| Available P (mg/kg) | Planting | 23.5 | 19.0 | 34.0 | 25.7 | 44.0 | 20.5 | 51.7 | 38.3 | 34.5 | 24.0 | 24.5 | 27.7 |
| | Silking | 79.0 | 69.5 | 72.0 | 73.5 | 41.0 | 28.0 | 62.5 | 43.8 | 39.0 | 26.0 | 61.0 | 42.0 |
| | Harvest | 33.9 | 17.5 | 45.5 | 32.2 | 32.0 | 23.0 | 48.5 | 34.5 | 49.5 | 36.5 | 50.0 | 45.3 |
| | P value | | | | <0.001 | | | | 0.071 | | | | <0.001 |
| Soil pH | Planting | 4.8 | 5.1 | 5.5 | 5.1 | 5.1 | 4.9 | 5.9 | 5.3 | 4.8 | 4.7 | 5.4 | 5.0 |
| | Silking | 4.8 | 4.7 | 5.4 | 5.0 | 5.3 | 5.0 | 5.7 | 5.3 | 5.1 | 4.8 | 5.6 | 5.2 |
| | Harvest | 5.1 | 4.9 | 5.8 | 5.3 | 5.1 | 4.9 | 5.8 | 5.3 | 5.1 | 4.9 | 5.6 | 5.2 |
| | P value | | | | 0.021 | | | | 0.399 | | | | <0.001 |
| Effective CEC | Planting | 3.0 | 4.2 | 4.2 | 3.8 | 4.0 | 2.9 | 8.3 | 4.9 | 3.8 | 3.1 | 7.1 | 4.7 |
| | Silking | 2.3 | 2.0 | 4.8 | 3.0 | 5.5 | 3.3 | 8.6 | 5.8 | 3.4 | 2.5 | 6.5 | 4.1 |
| | Harvest | 3.3 | 2.4 | 6.3 | 4.0 | 4.9 | 3.3 | 8.9 | 5.7 | 5.4 | 3.9 | 7.7 | 5.7 |
| | P value | | | | 0.006 | | | | 0.074 | | | | <0.001 |

LR – long rain cropping, SR – short rain cropping season, CEC –cation exchange capacity

### 5.4.2 Populations of *Aspergillus* and *Fusarium* spp. in soils

*Aspergillus*, *Penicillium* and *Fusarium* were the most frequent in soils samples (Figure 5.2). Their populations, however, did not vary significantly ($P > 0.05$) between the push-pull and non-push-pull cropping systems (Table 5.2). Population of *Aspergillus* spp. was significantly ($P = 0.037$) different across seasons and only significantly ($P = 0.011$) varied among counties during the 2017 long rain cropping season. The frequency of *Fusarium* spp. was significantly ($P < 0.05$) reduced by push-pull cropping system during the 2017 long rains and 2018 long rains cropping seasons ($P < 0.05$) compared to non-push-pull. Population of *Fusarium* spp. varied significantly between push-pull and non-push-pull only during the long rain 2018 cropping season ($P = 0.011$), and significantly ($P = 0.05$) varied across seasons.

The most frequent *Aspergillus* spp. in order of decreasing frequency were *Aspergillus* section *Fumigati* (67-93%), *A. niger* (13-93%) and *A. flavus* (0-33%). The average frequency of *A. flavus* in soils was less than 1% (Table 5.3, Figure 5.3) and the populations were not significantly different between push-pull and non-push-pull cropping systems ($P > 0.05$) (Figure 5.4). The population of *A. flavus* and *A. parasiticus* was low (< 50 CFU/g). Other *Aspergillus* spp. isolated included *A. parasiticus*, *A. ostianus*, *A. ochraceous*, and *A. carbonarious*. *Fusarium oxysporum* was the only frequent *Fusarium* spp. in soil samples collected from push-pull (26.9%) and non-push-pull (35.8%) (Table 5.3, Figure 5.3). The frequency of isolation of *F. oxysporum* did not differ significantly among soil samples collected from push-pull and non-push-pull, across the three sampling regimes and significantly different among cropping seasons ($P > 0.05$) (Figure 5.5). *Fusarium verticillioides* was isolated from very few samples (< 5%). Other *Fusarium* spp. isolated in much lower frequencies were *F. equiseti* and *F. subglutinans.*

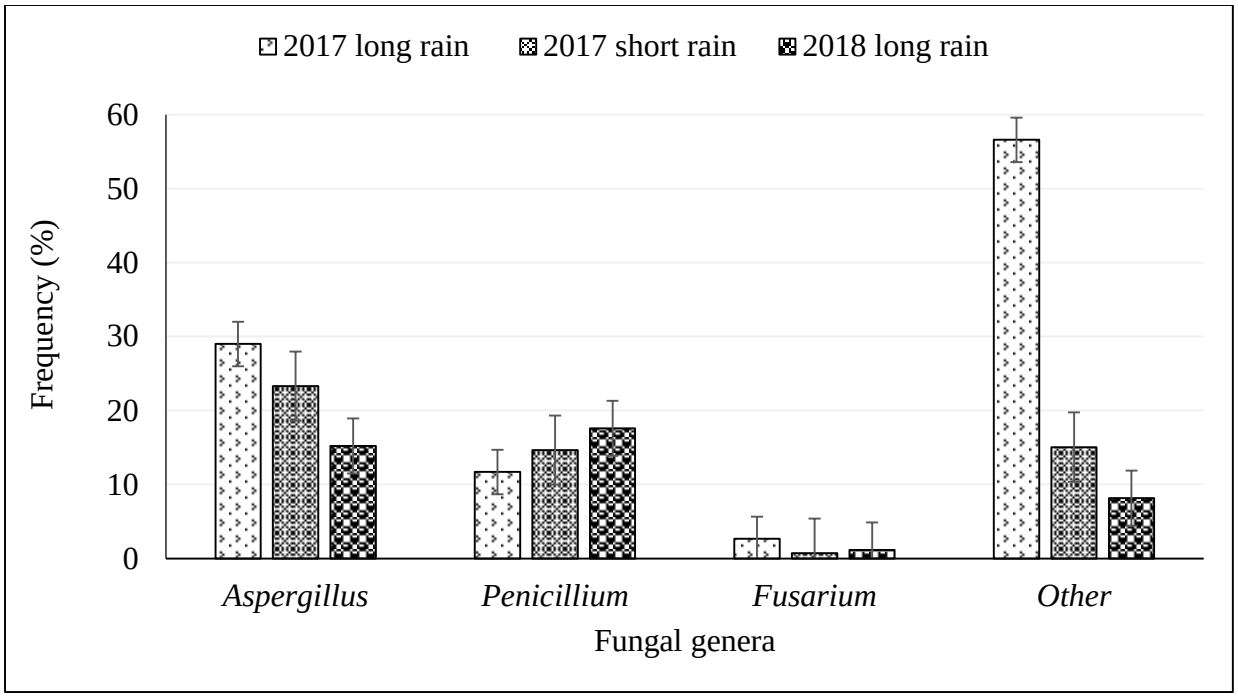


Figure 5. 2: Frequency (%) of isolation of different fungal genera from soil collected during three cropping seasons from push-pull and non-push-pull maize fields in western Kenya (Error bars represent the standard error of the mean)

Table 5. 2: Population (CFU/g) of major fungal genera isolated from soil at different sampling stages during three cropping seasons in western Kenya

| Season | Fungal genera | Planting | Silking | Harvest | Mean | P value |
|---|---|---|---|---|---|---|
| LR2017 | *Aspergillus* | 1901a | 1183b | 671c | 1256 | <0.001 |
| | *Fusarium* | 60c | 142a | 93b | 98 | <0.001 |
| | *Penicillium* | 502a | 312b | 514a | 441 | <0.001 |
| | Other | 1318c | 2356b | 2605a | 2087 | <0.001 |
| SR2017 | *Aspergillus* | 808a | 1143a | 1046a | 1000 | 0.617 |
| | *Fusarium* | 2c | 98a | 20b | 41 | <0.001 |
| | *Penicillium* | 146c | 935b | 1099a | 735 | <0.001 |
| | Other | 700a | 630a | 630a | 651 | 0.942 |
| LR2018 | *Aspergillus* | 819a | 788a | 585a | 730 | 0.607 |
| | *Fusarium* | 48a | 45a | 46a | 46 | 0.153 |
| | *Penicillium* | 1387a | 1042a | 663a | 1031 | 0.158 |
| | Other | 516 | 389aa | 323a | 409 | 0.632 |

CFU – colony forming units per gram, LR – long rain, SR – short rain, PPT – push-pull technology, NPPT – non-push-pull technology, P – calculated 95% probability value
Values followed by the same letter along the same row are not significantly different

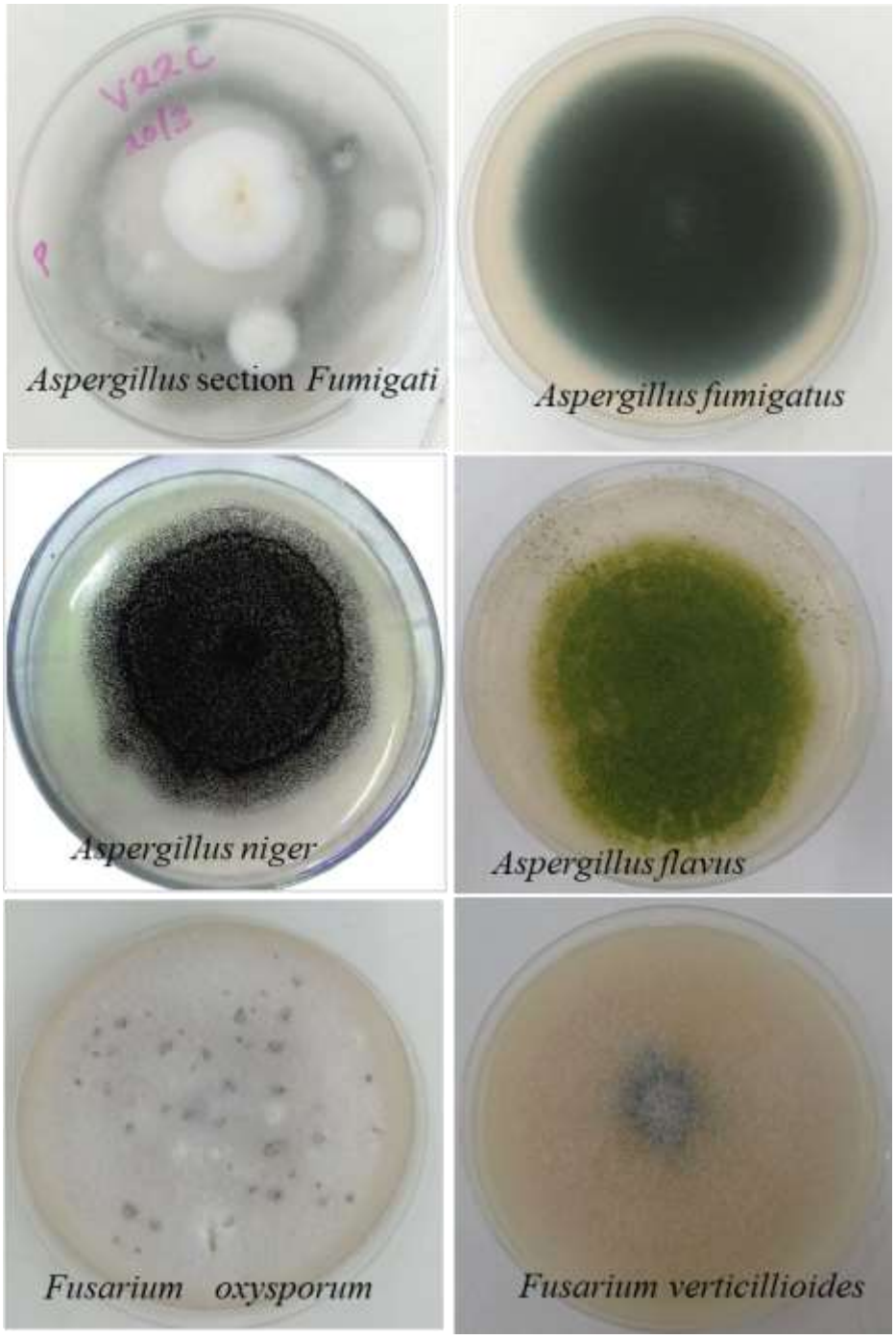

Figure 5. 3: Colonies of frequently isolated *Aspergillus* and *Fusarium* spp.

Table 5. 3: Frequency (%) of isolation of different *Aspergillus* and *Fusarium* spp. isolated from soil samples collected during three cropping seasons in push-pull and non-push-pull farms in western Kenya

| Season | Push-pull | | | Non-push-pull | | | P value (CS) |
|---|---|---|---|---|---|---|---|
| | Planting | Silking | Harvest | Planting | Silking | Harvest | |
| 2017 long rains | | | | | | | |
| *Aspergillus* section *Fumigati* | 23.3 | 9.5 | 6.5 | 18.1 | 15.4 | 9.3 | 0.110 |
| *A. flavus* | 0.2 | 0.4 | 0.8 | 1.8 | 1.3 | 0.1 | 0.512 |
| *A. niger* | 4.4 | 5.9 | 3.1 | 5.2 | 5.1 | 6.5 | 0.912 |
| Aspergilli | 15.0 | 6.8 | 4.9 | 13.7 | 9.3 | 6.0 | 0.006 |
| *F. oxysporum* | 1.2 | 4.2 | 1.4 | 2.3 | 2.2 | 2.5 | 0.101 |
| *F. verticillioides* | 0.1 | 0.1 | 0.0 | 0.2 | 0.0 | 0.0 | 0.088 |
| Fusaria | 0.4 | 0.5 | 0.4 | 0.0 | 0.4 | 0.0 | 0.566 |
| 2017 short rains | | | | | | | |
| *Aspergillus* section *Fumigati* | 24.4 | 8.7 | 11.8 | 17.3 | 14.5 | 14.1 | 0.123 |
| *A. flavus* | 0.0 | 0.8 | 0.3 | 0.1 | 0.2 | 0.0 | 0.152 |
| *A. niger* | 0.6 | 3.9 | 3.4 | 0.7 | 2.1 | 3.3 | 0.014 |
| Aspergilli | 6.3 | 3.5 | 5.9 | 6.3 | 6.3 | 4.7 | 0.128 |
| *F. oxysporum* | 0.1 | 1.0 | 0.2 | 0.2 | 0.3 | 0.6 | 0.271 |
| *F. verticillioides* | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.089 |
| Fusaria | 0.0 | 0.1 | 0.2 | 0.0 | 0.2 | 0.0 | 0.128 |
| 2018 long rains | | | | | | | |
| *Aspergillus* section *Fumigati* | 6.2 | 5.9 | 8.7 | 10.1 | 9.4 | 8.4 | 0.980 |
| *A. flavus* | 0.3 | 0.5 | 0.2 | 0.5 | 0.4 | 0.1 | 0.308 |
| *A. niger* | 1.8 | 3.6 | 4.1 | 2.0 | 3.4 | 3.2 | 0.396 |
| Aspergilli | 3.5 | 3.2 | 5.0 | 2.7 | 4.7 | 3.0 | 0.606 |
| *F. oxysporum* | 0.2 | 0.6 | 0.1 | 0.5 | 1.6 | 1.7 | 0.330 |
| *F. verticillioides* | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.9 | <0.001 |
| Fusaria | 0.0 | 0.0 | 0.1 | 0.5 | 0.1 | 0.1 | 0.522 |

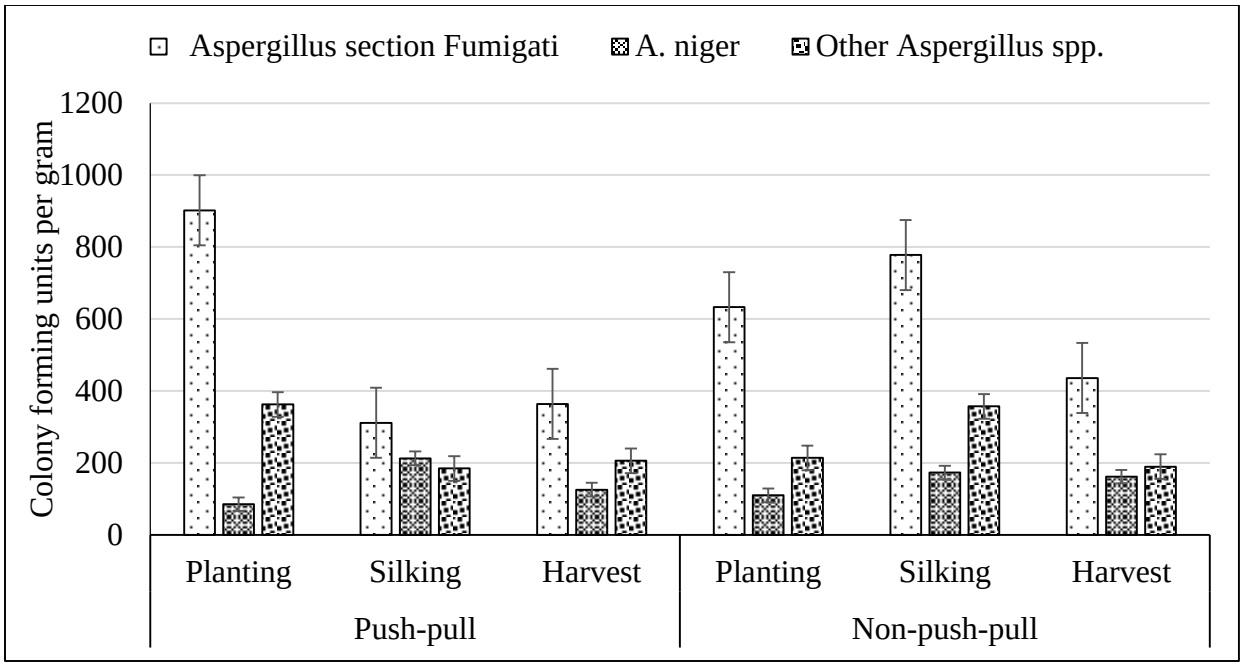


Figure 5. 4: Population (CFU/g) of *Aspergillus* spp. isolated from soil collected from push-pull and non-push-pull farms at different sampling stages during three cropping seasons in western Kenya (Error bars represent the standard error of the mean)

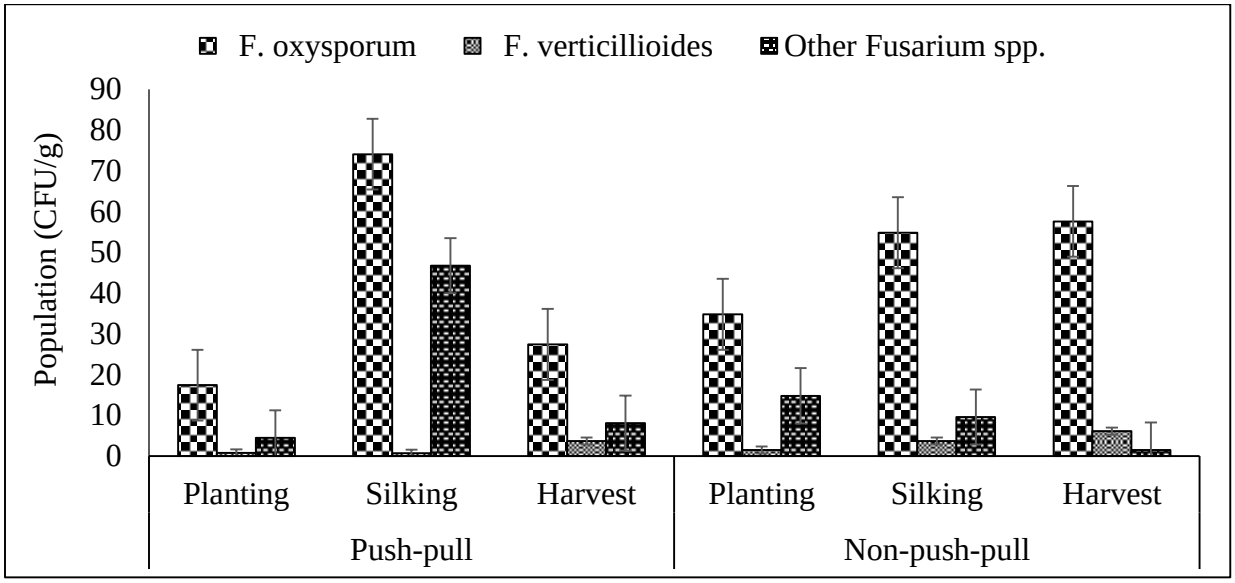


Figure 5. 5: Population (CFU/g) of *Fusarium* spp. isolated from soil collected from push-pull and non-push-pull farms at different sampling stages during three cropping seasons in western Kenya (Error bars represent the standard error of the mean)

### 5.4.3 Correlation among levels of soil nutrients and populations of *Aspergillus* and *Fusarium* spp. isolated from soils

Levels of organic C, total N, available P, pH and ECEC had no influence on the frequency and populations of *Fusarium* spp. and *Aspergillus* section *Fumigati*. The frequency of isolation of the *Aspergillus* spp. had positive correlation with pH and ECEC ($P < 0.05$) (Table 5.6). There were positive and significant correlations between the frequency of isolation of *A. flavus* and *A. niger* with levels of carbon, nitrogen, phosphorus, ECEC and pH ($P < 0.05$) (Table 5.7). The levels of organic carbon, however, had negative correlation with the total fungal population ($P < 0.05$) (Table 5.7). The populations of *A. flavus* and *A. niger* significantly increased ($P < 0.05$) with increased levels of carbon, phosphorus, ECEC and pH (Table 5.7).

Table 5. 4: Correlations among frequency of isolation of different fungi and levels of selected fertility parameters in soil samples collected over three cropping seasons at three sampling sites in push-pull and non-push-pull

| | Organic C (g/kg) | Total N (g/kg) | Available P (mg/kg) | ECEC | pH |
|---|---|---|---|---|---|
| *Aspergillus* | 0.043 | 0.049 | -0.061 | 0.137* | 0.191** |
| A. *flavus* | 0.134* | 0.081 | 0.152* | 0.224** | 0.225** |
| A. *niger* | 0.183* | 0.122* | 0.165** | 0.210** | 0.245** |
| Aspergilli | 0.047 | 0.011 | 0.083 | 0.283** | 0.309** |
| *Penicillium* | 0.018 | -0.005 | -0.026 | 0.166* | 0.068 |
| Other fungi | 0.086 | 0.099 | -0.089 | -0.223** | 0.095 |

* - significant at 0.05, ** - significant at 0.001, *** - significant at 0.0001; ECEC – effective cation exchange capacity

Table 5. 5: Correlations between populations of different fungi and levels of selected fertility parameters in soil samples collected over three cropping seasons at three sampling sites in push-pull and non-push-pull farms

| | Organic C (g/kg) | Total N (g/kg) | Available P (mg/kg) | ECEC | pH |
|---|---|---|---|---|---|
| *Aspergillus* | -0.055 | -0.023 | -.059 | 0.207** | 0.148* |
| A. *flavus* | 0.127* | 0.081 | 0.177** | 0.243** | 0.243** |
| A. *niger* | 0.154* | 0.093 | 0.226** | 0.232** | 0.250** |
| Aspergilli | 0.058 | 0.065 | 0.151* | 0.042 | 0.370** |
| *Penicillium* | -0.099 | -0.028 | -0.039 | 0.028 | 0.139* |
| Other fungi | -0.013 | 0.001 | -0.140 | -0.231** | -0.112 |

* - significant at 0.05, ** - significant at 0.001, *** - significant at 0.0001; ECEC – effective cation exchange capacity, CFU – colony forming units

## 5.5 Discussion

Organic carbon, nitrogen, phosphorus, pH and cation exchange capacity are important nutrition parameters in agricultural soils. When cereals are grown continuously for long periods, the levels of these nutrients are reduced drastically (Shah et al., 2003). Inadequacy of levels of pH, carbon and nitrogen have been shown to increase populations of pathogenic fungi (Kanwal et al., 2017). The results of this study showed no improvement in the levels of pH, ECEC, organic C, total N, and available P under the push-pull. In addition, the levels of the five fertility parameters did not differ between the starting (LR2017) and the last (LR2018) cropping seasons of the study.

Compared to phosphorus, nitrogen was the most limiting of the two nutrients across the seasons because 98% of soil samples had less than 0.20% total nitrogen. The findings concur with a previous study by (Vanlauwe et al., 2008). In fact, the range of values for organic C, total N and pH were similar between the current study and the study by Vanlauwe et al. (2008). This implied that though push-pull cropping system has direct impact on stemborer and fall armyworm infestation, ear rots, striga weed and grain yield (Khan et al., 2010; Midega et al., 2018; Owuor et al., 2018), the cropping system has minimal detectable impact on overall soil fertility status.

Kifuko-Koech et al. (2012) suggested that the reason for minimal effect of push-pull cropping system on soil fertility was absence of adequate levels of both nitrogen and phosphorus, which was the case in the current study. The study showed that when phosphorus was added to the soil in adequate levels, established desmodium in push-pull cropping systems substituted for inorganic nitrogen fertilization and crop growth was enhanced. Vanlauwe et al. (2008) associated low detectable soil fertility in push-pull soil samples with improperly maintained desmodium and desmodium that is not well established even though push-pull plots were old enough to cause detectable changes in nutrient levels (Vanlauwe et al., 2008). This might have been because the

desmodium dried off most of the seasons due to drought and therefore it was rarely fully established. Also, the study sites experience erratic rainfall and prolonged drought, which lowers the efficiency of desmodium because it dries up in patches and farmers keep gapping at the start of almost each season (Vanlauwe et al., 2008). The efficiency in increasing soil nutrition of the cropping system is also affected by the fact that farmers might not give enough time for desmodium and napier/Brachiaria to establish, because they concentrate on associated benefits of the cropping system such as fodder (Kifuko-Koech et al., 2012; Vanlauwe et al., 2008). Nitrogen levels are greatly affected by post-sampling handling and storage (Horneck et al., 2011).

The prevalence and population of *A. flavus*, and *F. verticillioides*, the main producers of aflatoxin and fumonisin, respectively (Klich, 2007; Leslie & Summerell, 2006), were low and were not significantly reduced in soils under push-pull. The trend between the cropping systems was similar to that reported by an earlier study (Owuor et al., 2017). The populations of these fungal species were however, much lower in this study as compared to the previous study. The difference could have been because in the previous study, soil sampling was done from a shallower depth (6 cm) while in the current study the sampling was deeper (15 cm). Moreover, spores of *A. flavus* are hydrophobic and therefore would not be found beyond 6 cm soil depth (Horn, 2003). Properties of top soil enhance microbial diversity and activity (Sopialena et al., 2017). The populations reported in this study was, however, too low to be the source of *A. flavus* and *F. verticillioides* isolated from maize in the current study (3.4.4). The primary source of inocula for these two fungal species was therefore not only soil but also crop residues on the soil (Keller, 2011; Parry et al., 1993).

Infected alternative hosts could also have been possible sources of secondary inocula, although secondary sources of inocula have been reported not to play an important role in infection of wheat (Snijders & Perkowski, 1990). Some mycotoxigenic fungi are facultative pathogens of other crops

(Horn, 2003). Previous studies have also reported that soil microorganisms mainly colonize host plants rhizosphere and the rhizosphere influence the diversity and activity of microorganisms (Pandey et al., 2018). In the current study, rhizosphere was avoided during soil sampling. In addition to nutrient stress, the low populations of the fungi could have been due to water and temperature stress due to climatic change (Pandey et al., 2018).

Increase in the levels of carbon, phosphorus, pH and ECEC significantly increased the populations and frequency of isolation of *A. flavus* and *A. niger*. Augusto et al. (2018) reported carbon and nitrogen as the limiting nutrients for soil organisms, hence the correlation reported in the current study. Additionally, organic carbon, pH and nitrogen had significant negative correlation with total fungal population. This could be because the farmers continually used DAP (3.4.2), which is a mineral fertilizer, and could have negatively affected the availability of organic C, available P and total N thus increasing the population of pathogenic fungi in the soil as reported by Augusto et al. (2018) and Kanwal et al. (2017). The current study, however, could not associate this correlation to the cropping systems – push-pull or non-push-pull - because both cropping systems had inadequate levels of the nutrients, particularly total nitrogen and carbon. Blandino et al. (2008) reported a negative correlation between adequate nitrogen fertilization and levels of ear rots infection, aflatoxin and fumonisin contamination. They showed that balanced nitrogen application lowered the levels of mycotoxins. Moreover, majority of the spores of the toxigenic fungi in the soil could have lost viability over time due to lack of enough substrate (Horn, 2003).

### 5.6 Conclusions and recommendations

There was no detectable change in the levels of pH, ECEC organic C, total N and available P, in soil under push-pull in comparison with non-push-pull cropping system across the seasons,

sampling sites and sampling regimes. In addition, the populations of toxigenic fungi, particularly *F. verticillioides* and *A. flavus* were low and were not reduced in soils under push-pull cropping system. Therefore, the positive and significant correlation between the populations of *A. flavus* and *A. niger* with the levels of C, available P, pH and ECEC could not be associated with the cropping system from which the soil samples were collected. Follow up studies are recommended to isolate fungi from top 3 cm and crop debris to establish the source of fungal inocula that contaminate maize with mycotoxin as reported in previous studies.

# CHAPTER SIX

## EFFECTS OF DESMODIUM ROOT EXTRACTS ON GROWTH OF MYCOTOXIN PRODUCING *Aspergillus flavus* AND *Fusarium verticillioides*


### 6.1 Abstract

Maize grown under push-pull cropping system has been reported to contain lower populations of *Aspergillus flavus* and *Fusarium verticillioides*, as well as lower concentrations of fumonisin and aflatoxin. In this study, the *in vitro* effect of desmodium root extracts on spore germination and mycelial growth of toxigenic *A. flavus* and *F. verticillioides* isolated from maize and soil was determined. Mycotoxin free maize was inoculated with spores of *A. flavus* and *F. verticillioides* and potential of the fungi to produce aflatoxin and fumonisin, respectively, was determined by ELISA. Dried and ground desmodium roots were extracted with methanol and dichloromethane and concentrated with a vacuum rotary evaporator. Spores from seven days old toxigenic *A. flavus* and *F. verticillioides* cultures were cultured on potato dextrose agar (PDA) amended with the crude extract. All the *F. verticillioides* isolates produced high fumonisin levels between 2804 and 599,741 µg/kg, while *A. flavus* isolates produced aflatoxin levels between 1.0 and 199,184 µg/kg. The root extracts showed reduction in proportion of germinated spores by 9.6% for *A. flavus* and 43.8% for *F. verticillioides*, and resulted in significant reduction in radial growth of *A. flavus* by 14.5% and *F. verticillioides* by 57%. These results suggested reduced spore germination and radial growth of toxigenic fungi in push-pull soil as a mechanism of reducing infection of maize by mycotoxigenic fungi during growth in the field.



**Key words:** aflatoxin, *Aspergillus flavus*, Desmodium, fumonisin, *Fusarium verticillioides*, push-pull, root extracts

## 6.2 Introduction

Mycotoxins are secondary metabolites produced by certain fungi that infect cereals such as maize (Miller, 2008; Negedu et al., 2011; WHO, 2018). The amount of mycotoxin is dependent on multiple factors, mainly environmental (moisture, humidity, temperature) and nutritional parameters during the development of the fungi (Miller, 2008). The fungi that produces mycotoxins are also responsible for maize ear rots infections (Schmaile III & Munkvold, 2009). Aflatoxin and fumonisin are the most economically important mycotoxins of maize in sub-Saharan Africa (Lewis et al., 2005; Mutegi, Cotty, & Bandyopadhyay, 2018; Mutiga et al., 2015; Kedera et al., 1999).

*Aspergillus flavus* and *F. verticillioides* are the major producers of aflatoxin and fumonisin, respectively (IARC, 1972; Klich, 2007a; Samson & Varga, 2009; Leslie & Summerell, 2006). *Aspergillus flavus* is an opportunistic pathogen of maize and grows as yellow-green spore masses (Schoeman, 2012; Rodrigues et al., 2011). *Aspergillus flavus* mainly leads to deterioration of maize quality in tropical areas undergoing the negative impact of climate change (Cotty & Jaime-Garcia, 2007). *Fusarium verticillioides* is a cosmopolitan pathogen of maize and its growth on maize is characterized by pinkish to violet moldiness of the grain (Leslie & Summerell, 2006). *Fusarium verticillioides* persists in host residues found on or in the soil upon mechanical incorporation for up to 900 days depending on the prevailing climatic conditions.

There are a couple of reports on incidence of aflatoxin and fumonisin contamination in maize in different counties of western Kenya, with scanty information on mitigation strategies (Kedera et al., 1999; Mutiga et al., 2014, 2015; Owuor et al; 2018). Owuor et al. (2018) reported that push-pull has potential to reduce ear rot and contamination of maize with mycotoxins associated with rotting. The mechanism of action is however, not understood.

Studies have reported that plant root-exudates influence the fungal community in the soil (Broeckling et al., 2008; Yang et al., 2014). This mainly happens through maintaining the population of resident fungi and inhibiting existence of non-residence fungi. The relationship between soil microorganisms and plants are very specific (Boivin et al., 2016; Steinkellner et al., 2007; Sullia, 1973). Several *in vitro* studies have shown that plant extracts have inhibitory activity against fungal plant pathogens (Muthomi et al., 2017; Njoki, Okoth, & Wachira, 2017; Okumu et al., 2019). The activity could be attributed to presence of bioactive chemicals such as flavonoids and alkaloids, which inhibit spore germination, modification of hyphae and modification of the structure of the fungal mycelia (Tabassum & Vidyasagar, 2013). Roots of mature desmodium produce C-glycosyl flavonoid exudates (Hooper et al., 2015). In this study, the *in vitro* activity of desmodium root extracts against toxigenic *Aspergillus flavus* and *Fusarium verticillioides* isolated from maize and soil, with the aim of elucidating the mechanisms involved in reduction of ear rots and mycotoxins in maize under push-pull cropping system was determined.

## 6.3 Materials and Methods

### 6.3.1 Determination of aflatoxin and fumonisin production potential of *Aspergillus flavus* and *Fusarium verticillioides*

One hundred and five isolates of *A. flavus* (53 = push-pull, 52 = non-push-pull) and 100 isolates of *F. verticillioides* (49 = push-pull, 45 = non-push-pull) recovered from maize and soils from western Kenya were tested for aflatoxin and fumonisin production potential, respectively. Twenty one of isolates tested for aflatoxin production were from soil and 84 were from maize, while all isolates tested for fumonisin production were from maize. The isolates were tested for mycotoxin production by inoculating 10 g of sterile mycotoxin free maize grains in 40 ml glass vials with 500 µl of spores ($10^8$) under aseptic conditions and incubated at 25°C for seven days to allow maximum

colonization of the maize. The maize was first tested for aflatoxin and fumonisin levels as described in section 3.3.3 and sterilized by autoclaving at 121°C and 15psi for 20 minutes. The colonized maize was blended with 50 ml of 70% methanol for *A. flavus* and 20 ml of 90% methanol for *F. verticillioides* isolates. After settling, the supernatant was filtered through a funnel lined with Whatman No. 1 filter paper and tested for aflatoxin and fumonisin levels as described in Section 3.3.3.

Aflatoxin and fumonisin levels were determined and interpreted as described in section 3.3.3. Six randomly selected aflatoxin producing isolates (3 = push-pull, 3 = non-push-pull) and six (4 = push-pull, 2 = non-push-pull) fumonisin producing isolates were purified by spreading 100 µl of $10^6$ dilution of spores on PDA and incubating at 25°C. The plates were checked daily for growth and distinct colonies from an individual spore were selected, sub-cultured on PDA and incubated at 25°C for seven days. Spores from the seven-day old cultures were used to test the antifungal activity of desmodium root extracts.

### 6.3.2 Preparation of desmodium root extracts

Desmodium root extracts were prepared following the method by (Ahmed et al., 2014) with modifications. Fresh roots of *D. intortum* and *D. uncinatum* were collected by pickaxe digging from the demonstration plots at the International Centre of Insect Physiology and Ecology (icipe) Thomas Odhiambo Campus Mbita campus. The roots were thoroughly washed under running tap water, chopped into one centimeter pieces, followed by surface sterilization in 1.3% sodium hypochlorite and rinsed with distilled water. The roots were then aseptically air-dried in the laboratory for one week and finely ground in a blender (Mika MNB1001 - Nutriblast Blender, 900W – Black). Two hundred grams of the powder of desmodium roots was extracted with 1000

ml of dichloromethane: methanol (1:1 v/v) by soaking and frequently shaking the mixture for 24 hours. The extract was passed through cotton wool and filtered through Whatman No.1 filter paper. The filtrate was concentrated using a vacuum rotary evaporator (Stuart, RE400/CO, SA) to 100 ml and the concentrated extract stored in a refrigerator at 4°C until use.

### 6.3.3 Determination of the effect of desmodium root extracts on mycelial growth of toxigenic *Aspergillus flavus* and *Fusarium verticillioides*

The ability of the desmodium root extract to inhibit fungal growth was tested using poisoned food technique ((Al-Samarrai, Singh, & Syarhabil, 2012) with modifications. Potato dextrose agar autoclaved for 20 minutes at 15 psi was cooled to 45°C and amended with 50 mg of chloramphenicol and streptomycin. The concentrated crude extract was mixed with the molten PDA in the ratio of one ml of methanol extract to 20 ml media (v/v). Control plates were incorporated with methanol only. The molten media was then stirred on a magnetic stirrer to uniformly distribute the extract and dispensed aseptically into sterile petri-dishes. The media was allowed to set and the methanol to evaporate in the biosafety cabinet overnight. The plates were point inoculated at the center with spores at the periphery of seven-day old purified cultures of six (3 = push-pull, 3 = non-push-pull) aflatoxin producing *A. flavus* isolates and six (4 = push-pull, 2 = non-push-pull) fumonisin producing *F. verticillioides* isolates. Each treatment was replicated thrice and incubated at 25°C. This experiment was repeated twice. Diameters of the fungal colonies were measured at the second, fourth and sixth day after incubation. Antifungal activity was determined as inhibition of radial mycelial growth by calculating the percentage reduction in fungal radial growth as follows:

$$\%\ \text{inhibition} = \frac{(\text{dc} - \text{dt})}{\text{dc}} \times 100$$

Where dc was the diameter of the fungal colony without extract, and dt was diameter of fungal colony in the plates with extract.

### 6.3.4 Determination of the effect of desmodium root extracts on *Aspergillus flavus* and *Fusarium verticillioides* spore germination and germ tube growth

Spores of *A. flavus* and *F. verticillioides* were harvested from seven-day old PDA cultures by adding five ml of 0.1% Tween 80 (v/v) solution into the plates. One milliliter of the spore suspension was transferred to an Eppendorf tube and serially diluted to $10^2$/ml spore concentration. One hundred microliters of the diluted spore suspension were spread on PDA plates amended with crude desmodium root extract in the ratio of one ml extract to 20 ml media (v/v). Control plates contained PDA amended with methanol only and each treatment was replicated twice. The media was allowed to set and the methanol to evaporate in the biosafety cabinet overnight. The plates were incubated at 25°C and observations made four, six, eight and ten hours after plating. The proportion of germinated spores was counted in open plates under the microscope at ×400 in 10 fields of view, and the length of germ tube was measured using a calibrated optical micrometer. Hyphae and germ tubes were observed for growth and presence of deformities. A drop of lactophenol cotton blue dye was placed on the surface of the inoculated media, a cover slip placed on top and observation done under a compound light microscope at ×40 magnification. The proportion of germinated spores was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{No.of germinated spores}}{\text{Total number of spores}} \times 100$$

Percentage inhibition of spore germination was calculated using the formula:

$$\text{Germination inhibition (\%)} = \frac{\text{Germinated spores without extract} - \text{Germinated spores with extract}}{\text{Germinated spores without extract}} \times 100$$

Percentage inhibition of germ tube elongation was calculated using the formula:

$$\text{Inhibition of elongation (\%)} = \frac{\text{Germ tube length without extract} - \text{Germ tube length with extract}}{\text{Germ tube length without extract}} \times 100$$

### 6.3.5 Data analyses

Data on aflatoxin and fumonisin levels produced by test isolates was described using measures of central tendency using descriptive statistics procedure in SPSS version 22. Aflatoxin levels were categorized into below limit of detection, less than 10 and greater than 10, and association among the levels produced by isolate and cropping system from which they were isolated was determined by Chi-square test. Means of colony diameter were compared between treatments at different incubation time by Analysis of Variance (ANOVA) tested at 5% probability in R Studio version 3.5.3. Statistical differences in means of proportion of germinated spores and germ tube length in the plates with and without extract after different hours of incubation were also determined by ANOVA in R studio and means separated using Fisher's LSD. Data that was not normally distributed was transformed before analysis by first changing the percentages to proportions and then using the arcsine transformation.

## 6.4 Results

### 6.4.1 Aflatoxin and fumonisin production by *Aspergillus flavus* and *Fusarium verticillioides*

Maize colonized by *A. flavus* and *F. verticillioides* isolates is shown in Figure 6.1. Ninety four percent of *A. flavus* isolates recovered from both push-pull and non-push-pull maize and soil produced detectable levels of aflatoxin. There was, however, no significant (P = 0.266) difference between the aflatoxin levels produced by isolates recovered from push-pull and those recovered from non-push-pull cropping system. Aflatoxin potential of the *A. flavus* isolates ranged between

1.0 and 199,184 µg/kg (Table 6.1). Six percent of *A. flavus* isolates did not produce aflatoxin. On the contrary, all the *F. verticillioides* isolates produced high fumonisin levels between 2804 and 599,741 µg/kg (Table 6.1). The fumonisin production potential did not differ between isolates from push-pull and those from non-push-pull (P = 0.757).

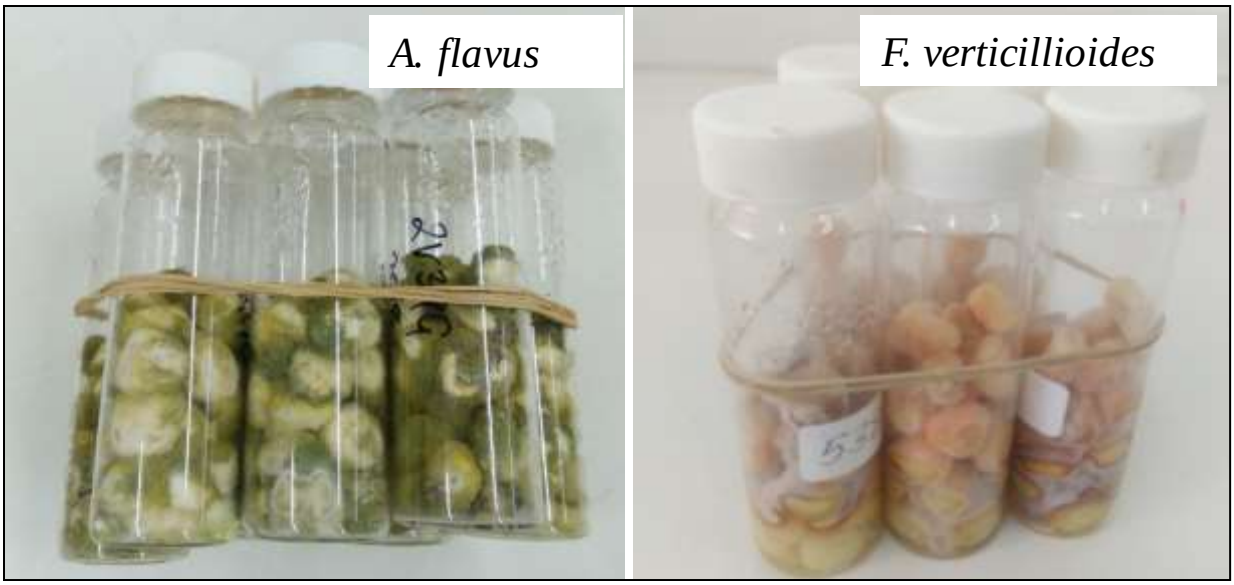


Figure 6. 1: Maize grains colonized by *A. flavus* and *F. verticillioides* after incubation for seven days at 25°C.

Table 6. 1: Aflatoxin levels (µg/kg) produced in clean maize by *A. flavus* and fumonisin levels (µg/kg) produced by *F. verticillioides* isolated from maize and soil under push-pull and non-push-pull cropping systems

| Range | Fumonisin | | | Aflatoxin | | |
|---|---|---|---|---|---|---|
| | Push-pull (n = 49) | Non-push-pull (n = 45) | Overall (n = 94) | Push-pull (n = 53) | Non-push-pull (n = 52) | Overall (n = 105) |
| Minimum | 2,929.4 | 2,803.8 | 2,803.8 | < LOD | < LOD | < LOD |
| Maximum | 521,482.4 | 599,740.6 | 599,740.6 | 199,184.3 | 10,433.1 | 199,184.3 |
| Median | 213,058.3 | 143,563.7 | 178,625.9 | 7.2 | 4.9 | 5.1 |

LOD – lower limit of detection (1 µg/kg for aflatoxin and 100 µg/kg for fumonisin)

### 6.4.2 Effect of desmodium root extracts on mycelial growth

Crude *D. intortum* and *D. uncinatum* extracts significantly ($P < 0.001$) reduced radial growth of *A. flavus* and *F. verticillioides* isolates (Figure 6.2, Table 6.2). The reduction was also significant across days after incubation ($P < 0.05$). There was significant ($P < 0.05$) interaction between presence of extract in the media and days of incubation. Reduction of radial growth of *A. flavus* was, however, not significantly different ($P = 0.059$) between the extracts of *D. intortum* and *D. uncinatum*. The reduction in radial growth of *F. verticillioides* and *A. flavus* was by 53-61% and 12 – 17%, respectively (Table 6.3). The percentage reduction in radial growth of the fungal colonies decreased with increase in days after incubation for most isolates of *F. verticillioides*. Percentage reduction of *A. flavus* and *F. verticillioides* growth was significantly ($P < 0.05$) decreased across days of incubation. *Desmodium uncinatum* root extract was significantly ($P < 0.05$) more effective in reduction of *F. verticillioides* radial growth compared to *D. intortum* extract. Both *D. intortum* and *D. uncinatum* caused significantly ($P < 0.001$) more reduction in colony diameter of *F. verticillioides* than that of *A. flavus*.

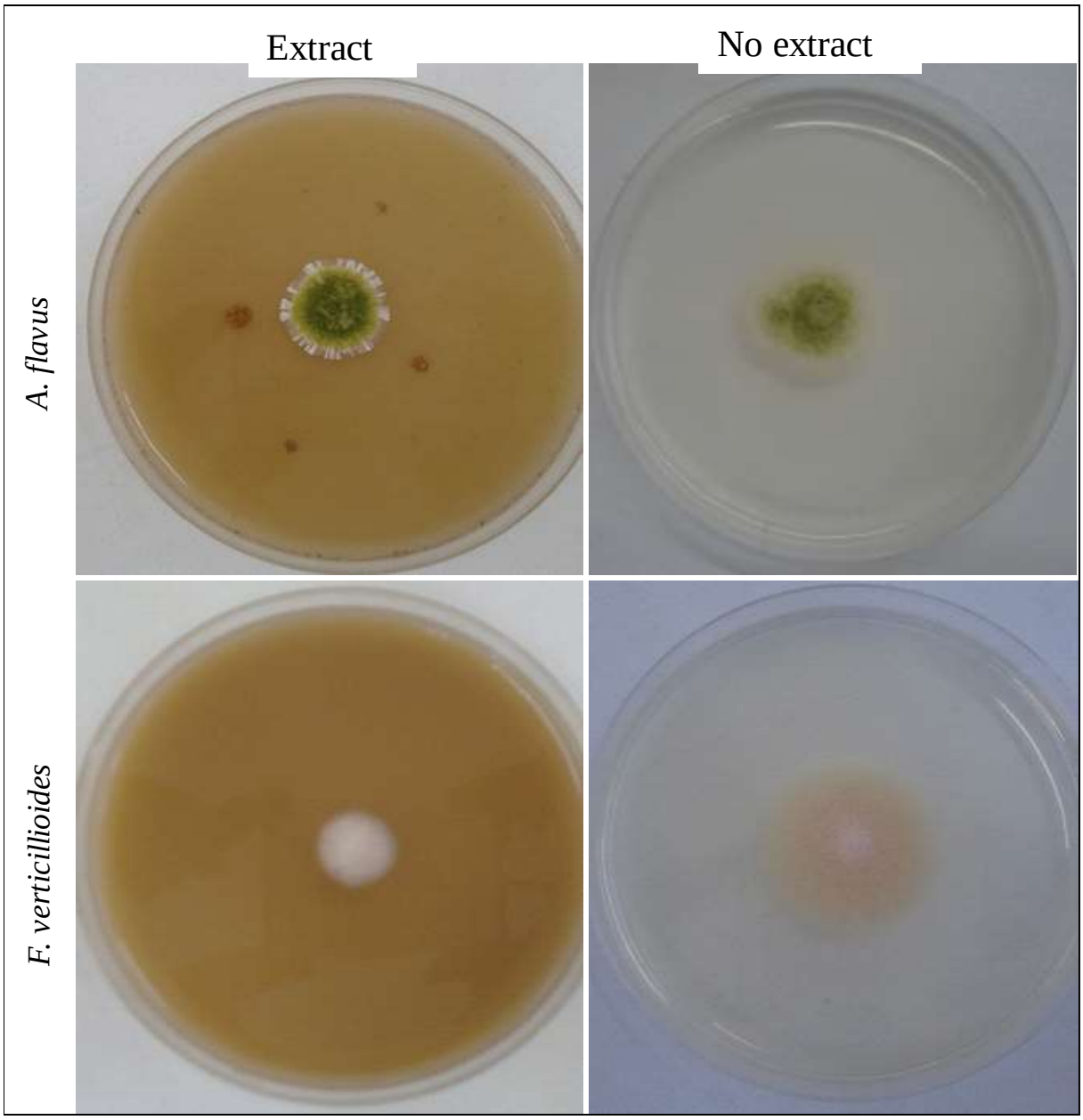


Figure 6. 2: Four days old cultures of *A. flavus* and *F. verticillioides* plated on PDA treated with desmodium root extract and PDA without the extract

Table 6. 2: Colony diameter (cm) of *A. flavus* and *F. verticillioides* grown on PDA amended with root extracts from two Desmodium species and incubated for two, four and six days

| Fungal species | Isolate | Days incubated | *D. intortum* | | *D. uncinatum* | |
|---|---|---|---|---|---|---|
| | | | Extract | No extract | Extract | No extract |
| *A. flavus* | 81B | Two | 1.1±0.0 | 1.4±0.1 | 1.4±0.1 | 1.0±0.0 |
| | | Four | 2.5±0.1 | 3.1±0.0 | 2.8±0.0 | 2.6±0.0 |
| | | Six | 4.1±0.2 | 5.3±0.0 | 4.6±0.0 | 4.3±0.0 |
| | 151D | Two | 1.1±0.1 | 1.2±0.0 | 1.3±0.1 | 1.0±0.1 |
| | | Four | 2.5±0.2 | 2.9±0.0 | 2.4±0.1 | 2.3±0.0 |
| | | Six | 4.0±0.3 | 5.0±0.0 | 3.9±0.0 | 3.7±0.1 |
| | 105A | Two | 1.5±0.0 | 2.0±0.0 | 1.9±0.1 | 1.2±0.0 |
| | | Four | 3.5±0.1 | 4.1±0.0 | 2.4±0.0 | 2.3±0.1 |
| | | Six | 5.4±0.1 | 6.3±0.0 | 3.8±0.1 | 3.5±0.0 |
| | 2M35E | Two | 1.2±0.0 | 2.1±0.0 | 1.3±0.1 | 1.2±0.0 |
| | | Four | 3.8±0.0 | 4.3±0.1 | 2.8±0.0 | 2.6±0.0 |
| | | Six | 4.6±0.0 | 6.5±0.1 | 4.6±0.0 | 4.2±0.0 |
| | 379B | Two | 1.4±0.0 | 1.4±0.0 | 1.3±0.0 | 1.1±0.0 |
| | | Four | 2.9±0.1 | 3.3±0.1 | 2.8±0.0 | 2.5±0.1 |
| | | Six | 4.8±0.2 | 5.2±0.1 | 4.6±0.1 | 4.1±0.1 |
| | 479D | Two | 1.0±0.0 | 1.2±0.0 | 1.1±0.0 | 1.0±0.0 |
| | | Four | 2.4±0.0 | 2.8±0.0 | 2.2±0.0 | 2.1±0.0 |
| | | Six | 3.9±0.0 | 4.5±0.0 | 3.7±0.0 | 3.3±0.0 |
| P value | | | | <0.001 | | <0.001 |
| *F. verticillioides* | 561B | Two | 2.1±0.0 | 0.9±0.0 | 0.5±0.0 | 1.6±0.0 |
| | | Four | 4.2±0.0 | 2.2±0.0 | 1.3±0.1 | 3.2±0.0 |
| | | Six | 6.7±0.0 | 3.5±0.0 | 2.2±0.0 | 5.1±0.1 |
| | 552A | Two | 1.8±0.0 | 0.8±0.1 | 0.5±0.1 | 1.6±0.0 |
| | | Four | 3.6±0.0 | 1.8±0.1 | 1.4±0.0 | 3.3±0.0 |
| | | Six | 5.4±0.0 | 2.8±0.2 | 2.4±0.0 | 5.2±0.0 |
| | 581A | Two | 2.0±0.0 | 0.8±0.0 | 0.6±0.0 | 1.8±0.0 |
| | | Four | 3.8±0.0 | 2.0±0.0 | 1.2±0.0 | 3.2±0.0 |
| | | Six | 5.6±0.0 | 3.0±0.0 | 2.0±0.0 | 4.1±0.1 |
| | 538A | Two | 2.0±0.0 | 0.8±0.0 | 0.5±0.0 | 1.6±0.0 |
| | | Four | 3.8±0.0 | 1.9±0.0 | 1.2±0.0 | 3.0±0.0 |
| | | Six | 5.7±0.1 | 3.0±0.0 | 1.9±0.0 | 4.4±0.0 |
| | 519A | Two | 2.0±0.1 | 0.8±0.0 | 0.6±0.0 | 1.8±0.0 |
| | | Four | 4.2±0.0 | 1.9±0.0 | 1.4±0.1 | 3.3±0.0 |
| | | Six | 6.6±0.0 | 2.9±0.0 | 2.3±0.0 | 5.3±0.0 |
| | 601A | Two | 2.0±0.0 | 0.8±0.0 | 0.5±0.1 | 1.6±0.0 |
| | | Four | 4.1±0.0 | 1.9±0.0 | 1.4±0.0 | 3.3±0.0 |
| | | Six | 6.6±0.0 | 3.0±0.0 | 2.3±0.0 | 5.4±0.0 |
| P value | | | | <0.001 | | <0.001 |

P – calculated 95% probability value

Table 6. 3: Inhibition (%) on colony diameter of *A. flavus* and *F. verticillioides* isolates grown on PDA amended with root extracts from two desmodium species and incubated for two, four and six days

| Isolate | *D. intortum* | | | | *D. uncinatum* | | | |
|---|---|---|---|---|---|---|---|---|
| | Two | Four | Six | Mean | Two | Four | Six | Mean |
| *A. flavus* | | | | | | | | |
| 81B | 21.4 | 19.4 | 22.6 | 21.1±0.4 | 28.6 | 7.1 | 6.5 | 14.1±2.2 |
| 151D | 8.3 | 13.8 | 20.0 | 14.0±2.0 | 23.1 | 4.2 | 5.1 | 10.8±2.2 |
| 105A | 25.0 | 14.6 | 14.3 | 18.0±1.2 | 36.8 | 4.2 | 7.9 | 16.3±2.9 |
| 2M35E | 42.9 | 11.6 | 29.2 | 27.9±2.4 | 7.7 | 7.1 | 8.7 | 7.8±0.3 |
| 379B | 0.0 | 12.1 | 7.7 | 6.6±0.0 | 15.4 | 10.7 | 10.9 | 12.3±0.7 |
| 479D | 16.7 | 14.3 | 13.3 | 14.8±0.4 | 9.1 | 4.5 | 10.8 | 8.1±1.1 |
| P value | | | | <0.001 | | | | <0.001 |
| *F. verticillioides* | | | | | | | | |
| 561B | 57.1 | 47.6 | 47.8 | 50.8±0.7 | 68.8 | 59.4 | 56.9 | 61.7±0.8 |
| 552A | 55.6 | 50.0 | 48.1 | 51.2±0.5 | 68.8 | 57.6 | 53.8 | 60.1±0.9 |
| 581A | 60.0 | 47.4 | 46.4 | 51.3±1.0 | 66.7 | 62.5 | 51.2 | 60.1±1.0 |
| 538A | 60.0 | 50.0 | 47.4 | 52.5±0.9 | 68.8 | 60.0 | 56.8 | 61.9±0.7 |
| 519A | 60.0 | 54.8 | 56.1 | 56.9±.04 | 66.7 | 57.6 | 56.6 | 60.3±0.7 |
| 601A | 60.0 | 53.7 | 54.5 | 56.1±0.4 | 68.8 | 57.6 | 57.4 | 61.2±0.8 |
| P value | | | | <0.001 | | | | <0.001 |

P – calculated 95% probability value

### 6.4.3 Effect of desmodium root extract on spore germination and germ tube growth

*Aspergillus flavus* and *F. verticillioides* spores did not germinate until after six hours of incubation (Figure 6.3, 6.4). The proportion of germinated spores of *A. flavus* was significantly (P = 0.001) reduced by the extract after ten hours of incubation (Table 6.4, Figure 6.3). By the tenth hour of incubation, the germ tubes had started developing into hyphae. Treatment of PDA media with desmodium crude extract showed 3.2 - 11.4% inhibition of *A. flavus* spore germination and 16.1% - 48.3% inhibition of germ tube elongation, and the extent of inhibition increased with increase in time of incubation (Table 6.4).

The proportion of germinated *F. verticillioides* spores and length of germ tubes after six and eight hours of incubation were significantly (P < 0.05) reduced by the extract (Figure 6.4, Table 6.4).

Desmodium root extract showed up to 50.0% and 43% inhibition of *F. verticillioides* spore germination and germ tube elongation, respectively. After ten hours of incubation, over 95% of spores in the extract-treated plate had germinated but the germ-tubes were still short, and the hyphae had not started branching. In the plates without extract, however, there was 100% spore germination and hyphae were fully formed and branched.

Table 6. 4: Proportion (%) of germinated spores and germ tube length (µm) of *A. flavus* and *F. verticillioides* grown on PDA amended with root extracts from *D. intortum* and incubated for six, eight and ten hours

| Treatment | Germinated spores (%) [b] | | | Germ tube length (µm) [b] | | |
|---|---|---|---|---|---|---|
| | Six | Eight | Ten | Six | Eight | Ten |
| *A. flavus* | | | | | | |
| Extract | 8.2±1.6 | 32.3±2.9 | 86.2±3.0 | 3.3±0.3 | 9.4±0.6 | 23.0±3.3 |
| No extract | 8.5±1.3 | 37.7±3.0 | 97.3±1.7 | 3.9±0.6 | 11.4±0.9 | 44.5±4.4 |
| Reduction (%) | 3.2 | 14.2 | 11.4 | 16.1 | 17.6 | 48.3 |
| P value | 0.913 | 0.392 | 0.001 | 0.219 | 0.099 | 0.017 |
| *F. verticillioides* | | | | | | |
| Extract | 5.9±0.9 | 43.2±3.9 | | 10.7±1.2 | 21.3±1.4 | |
| No extract | 9.4±2.2 | 86.4±2.5 | | 14.1±1.2 | 37.4±2.1 | |
| Reduction (%) | 37.5 | 50.0 | | 24.1 | 43.1 | |
| P value | 0.021 | < 0.001 | | 0.044 | < 0.001 | |

[b] – mean ± standard error of mean

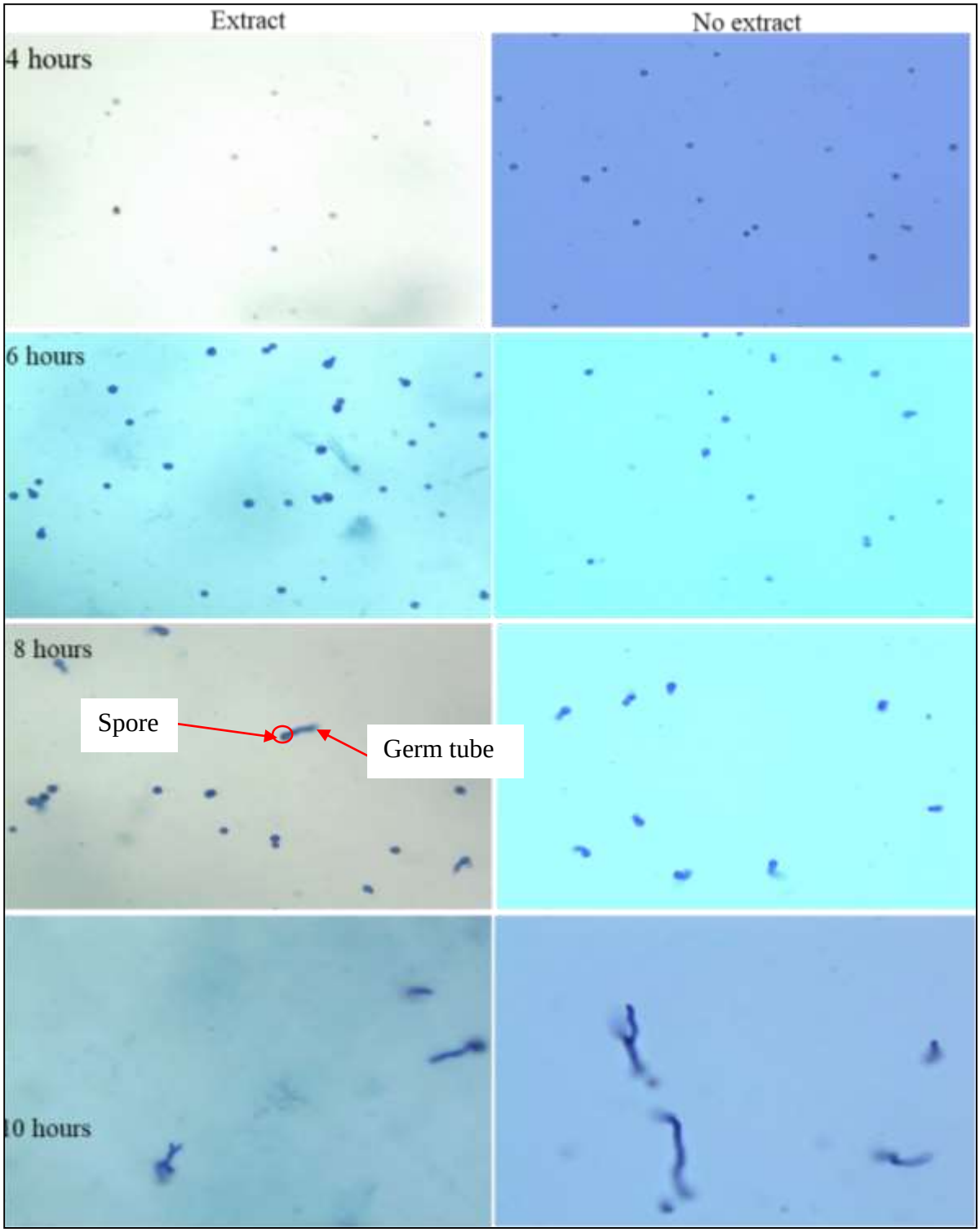


Figure 6. 3: Spores and germ tubes of *A. flavus* grown on PDA amended with *D. intortum* root extract and controls after incubation for four, six, eight and ten hours

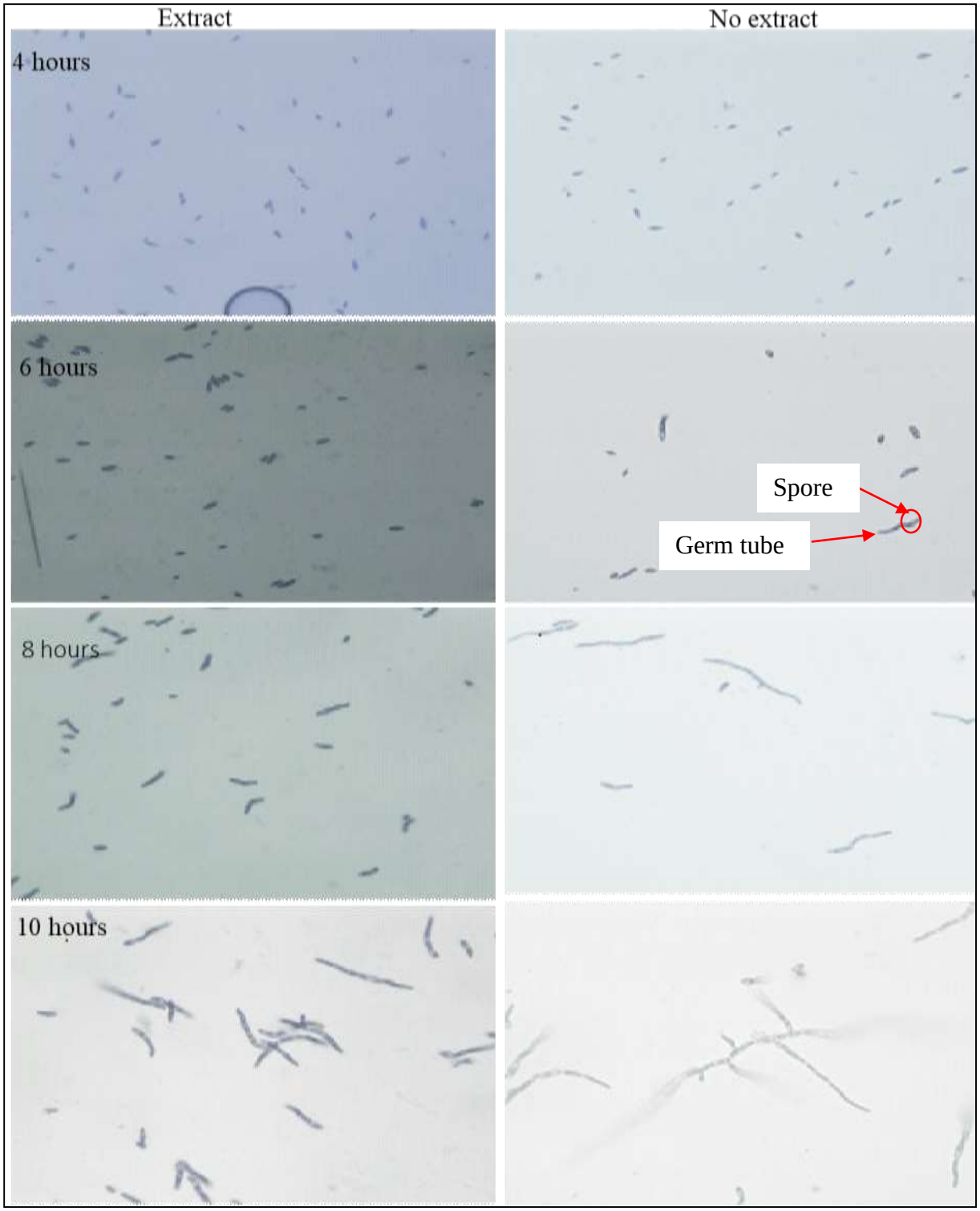


Figure 6. 4: Spores and germ tubes of *F. verticillioides* grown on PDA amended with *D. intortum* root extract and controls after incubation for four, six, eight and ten hours

## 6.5 Discussion

The results of this study showed that all the *F. verticillioides* from maize had high fumonisin production of above 2,000 µg/kg and up to 599,741 µg/kg. This is an indicator of the high risk of exposure of consumers of maize to high levels of fumonisin from *F. verticillioides* (Leslie & Summerell, 2006). The fumonisin levels reported here could be due to the ideal conditions of incubation of the maize. In spite of being a field toxin, high populations of *F. verticillioides* in physiologically mature maize could also imply high levels of fumonisin during post-harvest handling and storage, especially if the maize is exposed to temperature, humidity and moisture that is favorable for the fungal proliferation and fumonisin production (Fountain et al., 2014; Miller, 2008). *Fusarium verticillioides* grow and produce fumonisin at temperatures between 20 and 25°C at moderate water activity (Leggieri et al., 2019; Samapundo et al., 2005). Unfortunately, the average temperature and precipitation pattern in western offer these conditions (Jaetzold et al., 2009). Fumonisin production under field conditions is increased by drought stress (Leslie & Summerrel, 2006).

Unlike *F. verticillioides*, not all tested *A. flavus* isolates produced aflatoxin. Six percent of *A. flavus* isolates did not produce aflatoxin, 53% produced low levels of up to 10 µg/kg while 41% produced levels between 10 and 199,184 µg/kg. Even though *A. flavus* has been reported in low frequency and abundance in previous studies (Owuor et al., 2018), further infection and aflatoxin production could increase due to mishandling and storage under high moisture content, temperature and humidity, given that aflatoxin is a storage as opposed to field toxin (Fountain et al., 2014). In the field, *A. flavus* is an opportunistic pathogen that infect when maize weakened by factors such as drought stress, nutrient stress and physical damage ( Abbas et al., 2006; Hocking, 2006; Klich, 2007a). *Aspergillus flavus* is favored by hot and dry climatic conditions characterized by temperatures of 25-42°C and low moisture content, but, the optimum temperature for aflatoxin

production is 28-30°C, at high water activity (≥ 0.95 $a_w$) (Leggieri et al., 2019; Sanchis & Magan, 2004).

Both *D. intortum* and *D. uncinatum* root extracts showed significant reduction in radial growth of *F. verticillioides* cultures and a lesser extent of inhibition of *A. flavus*. However, the difference in activity between the extracts of the two *Desmodium* spp. cannot emphasized on because the extracts were used in crude state and the amount of extract per unit volume of medium was not standardized. The mechanism of inhibition of radial growth of the fungal pathogens was through slowed germination and inhibition of germ tube and hyphae elongation. Previous studies reported reduced rotting of maize grown in the push-pull cropping (Owuor et al., 2018) and lower levels of mycotoxins, especially aflatoxin and fumonisin. The findings of the current study imply possible reduction in rate of spore germination and mycelial growth of toxigenic *A. flavus* and *F. verticillioides* in soil as the mechanism by which the cropping system reduced the populations of the infection of maize by the two fungi and consequently the levels of associated mycotoxins. To my knowledge, there are no previous studies reporting on antifungal activities of *D. intortum* and *D. uncinatum* root extracts against plant pathogens. However, leaf extracts of *D. heterocarpon* have been reported to have antifungal activity against some fungi, which included *A. niger* (Arora et al., 2014).

The two *Desmodium* spp. used in this study are those that farmers use in push-pull cropping system and their roots have been reported to produce root exudates that contain several C-glycosyl flavonoids into the rhizosphere, some of which have been aassociated with suicidal germination of striga weed (Hooper et al., 2015). Some glycosylated flavonoids have also been associated with antifungal self-defense of mango fruits against decay-causing fungal pathogens (Sudheeran et al., 2019). Tapas, Sakarkar, & Kakde (2008) and Babu et al. (2016) reported that some groups of C-

glycosyl flavonoids extracted from plant roots are active against certain fungi. Therefore, it is possible that some of the flavonoids that constitute desmodium root exudates released into the soil are antifungal against *F. verticillioides* and *A. flavus*. The chemicals affect fungal growth by disrupting fungal cell membranes and inhibition of respiration, which may lead to cell death (Chen et al., 2018; Ren et al., 2020). The functioning of cell membranes is disrupted through interruption of the ergosterol content of the cell membranes, while respiration is disrupted by interruption of NADH oxidase and SDH activities of the process. It has also been reported that some plant extracts antifungal activity is through reduction in expression of mycotoxigenic genes in their biosynthesis pathway (Hu et al., 2017).

Antifungal compounds from root extracts have been reported to mainly work through foliar application (Muthomi et al., 2017a; Muthomi et al., 2017b). In such cases, active compounds require to be formulated and commercialized as bio-fungicides. Such kinds of fungicides work through contact or systemically once sprayed on the crops. On the other hand, desmodium root exudates do not require extraction and formulation because they are released directly into the soil where they inhibit proliferation of the fungi. Hence once adopted as a strategy for mycotoxin control, push-pull cropping system mechanism of action would be reduction of primary inocula of the fungi in the soil. The observed activity of the extract could be attributed to presence of bioactive chemicals such as flavonoids and alkaloids, which inhibit spore germination and modify the structure of the fungal mycelia (Tabassum & Vidyasagar, 2013). Different plant extracts target pathogens through different ways including alteration of cell wall and cell membrane, which interferes with metabolic processes such as electron transport and nutrient absorption.

Previous studies have reported that leaf extracts of some plants may also have antifungal activity against toxigenic fungi such as *A. flavus* and *F. verticillioides*, and are sometimes more active than

root extracts (Mahesh & Satish, 2008). During growth and cutting of desmodium foliage, leaves and stems fall on the ground and rot, where they act as mulch and source of organic carbon. Though the effect of the desmodium foliage on microbial growth has not been studied, it is possible that while they decompose, they produce chemicals that inhibit proliferation of fungi such as *A. flavus* and *F. verticillioides* and/or encourage multiplication of beneficial microorganisms. This, however, requires to be studied.

### 6.6 Conclusion and recommendations

Extracts of the two *Desmodium* spp. showed inhibition of germination and radial growth of toxigenic *A. flavus* and *F. verticillioides* isolated from maize and soil in percentages that imply they have potential to reduce the level of inocula of the two fungi in the push-pull soil through reduced proliferation. The reduction was, however, significantly higher against *F. verticillioides* than *A. flavus*, as demonstrated by the differences in *in vitro* antifungal activity test. Through reduction of fungal inocula in the soil, the populations of the fungi infecting maize during growth would be reduced and consequently pre-harvest contamination of maize with aflatoxin and fumonisin. This could be concluded as one of the mechanisms by which push-pull cropping system reduces the occurrence of maize ear rots and ear rot fungi. Further studies of the extracts to fractionate the crude extract and identify the antifungal components are recommended. *In situ* studies of the effect of desmodium root exudates on the population of the two fungal species are also recommended in order to determine the extent of activity of exudates as well as determine if the exudates have stimulatory activity towards beneficial microorganisms.

# CHAPTER SEVEN
## GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### 7.1 General discussion

Most socio-economic and agronomic practices were similar between push-pull and non-push-pull farmers maize farmers. This implies that the differences between push-pull and non-push-pull cropping systems in terms of contamination of maize with mycotoxin-producing fungi, ear rot disease and mycotoxins reported in the current study were mainly due to the cropping system adopted and not farming practices. The difference between two cropping systems was intercropping of maize with desmodium and planting napier/Brachiaria grass at the border of the intercrop. Another implication of this finding was that the effect of socio-economic and agronomic practices shown was associated with both push-pull and non-push-pull farmers and affected mycotoxin levels in maize from both cropping systems.

The study also reported that majority of the respondents were resource constrained, as implied by the low average annual income recorded. The low annual income could mainly be from sale of surplus food produce, because they did not have off-farm income. Resource constrained push-pull farmers would prioritize desmodium and napier/Brachiaria grass as fodder for sale and therefore not follow proper cutting regimes. This would reduce the efficiency of the cropping system in controlling stemborer, fall armyworm, striga weed and replenishment of soil nutrition (Kifuko-Koech et al., 2012). This probably contributed to the insignificant soil nutrient levels under push-pull cropping system during the study period, which agreed with the findings by Vanlauwe et al. (2008). Soil samples collected from both cropping systems had inadequate organic carbon, total nitrogen, and available phosphorus levels. Kifuko-Koech et al. (2012) reported that adequate supply of phosphorus under push-pull would better establish desmodium, which in turn would fix enough nitrogen to enhance crop growth. In the case of the current study, either DAP was not

adequately applied to supply enough phosphorus or desmodium was not properly established and maintained to cause detectable impact in nutrient levels in the soil. Regardless, desmodium significantly reduced insect damage, ear rot incidence, ear rots fungi, aflatoxin and fumonisin in maize under push-pull cropping system.

Both push-pull and non-push-pull farmers identified stemborer as the main insect pest of maize. This was confirmed by the field data collected which showed stemborer damage in both push-pull and non-push-pull. However, the field data reported significantly reduced stemborer damage in maize under the push-pull cropping system. The socio-economic and agronomic practices of farmers would have not been responsible for the differences in stemborer damage between push-pull and non-push-pull because all farmers had similar practices.

Fall armyworm was not highlighted as a major insect pest of maize during the household survey, because the first incidence of fall armyworm in the country was experienced in the cropping season following the survey season (MOA, 2017). Data from the current study showed that fall armyworm is an aggressive feeder and caused more foliage and ear damage than stemborer (Goergen et al., 2016; Overholt et al., 2001). Like stemborer, fall armyworm damage was significantly reduced under push-pull cropping system across the seasons. Moths of both insects are nocturnal and therefore not easily targeted by conventional insecticides (Kfir et al., 2002). However, the moths are easily targeted by push-pull cropping system because of its behavior modifying mechanisms; desmodium produce a set of chemicals that 'push' away the gravid moths which are simultaneously 'pulled' by chemicals produced by the border crop napier/Brachiaria grass (Cook et al., 2007). That was probably why even though majority of the non-push-pull farmers mentioned stemborers as the commonest insect in the farm, the farmers had no effective mechanism of control

and therefore the insects still caused significant damage to maize as indicated by the current findings.

The damage caused by both stemborer and fall armyworm significantly varied across seasons under both cropping systems. This could be because the extent of insect damage is usually influenced by the climatic conditions during a season (Sarmento et al., 2002; Viegas et al., 2016). Insects are known as vectors for ear rot and mycotoxin producing fungi (Sobek & Munkvold, 1999). In the current study, this was supported by the significant positive correlation between stemborer and fall armyworm damage of maize and incidence of *Fusarium* and total ear rot. Insect damage can also cause ear rots by leaving open wounds on the ears, through which fungi dispersed by wind infect the ears (Dowd, 2003; Munkvold et al., 1997). However, insect damage is not the only route of infection by ear rot fungi. It can also happen systemically through the roots and stem and fungi dispersed into the air can infect developing kernels through the silk (Parsons & Munkvold, 2012; Thompson et al., 2018).

Farmers' knowledge of ear rots was significantly lower for push-pull respondents compared to the non-push-pull counterparts. This was supported by the reported reduction of ear rots, especially *Fusarium* ear rot, in maize under push-pull cropping system. This implies that absence or minimal infection of maize under push-pull by ear rots was responsible to lack of awareness by push-pull farmers about ear rots. Push-pull has previously been observed to reduce the incidence of ear rots in maize (Owuor et al., 2018). This was also supported by significant reduction in the populations of ear rot fungi, *F. verticillioides* and *A. flavus* under push-pull cropping system both in the maize collected during the survey and the current three-season study. Push-pull cropping system resulted in reduction in fumonisin contamination in maize. *Fusarium verticillioides* was the most prevalent fungi in maize samples collected during the survey and those collected during the three-season

study, a trend that concurs with the findings of previous studies (Alakonya et al., 2009; Kedera et al., 1999).

*Aspergillus flavus* showed no physical damage to the maize kernels and it was isolated in significantly lower frequency and population in maize under the push-pull cropping system. This agreed with the findings of Schoeman (2012) who reported that *Aspergillus* ear rot causes little damage on kernels that it would be rarely visible. The frequency and populations of *A. flavus* were also very low in soils. Therefore, soil could not have acted as source of *A. flavus* inocula. The zero incidence of *Aspergillus* ear rot could also be supported by the fact that *A. flavus* is a passive pathogen, that infects crops weakened by insect infestation, drought, poor soil nutrition and disease. The population and frequency of *A. flavus* in maize in this study was very low. *Fusarium verticillioides* is the main producer of fumonisin while *A. flavus* is the main producer of aflatoxin in maize (Klich, 2007b; Leslie & Summerell, 2006).

Although significantly higher proportion of push-pull farmers practiced minimum tillage by use of hand hoe, a practice associated with accumulation of saprophytic fungi inocula in the soil (Flett et al., 1998), maize samples from push-pull cropping system had significantly lower populations of *F. verticillioides* and *A. flavus*. This implied that push-pull cropping system had mechanisms that inhibited the build-up of toxigenic fungi, and that was why even though both aflatoxin and fumonisin contamination of maize were associated with use of DAP at planting, a practice undertaken by both push-pull and non-push-pull farmers, maize from push-pull fields had significantly lower levels of *A. flavus*, *F. verticillioides*, aflatoxin, and fumonisin. However, previous studies reported that use of mineral fertilizers increase populations of toxigenic fungi and contamination of maize with associated mycotoxins. The impact of minimum tillage could also

have been masked by the fact that push-pull farmers removed stovers from the farm and did not plough-in post-harvest (Keller, 2011; Njeru et al., 2016).

This study showed no improvement in soil nutrition under the push-pull cropping system, and therefore the observed reduction in ear rots, ear rot fungi and mycotoxins were not associated with levels of nutrients in the soil. The populations of ear rot fungi were not reduced in the push-pull soil, implying that soil was not the maize source of *A. flavus* and *F. verticillioides* isolated from physiologically mature maize grains. Possibly, the maize stovers left on the soil surface after harvest were the source of the high *F. verticillioides* population isolated from physiologically mature maize (Njeru et al., 2016; Tanveer et al., 2017).

Desmodium foliage controls stemborer and fall armyworm by production of repugnant smell that 'push' them away while the moths are simultaneously 'pulled' by napier/Brachiaria grass at the border of the plot (Khan et al., 2000; Midega et al., 2018). The border crop, however, has characteristics that cause high mortality of the larvae (Khan et al., 2000). Desmodium roots produce several flavonoids into the rhizosphere (Hooper et al., 2015). Some categories of flavonoids have been reported to possess antifungal activity (Babu et al., 2016; Tapas et al., 2008). In the case of the current study, the fungi would be *F. verticillioides* which caused *Fusarium* ear rot and produced fumonisin and *A. flavus* which asymptomatically infected the maize and produced aflatoxin. Presence of mechanisms for management of mycotoxin contamination of maize under push-pull cropping system would be the only explanation why despite push-pull respondents practicing minimum tillage, not doing crop rotation and leaving maize stovers in the field after harvest, there were lower levels of contamination of maize with aflatoxin and fumonisin at harvest.

## 7.2 Conclusions

Good agricultural practices are encouraged to avoid preservation of maize stovers from previous cropping season in the farm until subsequent seasons, because it encourages survival of saprophytic fungi, which would act as primary source of infection On the contrary, farming practices that involve removing, burying or destroying harvested maize stovers are likely to reduce the amount of saprophytic fungi inocula during subsequent seasons. Many fungi are saprophytic and survive in crop residues for more than one cropping season. Proper handling of maize stovers after harvest is therefore an important aspect in managing mycotoxin contamination of maize.

Agricultural practices that preserve crop residues and those that stress the crop increased the odds for aflatoxin and fumonisin contamination of maize, in both push-pull and non-push-pull cropping systems. However, application of DAP at planting was the most important agricultural practice because it was positively and significantly associated with both aflatoxin and fumonisin levels. This was probably because the fertilizer was applied in lower or higher quantities than required which stressed the crop and pre-disposed it to opportunistic infection. The associations were however weak, and therefore this could not have been the only factor that influenced the levels of the two mycotoxins. Despite bad agricultural practices by push-pull farmers, there still was lower populations of *F. verticillioides* and *A. flavus* as well as levels of fumonisin and aflatoxin. This could have been because push-pull cropping system had complex mechanisms that reduced the occurrence of ear rots and mycotoxins. Reduction of occurrence of ear rots, particularly *Fusarium* ear rot which happened was attributed to reduced insect damage. Repulsion of stemborer and fall armyworm by desmodium had a direct impact on the amount of fungal inocula that would infect the maize ears as a result of wounding. Reduced stemborer and fall armyworm damage also results in increased maize grain yield, and minimized yield losses attributed to damage of maize by the two insects.

There was no association between the levels of nutrients tested and the cropping system. However, the association between the nutrients and populations of *A. flavus* and *A. niger* implied that fertilization should be done carefully, as previous studies have indicated application of insufficient nitrogen and organic matter would favor proliferation of mycotoxigenic fungi and their associated mycotoxins.

The mechanisms of ear rots and mycotoxin management under push-pull cropping system were identified as management of stemborer and fall armyworm which significantly reduced the incidence of ear rots, ear rot fungi and associated mycotoxins and reduced populations of mycotoxin-producing fungi by inhibition of their spore germination and radial growth as demonstrated by the *in vitro* effect of crude desmodium root extracts on toxigenic *A. flavus* and *F. verticillioides*. *In vitro* effect of desmodium root extracts on *A. flavus* and *F. verticillioides* showed that chemicals produced by desmodium into the rhizosphere significantly inhibit increase of the inocula of the two fungi. Consequently, this would reduce the occurrence of ear rots caused by these fungi and the levels of their associated mycotoxins.

There was statistically significant correlation among some agronomic factors, stemborer and fall armyworm damage, ear rots, ear rot fungi and aflatoxin and fumonisin levels in maize. The correlations were, however, weak as indicated by the low correlation coefficients of less than 0.7. This implied that none of the described mechanisms would individually work to reduce mycotoxins to safe levels. Instead, they jointly contributed to management of ear rot fungi and mycotoxin contamination of maize. The positive and significant correlation between *A. flavus* and *F. verticillioides* and aflatoxin and fumonisin levels, respectively, implied that occurrence of mycotoxin-producing fungi in maize can be used as an indicator of the extent of exposure to associated mycotoxin.

### 7.3 Recommendations

Based on the findings of the current study, the following recommendations were drawn:

i. Training maize farmers on mycotoxin awareness and good agricultural practices such as removal or burying of stovers after harvest, rotation with non-cereal crops, harvesting of stovers for hay instead of directly grazing the livestock in the farm, sorting out rotten ears, proper disposal of rotten maize.

ii. Maize farmers should be trained and encouraged to keep desmodium and border grass in their push-pull plots well maintained and properly spaced to maximize their benefits in management of stemborer and fall armyworm damage, ear rots, soil nutrition, aflatoxin and fumonisin contamination of maize.

iii. Promotion and implementation of push-pull cropping system as a component of integrated management approach for maize ear rots and mycotoxin contamination of maize in as many areas as possible including aflatoxin hotspots in Kenya, for example lower Eastern and Central Kenya.

iv. *In situ* studies to determine the effect of desmodium root exudates on the populations of mycotoxigenic fungi in soil under push-pull cropping system, to complement the current *in vitro* study.

v. Further studies are recommended to fractionate desmodium root extracts and determine the chemical components with antifungal activity against *A. flavus* and *F. verticillioides*.

vi. Long term studies on the effect of push-pull cropping system on the population of stemborer and fall armyworm to evaluate their potential for resistance.

## REFERENCES


Abbas, H. K., Wilkinson, J. R., Zablotowicz, R. M., Accinelli, C., Abel, C. A., Bruns, H. A., & Weaver, M. A. (2009). Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. *Toxin Reviews*, *28*(2–3), 142–153. https://doi.org/10.1080/15569540903081590

Abbas, Hamed K., Zablotowicz, R. M., Bruns, H. A., & Abel, C. A. (2006). Biocontrol of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. *Biocontrol Science and Technology*, *16*(5), 437–449. https://doi.org/10.1080/09583150500532477

Afolabi, C. G., Bandyopaghyay, R., Leslie, J. F., & Ekpo, E. J. A. (2006). Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. *Journal of Food Protection*, *69*(8), 2019–2023. https://doi.org/10.4315/0362-028X-69.8.2019

Ahmad, W., Farmanullah, Shah, Z., Jamal, M., & Shah, K. A. (2014). Recovery of organic fertility in degraded soil through fertilization and crop rotation. *Journal of the Saudi Society of Agricultural Sciences*, *13*(2), 92–99. https://doi.org/10.1016/J.JSSAS.2013.01.007

Ahmed, D., Kumar, V., Verma, A., Gupta, P. S., Kumar, H., Dhingra, V., … Sharma, M. (2014). Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of Albizzia Lebbeck Benth. stem bark (ALEx) on streptozotocin induced diabetic rats. *BMC Complementary and Alternative Medicine*, *14*(1), 243. https://doi.org/10.1186/1472-6882-14-243

Ajala, O. S., & Saxena, N. K. (1994). Interrelationship among *Chilo partellus* (Swinhoe)damage parameters and their contribution to grain yield reduction in maize (*Zea mays* L.). *Applied Entomology and Zoology*, *29*(4), 469–476. https://doi.org/10.1303/aez.29.469

Al-Samarrai, G., Singh, H., & Syarhabil, M. (2012). Evaluating eco-friendly botanicals (natural

plant extracts) as alternatives to synthetic fungicides. *Annals of Agricultural and Environmental Medicine*, *19*(4), 673–676.

Alakonya, A. E., Monda, E. O., & Ajanga, S. (2009). Fumonisin B1 and aflatoxin B1 levels in Kenyan maize. *Journal of Plant Pathology*, *91*(2), 459–464.

Anderson, J. M., & Ingram, J. S. I. (1993). *Tropical soil biology and fertility: A Handbook of Methods*. *CAB International* (Second edi). Wallingford, Oxon, UK, Oxon, UK: CAB International. https://doi.org/10.2307/2261129

Andersson, J., & Halvarsson, M. (2011). *The economic consequences of Striga hermonthica in maize production in Western Kenya*. *Dept. of Economics*.

Arino, A., Herrera, M., Juan, T., Estopanan, G., Carraminana, J. J., Rota, C., & Herrera, A. (2009). Influence of agricultural practices on the contamination of maize by fumonisin mycotoxins. *Journal of Food Protection*, *72*(4), 898–902. https://doi.org/10.4315/0362-028X-72.4.898

Arora, S., Yadav, V., Kumar, P., & Kumar, D. (2014). Antimicrobial studies of leaf extracts from *Desmodium heterocarpon* (L.) DC. *Medicinal Plants*, *6*(3), 206–208. https://doi.org/10.5958/0975-6892.2014.00011.2

Atera, E. A., Ishii, T., Onyango, J. C., Itoh, K., & Azuma, T. (2013). Striga infestation in Kenya: Status, distribution and management options. *Sustainable Agriculture Research*, *2*(2), 99. https://doi.org/10.5539/sar.v2n2p99

Augusto, T., Souza, F. De, & Freitas, H. (2018). Long-term effects of fertilization on soil organism diversity. In *Sustainable Agriculture Reviews* (pp. 211–247). Springer International Publishing. https://doi.org/10.1007/978-3-319-90309-5

Babu, B. G., Naveen Kumar, A. D., Badana, A., Kumari, S., Jha, A., & Malla, R. R. (2016). Effect of C-glycosyl flavone from *Urginea Indica* on antibiotic induced microbial cell death.

*International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(5), 296–305.

Beattie, S., Schwarz, P. B., Horsley, R., Barr, J., & Casper, H. H. (1998). The effect of grain storage conditions on the viability of *Fusarium* and deoxynivalenol production in infested malting barley. *Journal of Food Protection*, *61*(1), 103–106. https://doi.org/10.4315/0362-028X-61.1.103

Bennett, J. W., & Klich, M. (2013). Mycotoxins. *Clinical Microbiology Reviews*, *16*(3), 497–516. https://doi.org/10.1128/CMR.16.3.497

Berner, D. K., Kling, J. G., & Singh, B. B. (1995). Striga research and control: A perspective from Africa. *Plant Disease*, *79*(7), 652–660. https://doi.org/10.1210/jcem-73-4-907

Bigirwa G., Kaaya A.N., Sserurwa G., Adipala E., O. S. (2007). Incidence and severity of maize ear rots and factors responsible for their occurrence in Uganda. *Journal of Applied Sciences*, *23*(7), 3780–3785.

Bigirwa, G., Kaaya, A. N., Sseruwu, G., Adipala, E., & Okanya, S. (2007). Incidence and severity of maize ear rots and factors responsible for their occurrence in Uganda. *Journal of Applied Sciences*, *7*(23), 3780–3785. https://doi.org/10.3923/jas.2007.3780.3785

Bigirwa, G., Sseruwu, G., Kaaya, A. N., Adipala, E., & Okanya, S. (2006). Fungal microflora causing maize ear rots in Uganda and associated aflatoxins. *Journal of Biological Sciences*, *6*(3), 540–546. https://doi.org/10.3923/jbs.2006.540.546

Biselli, S., Hartig, L., Wegner, H., & Hummert, C. (2004). Analysis of *Fusarium* toxins using LC/MS-MS: Application to various food and feed matrices. *LC-GC Europe*.

Blandino, M., Reyneri, A., & Vanara, F. (2008). Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. *Crop Protection*, *27*(2), 222–230. https://doi.org/10.1016/j.cropro.2007.05.008

Boivin, S., Fonouni-Farde, C., & Frugier, F. (2016). How auxin and cytokinin phytohormones modulate root microbe interactions. *Frontiers in Plant Science, 7*, 1240. https://doi.org/10.3389/fpls.2016.01240

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001, October). Production of plant secondary metabolites: A historical perspective. *Plant Science*. https://doi.org/10.1016/S0168-9452(01)00490-3

Bowers, E., Hellmich, R., & Munkvold, G. (2013). Vip3Aa and Cry1Ab proteins in maize reduce *Fusarium* ear rot and fumonisins by deterring kernel injury from multiple Lepidopteran pests. *World Mycotoxin Journal*, *6*(2), 127–135. https://doi.org/10.3920/WMJ2012.1510

Bragg, P. E., Maust, M. D., & Panaccione, D. G. (2017). Ergot alkaloid biosynthesis in the maize (*Zea mays*) ergot fungus Claviceps gigantea. *J Agric Food Chem*, *65*(49), 10703–10710. https://doi.org/10.1021/acs.jafc.7b04272.

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology*, *74*(3), 738–744. https://doi.org/10.1128/AEM.02188-07

Broggi, L. E., Pacin, A. M., Gasparovic, A., Sacchi, C., Rothermel, A., Gallay, A., & Resnik, S. (2007). Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos Province, Argentina. *Mycotoxin Research*, *23*(2), 59–64. https://doi.org/10.1007/BF02946026

Bruns, H. A. (2003, January 8). Controlling aflatoxin and fumonisin in maize by crop management. *Journal of Toxicology - Toxin Reviews*. https://doi.org/10.1081/TXR-120024090

Burger, H.-M., Abel, S., & Gelderblom, W. C. (2017). Disruption of lipid rafts constituents and implications for the cancer promotion properties of the carcinogenic mycotoxin, fumonisin

B1. *Cancer Research*, *77*(22 Supplement), B02–B02. https://doi.org/10.1158/1538-7445.NEWFRONT17-B02

Bush, B. J., Carson, M. L., Cubeta, M. A., Hagler, W. M., & Payne, G. (2003). Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. *Phytopathology Journal*, (94), 88–93.

Calatayud, P.-A., Okuku, G., Musyoka, B., Khadioli, N., Ong'amo, G., & Le Ru, B. (2014). *Busseola segeta*, a potential new pest of maize in western Kenya. *Entomology, Ornithology & Herpetology*, *3*(3), 132. https://doi.org/10.4172/2161-0983.1000132

Camardo Leggieri, M., Giorni, P., Pietri, A., & Battilani, P. (2019). *Aspergillus flavus* and *Fusarium verticillioides* interaction: Modeling the Impact on Mycotoxin Production. *Frontiers in Microbiology*, *10*, 2653. https://doi.org/10.3389/fmicb.2019.02653

CAST (Council for Agricultural and Science Technology). (2003). *Mycotoxins: risks in plant, animal, and human systems*. Ames, Iowa, USA: Council for Agricultural Science and Technology.

Chen, C., Long, L., Zhang, F., Chen, Q., Chen, C., Yu, X., … Long, Z. (2018). Antifungal activity, main active components and mechanism of *Curcuma longa* extract against *Fusarium graminearum*. *PLoS ONE*, *13*(3). https://doi.org/10.1371/journal.pone.0194284

Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage: A review. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*. https://doi.org/10.1080/19440040903573032

CIMMYT. (2004). *Maize diseases: A guide for field identification*. (CIMMYT, Ed.) (4th ed.). Mexico: CIMMYT.

Clayton, E. E. (1927). *Diplodia* ear-rot disease of corn. *Journal of Agricultural Research*, *34*(4),

357–371.

Coker, R. (1997). *Mycotoxins and their control: constraints and opportunities*. *NRI Bulletin* (Vol. 73).

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, *52*(1), 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407

Cotty, P. J., & Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, *119*(1–2), 109–115.

De Groote, H. (2002). Maize yield losses from stemborers in kenya. *Insect Sci. Applic.*, *22*(2), 89–96.

De Vries, H. R., Maxwell, S. M., & Hendrickse, R. G. (1989). Foetal and neonatal exposure to aflatoxins. *Acta Paediatr Scand*, *78*(3), 373–378. https://doi.org/10.1111/j.1651-2227.1989.tb11095.x

Debenport, S. J., Assigbetse, K., Bayala, R., Chapuis-Lardy, L., Dick, R. P., & McSpadden Gardener, B. B. (2015). Association of shifting populations in the root zone microbiome of millet with enhanced crop productivity in the Sahel region (Africa). *Applied and Environmental Microbiology*, *81*(8), 2841–2851. https://doi.org/10.1128/AEM.04122-14

Degraeve, S., Madege, R. R., Audenaert, K., Kamala, A., Ortiz, J., Kimanya, M., … Haesaert, G. (2015). Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania. *Food Control*, *59*, 225–233. https://doi.org/10.1016/j.foodcont.2015.05.028

Dereje A, G. (2014). On farm pre harvest agronomic management practices of *Aspergillus* infection on groundnut in Abergelle, Tigray. *Journal of Plant Pathology & Microbiology*,

*05*(02). https://doi.org/10.4172/2157-7471.1000228

Dolezal, A. L., Shu, X., OBrian, G. R., Nielsen, D. M., Woloshuk, C. P., Boston, R. S., & Payne, G. A. (2014). *Aspergillus flavus* infection induces transcriptional and physical changes in developing maize kernels. *Frontiers in Microbiology*, *5*, 384. https://doi.org/10.3389/fmicb.2014.00384

Dowd, P. F. (2003, January 8). Insect management to facilitate preharvest mycotoxin management. *Journal of Toxicology - Toxin Reviews*. Taylor & Francis. https://doi.org/10.1081/TXR-120024097

Dragich, M., & Nelson, S. (2014). *Gibberella* and *Fusarium* ear rots of maize in Hawai'i. *Plant Disease*, *PD-102*(August), 1–8.

Duan, C., Qin, Z., Yang, Z., Li, W., Sun, S., Zhu, Z., & Wang, X. (2016). Identification of pathogenic *Fusarium* spp. causing maize ear rot and poten tial mycotoxin production in China. *Toxins*, *8*(6). https://doi.org/10.3390/toxins8060186

Edwards, S. G. (2004). Influence of agricultural practices on *Fusarium* infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. In *Toxicology Letters* (Vol. 153, pp. 29–35). https://doi.org/10.1016/j.toxlet.2004.04.022

Ezekiel, C. N., Atehnkeng, J., Odebode, A. C., & Bandyopadhyay, R. (2014). Distribution of aflatoxigenic *Aspergillus* section *Flavi* in commercial poultry feed in Nigeria. *International Journal of Food Microbiology*, *189*, 18–25. https://doi.org/10.1016/j.ijfoodmicro.2014.07.026

Fink-Gremmels, J. (2008). The role of mycotoxins in the health and performance of dairy cows. *The Veterinary Journal*, *176*(1), 84–92. https://doi.org/10.1016/j.tvjl.2007.12.034

Fink-Gremmels, Johanna. (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: A

review. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *25*(2), 172–180. https://doi.org/10.1080/02652030701823142

Flett, B. C., McLaren, N. W., & Wehner, F. C. (1998). Incidence of ear rot pathogens under alternating corn tillage practices. *Plant Disease*, *82*(7), 781–784. https://doi.org/10.1094/PDIS.1998.82.7.781

Flynn, R., & Idowu, J. (2015). Nitrogen fixation by legumes: Biological nitrogen fixation. Retrieved February 4, 2019, from https://aces.nmsu.edu/pubs/_a/A129/

Fountain, J. C., Scully, B. T., Ni, X., Kemerait, R. C., Lee, R. D., Chen, Z. Y., & Guo, B. (2014). Environmental influences on maize-*Aspergillus* flavus interactions and aflatoxin production. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2014.00040

Frank, J. H., Thomas, M. C., Yousten, A. A., Howard, F. W., Giblin-davis, R. M., Heppner, J. B., … Sourakov, A. (2008). Push-pull strategy for insect pest management. In *Encyclopedia of Entomology* (pp. 3074–3082). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-6359-6_3253

Fredalette, U. (2017). Companion "push-pull" crop system proves successful against fall armyworm in East Africa - African Farming. Retrieved May 10, 2019, from https://www.africanfarming.com/companion-push-pull-crop-systems-proves-successful-against-fall-armyworm-in-east-africa/

Frisvad, J. C., Samson, R. A., Houbraken, J. A. M. P., Kuijpers, A. F. A., & Frank, J. M. (2004). New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. *Studies in Mycology*, *50*(1), 45–61.

Gachengo, C. N., Vanlauwe, B., Palm, C. A., & Cadisch, G. (2004). Chemical characterisation of a standard set of organic materials. In *Modelling nutrient management in tropical cropping*

*systems ACIAR Proceedings No. 114* (pp. 48–53).

Gichana, A. (2014). Striga weed affecting production in Western Kenya | The Star, Kenya. Retrieved January 26, 2019, from https://www.the-star.co.ke/news/2014/08/12/striga-weed-affecting-production-in-western-kenya_c982301

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. *PLOS ONE*, *11*(10), 1–9. https://doi.org/10.1371/journal.pone.0165632

Govaerts, B., Mezzalama, M., Sayre, K. D., Crossa, J., Lichter, K., Troch, V., … Deckers, J. (2008). Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. *Applied Soil Ecology*, *38*(3), 197–210. https://doi.org/10.1016/j.apsoil.2007.10.009

Guo, B., Ji, X., Ni, X., Fountain, J. C., Li, H., Abbas, H. K., … Scully, B. T. (2017). Evaluation of maize inbred lines for resistance to pre-harvest aflatoxin and fumonisin contamination in the field. *Crop Journal*, *5*(3), 259–264. https://doi.org/10.1016/j.cj.2016.10.005

Gutleb, A. C., Morrison, E., & Murk, A. J. (2002). Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review. *Environmental Toxicology and Pharmacology*, *11*(3–4), 309–320.

Haschek, W. M., & Voss, K. A. (2013). Mycotoxins. In *Haschek and Rousseaux's Handbook of Toxicologic Pathology* (pp. 1187–1258). https://doi.org/10.1016/B978-0-12-415759-0.00039-X

Hassanali, A., Herren, H., Khan, Z. R., Pickett, J. A., & Woodcock, C. M. (2008). Integrated pest management: The push-pull approach for controlling insect pests and weeds of cereals, and

its potential for other agricultural systems including animal husbandry. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 611–621. https://doi.org/10.1098/rstb.2007.2173

Hassegawa, R. H., Fonseca, H., Fancelli, A. L., da Silva, V. N., Schammass, E. A., Reis, T. A., & Corrêa, B. (2008). Influence of macro- and micronutrient fertilization on fungal contamination and fumonisin production in corn grains. *Food Control*, *19*(1), 36–43. https://doi.org/10.1016/j.foodcont.2007.01.006

He, X. H., Critchley, C., & Bledsoe, C. (2003). Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). *Critical Reviews in Plant Sciences*, *22*(6), 531–567. https://doi.org/10.1080/713608315

Hocking, A. D. (2006). *Aspergillus* and related teleomorphs. *Food Spoilage Microorganisms*, 451–487. https://doi.org/10.1533/9781845691417.4.451

Hooper, A. M., Caulfield, J. C., Hao, B., Pickett, J. A., Midega, C. A. O., & Khan, Z. R. (2015). Isolation and identification of Desmodium root exudates from drought tolerant species used as intercrops against *Striga hermonthica*. *Phytochemistry*, *117*, 380–387. https://doi.org/10.1016/j.phytochem.2015.06.026

Horn, B. W. (2003, January 8). Ecology and population biology of aflatoxigenic fungi in soil. *Journal of Toxicology - Toxin Reviews*. Taylor & Francis. https://doi.org/10.1081/TXR-120024098

Horneck, D. A., Sullivan, D. M., Owen, J. S., & Hart, J. M. (2011). Soil test interpretation guide. *Oregon State University Extension Service*, (July), 1–12.

Hu, Y., Zhang, J., Kong, W., Zhao, G., & Yang, M. (2017). Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on

*Aspergillus flavus*. *Food Chemistry*, *220*, 1–8. https://doi.org/10.1016/j.foodchem.2016.09.179

Humber, R. A. (1997). Fungi. In *Manual of techniques in insect pathology* (pp. 153–185). https://doi.org/10.1016/B978-012432555-5/50011-7

IARC, (International Agency for Research onCancer). (1972). IARC monographs on the evaluation of carcinogenic risk of chemicals to man. *CAB Direct*, *1*, 184.

International Centre of Insect Physiology and Ecology (icipe). (2013). Determining the effect of stemborers on yields of cereal crops , principally maize and sorghum. *Integrated Systems for the Humid Tropics(Humidtropics)*, *254*(December).

International Centre of Insect Physiology and Ecology, (icipe). (2019). "Push-pull": A Platform technology for improving livelihoods of resource poor farmers. Retrieved May 16, 2019, from http://www.push-pull.net/

Ismail, S., Shindano, J., Nyirenda, D. B., Bandyopadhyay, R., & Akello, J. (2014). Does exposure to aflatoxin constrain efforts to reduce stunting in Zambia? *Institute of Development Studies*, *IDS Specia*(September), 5.

Iwouha, J.-P. (2017). Maize production – An interesting small business opportunity you should consider this year - Smallstarter Africa. Retrieved January 9, 2019, from http://www.smallstarter.com/browse-ideas/how-to-start-a-maize-farming-and-production-business-in-africa/

Jaetzold, R., Schmidt, H., Hornetz, B., Shisanya, C. (2009). *Farm management handbook of Kenya: Natural conditions and farm management information*. *Ministry of Agriculture* (Vol. Vol. II).

Jama, B., Palm, C. A., Buresh, R. J., Niang, A., Gachengo, C., Nziguheba, G., & Amadalo, B.

(2000). *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya: A review. *Agroforestry Systems*, *49*(2), 201–221. https://doi.org/10.1023/A:1006339025728

Jama, Bashir, & Van Straaten, P. (2006). Potential of East African phosphate rock deposits in integrated nutrient management strategies. *Anais Da Academia Brasileira de Ciências*, *78*(4), 781–790. https://doi.org/10.1590/S0001-37652006000400012

JECFA. (2018). *Safety evaluation of certain contaminants in food*. (World Health Organization, Ed.). Geneve: FAO/WHO. https://doi.org/10.1016/j.ijfoodmicro.2007.01.001

Jones, F. T. (2009). Qualitative and quantitative analysis of mycotoxins. *Comprehensive Reviews in Food Science and Food Safety*, *8*, 497–498. https://doi.org/10.1111/j.1949-8594.1909.tb03113.x

Jovaiš Iene ˙, J., Bakutis, B., Baliukoniene, V., Gerulis, G., Jovaišiene, J., Bakutis, B., … Gerulis, G. (2016). *Fusarium* and *Aspergillus* mycotoxins effects on dairy cow health, performance and the efficacy of anti-mycotoxin additive. *Polish Journal of Veterinary Sciences*, *19*(1), 79–87. https://doi.org/10.1515/pjvs-2016-0011

Kabak, B., Dobson, A. D. W., & Var, I. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition*, *46*(8), 593–619. https://doi.org/10.1080/10408390500436185

Kamala, A., Ortiz, J., Kimanya, M., Haesaert, G., Donoso, S., Tiisekwa, B., & De Meulenaer, B. (2015). Multiple mycotoxin co-occurrence in maize grown in three agro-ecological zones of Tanzania. *Food Control*, *54*, 208–215. https://doi.org/10.1016/j.foodcont.2015.02.002

Kang'ethe, E. (2011). Situation analysis: Improving food safety in the maize value chain in Kenya. Report prepared for FAO by Prof . Erastus Kang ' ethe College of Agriculture and Veterinary

Science University of Nairobi, (September), 1–89.

Kankonda, O. M., Akaibe, B. D., Ong'amo, G. O., & Le Ru, B. P. (2017). Diversity of lepidopteran stemborers and their parasitoids on maize and wild host plants in the rain forest of Kisangani, DR Congo. *Phytoparasitica*, *45*(1), 57–69. https://doi.org/10.1007/s12600-017-0561-6

Kanwal, A., Javaid, A., Mahmood, R., & Akhtar, N. (2017). Correlation between soil nutrients and soil-borne mycoflora in wheat-rice cropping system of punjab , pakistan. *The Journal of Animal and Plant Sciences*, *27*(4), 1256–1263.

Karagiannidis, N., Velemis, D., & Stavropoulos, N. (1997). Root colonization and spore population by VA-mycorrhizal fungi in four grapevine rootstocks. *Vitis*, *36*(2), 57–60.

Kedera, C. J., Plattner, R. D., & Desjardins, A. E. (1999). Incidence of *Fusarium* spp. and levels of fumonisin B1 in maize in western Kenya. *Applied and Environmental Microbiology*, *65*(1), 41–44.

Keller, M. D. (2011). *The Contribution of Within-Field Inoculum Sources of Gibberella zeae to Fusarium Head Blight in Winter Wheat and Barley*.

Kerr, R. B., Snapp, S., Chirwa, M., Shumba, L., & Msachi, R. (2007). Participatory research on legume diversification with Malawian smallholder farmers for improved human nutrition and soil fertility. *Experimental Agriculture*, *43*(4), 437–453. https://doi.org/10.1017/S0014479707005339

Keya, S., & Rubaihayo, P. (2013). *Progress in on-farm production and productivity in the East African community: 50 years after independence*. *Kilimo Trust Technical Paper No.8*.

Kfir, R., Overholt, W. A., Khan, Z. R., & Polaszek, A. (2002). Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annual Review of Entomology*, *47*, 701–731. https://doi.org/10.1146/annurev.ento.50.071803.130431

Khan, Z., Midega, C., Pittchar, J., Pickett, J., & Bruce, T. (2011). Push-pull technology: A conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. *International Journal of Agricultural Sustainability*, *9*(1), 162–170. https://doi.org/10.3763/ijas.2010.0558

Khan, Z. R., Hassanali, A., Overholt, W., Khamis, T. M., Hooper, A. M., Pickett, J. A., … Woodcock, C. M. (2002). Control of witchweed Striga hermonthica by intercropping with Desmodium spp., and the mechanism defined as allelopathic. *Journal of Chemical Ecology*, *28*(9), 1871–1885. https://doi.org/10.1023/A:1020525521180

Khan, Z. R., Hassanali, A., Pickett, J. A., Wadhams, L. J., & Muyekho, F. (2003). Strategies for control of cereal stemborers and striga weed in maize-based farming systems in eastern africa involving 'push-pull' and allelopathic tactics, respectively. In *African Crop Science Conference Proceedings* (Vol. 6, pp. 602–608). https://doi.org/10.1080/0013188970390209

Khan, Z. R., Midega, C. A. O., Amudavi, D. M., Hassanali, A., & Pickett, J. A. (2008). On-farm evaluation of the "push-pull" technology for the control of stemborers and striga weed on maize in western Kenya. *Field Crops Research*, *106*(3), 224–233. https://doi.org/10.1016/j.fcr.2007.12.002

Khan, Z. R., Midega, C. A. O., Bruce, T. J. A., Hooper, A. M., & Pickett, J. A. (2010). Exploiting phytochemicals for developing a "push-pull" crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany*, *61*(15), 4185–4196. https://doi.org/10.1093/jxb/erq229

Khan, Z. R., Midega, C. A. O., Njuguna, E. M., Amudavi, D. M., Wanyama, J. M., & Pickett, J. A. (2008). Economic performance of the "push-pull" technology for stemborer and Striga control in smallholder farming systems in western Kenya. *Crop Protection*, *27*(7), 1084–

1097. https://doi.org/10.1016/j.cropro.2008.01.005

Khan, Z. R., & Pickett, J. (2004). The "push-pull" strategy for stemborer management: A case study in exploiting biodiversity and chemical ecology: Ecological engineering for pest management. In G. M. Gurr & M. A. Wratten, Steve D Altieri (Eds.), *Advances in habitat management for arthropods* (pp. 155–164). Csiro Publishing.

Khan, Z. R., Pickett, J. A., Van Den Berg, J., Wadhams, L. J., & Woodcock, C. M. (2000). Exploiting chemical ecology and species diversity: Stem borer and striga control for maize and sorghum in Africa. In *Pest Management Science* (Vol. 56, pp. 957–962). John Wiley & Sons, Ltd. https://doi.org/10.1002/1526-4998(200011)56:11<957::AID-PS236>3.0.CO;2-T

Khan, Z. R., Pittchar, J. O., Midega, C. A. O., & Pickett, J. A. (2018). Push-pull farming system controls fall armyworm: Lessons from Africa. *Outlooks on Pest Management*, *29*(5), 220–224. https://doi.org/10.1564/v29_oct_09

Kibaara, B. W. (2005). *Technical efficiency in kenyan's maize production: An application of the stochastic frontier approach*. *Thesis*. Colorado State University, Fort Collins, Colorado.

Kibet, C. (2011). Major challenges facing Kenyan agricultural sector. Retrieved October 1, 2018, from https://tudelft.openresearch.net/page/10574/major-challenges-facing-kenyan-agricultural-sector

Kifuko-Koech, M. N. (2013). *Desmodium effect on soil fertility, Striga control and maize production in Busia and Siaya counties, western Kenya*. University of Eldoret.

Kifuko-Koech, M., Pypers, P., Okalebo, J. R., Othieno, C. O., Khan, Z. R., Pickett, J. A., … Vanlauwe, B. (2012). The impact of *Desmodium* spp. and cutting regimes on the agronomic and economic performance of Desmodium-maize intercropping system in western Kenya. *Field Crops Research*, *137*, 97–107. https://doi.org/10.1016/j.fcr.2012.08.007

Kifuko, M. N., Othieno, C. O., Okalebo, J. R., Kimenye, L. N., Ndung'u, K. W., & Kipkoech, A. K. (2007). Effect of combining organic residues with Minjingu phosphate rock on sorption and availability of phosphorus and maize production in acid soils of western Kenya. *Experimental Agriculture*, *43*(1), 51–66. https://doi.org/10.1017/S0014479706004121

Kimanya, M.E. (2015). The health impacts of mycotoxins in the eastern Africa region. *Current Opinion in Food Science*, *6*. https://doi.org/10.1016/j.cofs.2015.11.005

Kimanya, Martin E., De Meulenaer, B., Van Camp, J., Baert, K., & Kolsteren, P. (2012). Strategies to reduce exposure of fumonisins from complementary foods in rural Tanzania. *Maternal & Child Nutrition*, *8*(4), 503–511. https://doi.org/10.1111/j.1740-8709.2011.00337.x

Klich, M. A. (2007a). Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. A. review. *Mycoscience*, *48*(2), 71–80. https://doi.org/10.1007/s10267-006-0336-2

Klich, M. A. (2007b, November). *Aspergillus flavus*: The major producer of aflatoxin. *Molecular Plant Pathology*. https://doi.org/10.1111/j.1364-3703.2007.00436.x

Koenning, S., & Payne, G. (2018). Mycotoxins in corn. Retrieved January 15, 2019, from https://corn.ces.ncsu.edu/mycotoxins-in-corn/

Kountche, B. A., Al-Babili, S., & Haussmann, B. I. G. (2016). Striga: A Persistent problem on millets. In *Biotic stress resistance in millets* (pp. 173–203). Elsevier. https://doi.org/10.1016/B978-0-12-804549-7.00006-8

Krishisewa. (n.d.). Major diseases of maize and their management - Krishisewa. Retrieved October 8, 2019, from https://www.krishisewa.com/disease-management/288-dmaize.html

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., MacDonald, S., & Crews, C. (2008). Mycotoxin analysis: An update. *Food Additives and Contaminants - Part A Chemistry,*

*Analysis, Control, Exposure and Risk Assessment*, *25*(2), 152–163. https://doi.org/10.1080/02652030701765723

Krska, R., Welzig, E., Berthiller, F., Molinelli, A., & Mizaikoff, B. (2005). Advances in the analysis of mycotoxins and its quality assurance. *Food Additives and Contaminants*, *22*(4), 345–353. https://doi.org/10.1080/02652030500070192

Kushiro, M. (2008). Effects of milling and cooking processes on the deoxynivalenol content in wheat. *International Journal of Molecular Sciences*, *9*(11), 2127–2145. https://doi.org/10.3390/ijms9112127

Leslie, J. F., Bandyopadhyay, R., & Visconti, A. (2008). *Mycotoxins: Detection methods, management, public health and agricultural trade.* (J. F. Leslie, R. Bandyopadhyay, & A. Visconti, Eds.), *CABI Books* (Vol. 2). Wallingford: CABI. https://doi.org/10.1079/9781845930820.0000

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. Oxford: Blackwell Publishing.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., … Gupta, N. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives*, *113*(12), 1763–1767.

Louarn, G., Pereira-Lopès, E., Fustec, J., Mary, B., Voisin, A. S., de Faccio Carvalho, P. C., & Gastal, F. (2015). The amounts and dynamics of nitrogen transfer to grasses differ in alfalfa and white clover-based grass-legume mixtures as a result of rooting strategies and rhizodeposit quality. *Plant and Soil*, *389*(1–2), 289–305. https://doi.org/10.1007/s11104-014-2354-8

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., … Al., E. (2012, August 2). Defining the core *Arabidopsis thaliana* root microbiome. *Nature*. https://doi.org/10.1038/nature11237

Magan, Naresh, Hope, R., Cairns, V., & Aldred, D. (2003). Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. In *Epidemiology of Mycotoxin Producing Fungi* (pp. 723–730). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-017-1452-5_7

Mahapatra, R., Jampala, S. S. M., & Patel, D. R. (2015). Induction of systemic acquired resistance in *Zea mays* L. by *Aspergillus flavus* and *A. parasiticus* derived elicitors. *Archives of Phytopathology and Plant Protection*, *48*(2), 120–134. https://doi.org/10.1080/03235408.2014.884523

Mahesh, B., & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences*, *4*(S), 839–843.

Maina, A. W., Wagacha, J. M., Mwaura, F. B., Muthomi, J. W., & Woloshuk, C. P. (2016). Postharvest practices of maize farmers in Kaiti District, Kenya and the impact of hermetic storage on populations of *Aspergillus* Spp. and aflatoxin contamination. *Journal of Food Research*, *5*(6), 53. https://doi.org/10.5539/jfr.v5n6p53

Makokha, M., Odera, H., Maritim, H. ., Okalebo, J. R., & Iruria, D. M. (1999). Farmers' perceptions and adoption of soil management technologies in western Kenya. *African Crop Science Journal*, *7*(4), 549–558.

Manu, N., Opit, G. P., Osekre, E. A., Arthur, F. H., Mbata, G., Armstrong, P., … Campbell, J. F. (2019). Moisture content, insect pest infestation and mycotoxin levels of maize in markets in the northern region of Ghana. *Journal of Stored Products Research*, *80*, 10–20.

https://doi.org/10.1016/J.JSPR.2018.10.007

Marenya, P. P., & Barrett, C. B. (2009). State-conditional fertilizer yield response on western Kenyan farms. *American Journal of Agricultural Economics*, *91*(4), 991–1006. https://doi.org/10.1111/j.1467-8276.2009.01313.x

Massawe, C. R., Kaswende, J. S., Mbwaga, A. M., & Hella, J. P. (2002). On-farm verification of maize/cowpea intercropping on the control of Striga under subsistence farming. Integrated approaches to higher maize productivity in the new millennium. In *Proceedings of the Eastern and Southern Africa Regional Maize Conference. 5-11 Feb 2002. Nairobi (Kenya).* (pp. 165–167). CIMMYT.

Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H., & Bouwmeester, H. J. (2005). The Strigolactone germination stimulants of the plant-parasitic striga and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiology*, *139*, 920–934. https://doi.org/10.1104/pp.105.061382.920

Matusso, J., Mugwe, J., & Mucheru-Muna, M. (2014). Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of sub-Saharan Africa. *Research Journal of Agriculture and Environmental Management*, *3*(3), 162–174. https://doi.org/10.1039/b901621j

Mays, D. T. (2015). *Mycotoxin management in maize (Zea mays (L.)) damaged by lepidopteran pests*. Texas A & M Univeristy.

Mbuge, D. O., Negrini, R., Nyakundi, L. O., Kuate, S. P., Bandyopadhyay, R., Muiru, W. M., … Mezzenga, R. (2016). Application of superabsorbent polymers (SAP) as desiccants to dry maize and reduce aflatoxin contamination. *Journal of Food Science and Technology*, *53*(8), 3157–3165. https://doi.org/10.1007/s13197-016-2289-6

McGee, D. C. (1988). *Maize diseases. A reference source for seed technologists. CAB Direct*. St. Paul, Minnesota: APS Press.

McMaster, M. C. (2007). Advantages and disadvantages of HPLC. In *HPLC: A practical user's guide* (2nd ed.). John Wiley & Sons, Inc.

Medina, A., Akbar, A., Baazeem, A., Rodriguez, A., & Magan, N. (2017). Climate change , food security and mycotoxins : Do we know enough ? *Fungal Biology Reviews*, *31*(3), 143–154. https://doi.org/10.1016/j.fbr.2017.04.002

Medina, Á., Mateo, E. M., Valle-algarra, F. M., Mateo, F., Mateo, R., & Jiménez, M. (2008). Influence of nitrogen and carbon sources on the production of ochratoxin A by ochratoxigenic strains of *Aspergillus* spp . isolated from grapes. *International Journal of Food Microbiology*, *122*, 93–99. https://doi.org/10.1016/j.ijfoodmicro.2007.11.055

Mehl, H. L., Opoku, J., Malone, S., Kleczewski, N. M., Herbert, D. A., & Hamby, K. (2018). Relationship between invasive brown marmorated stink bug (*Halyomorpha halys*) and fumonisin contamination of field corn in the mid-Atlantic U.S. *Plant Disease*, PDIS-06-18-1115-RE. https://doi.org/10.1094/pdis-06-18-1115-re

Mesterházy, Á., Lemmens, M., & Reid, L. M. (2012). Breeding for resistance to ear rots caused by *Fusarium* spp. in maize - A review. *Plant Breeding*. https://doi.org/10.1111/j.1439-0523.2011.01936.x

Miano, D. W., Kimenju, J. W., Muiru, W. M., & Charles, K. A. (2011). Occurrence of common maize diseases in Kiambu, Embu and Nakuru counties of Kenya. In *Optimimization of Agricultural Value Chains for sustainable Development* (p. 179). Nairobi: Faculty of Agriculture, University of Nairobi.

Midega, C. A. O., Bruce, T. J. A., Pickett, J. A., & Khan, Z. R. (2015). Ecological management of

cereal stemborers in African smallholder agriculture through behavioural manipulation. *Ecological Entomology*, *40*(S1), 70–81. https://doi.org/10.1111/een.12216

Midega, C. A. O., Jonsson, M., Khan, Z. R., & Ekbom, B. (2014). Effects of landscape complexity and habitat management on stemborer colonization, parasitism and damage to maize. *Agriculture, Ecosystems and Environment*, *188*, 289–293. https://doi.org/10.1016/j.agee.2014.02.028

Midega, C. A. O., Khan, Z. R., Amudavi, D. M., Pittchar, J., & Pickett, J. A. (2010). Integrated management of Striga hermonthica and cereal stemborers in finger millet (*Eleusine coracana* (L.) Gaertn.) through intercropping with *Desmodium intortum*. *International Journal of Pest Management*, *56*(2), 145–151. https://doi.org/10.1080/09670870903248843

Midega, C. A. O., Khan, Z. R., Van Den Berg, J., Ogol, C. K. P. O., Pickett, J. A., & Wadhams, L. J. (2006). Maize stemborer predator activity under 'push – pull' system and Bt-maize: A potential component in managing Bt resistance. *International Journal of Pest Management*, *52*(1), 1–10. https://doi.org/10.1080/09670870600558650

Midega, C. A. O., Murage, A. W., Pittchar, J. O., & Khan, Z. R. (2016). Managing storage pests of maize: Farmers' knowledge, perceptions and practices in western Kenya. *Crop Protection*, *90*, 142–149. https://doi.org/10.1016/j.cropro.2016.08.033

Midega, C. A. O., Pittchar, J. O., Pickett, J. A., Hailu, G. W., & Khan, Z. R. (2018). A climate-adapted push-pull system effectively controls fall armyworm, *Spodoptera frugiperda* (J E Smith), in maize in East Africa. *Crop Protection*, *105*, 10–15. https://doi.org/10.1016/j.cropro.2017.11.003

Midega, C. A. O., Pittchar, J., Salifu, D., Pickett, J. A., & Khan, Z. R. (2013). Effects of mulching , N-fertilization and intercropping with *Desmodium uncinatum* on *Striga hermonthica*

infestation in maize. *Crop Protection*, *44*, 44–49. https://doi.org/10.1016/j.cropro.2012.10.018

Miller, J. D. (2008). Mycotoxins in small grains and maize: old problems, new challenges. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, *25*(2), 219–230. https://doi.org/10.1080/02652030701744520

MOA, (Ministry of Agriculture and Irrigation). (2017). *Status of the fall army worm ( FAW ) in Kenya*.

Mohamed, A. B. (2016). *Population of Aspergillus section Flavi and aflatoxin contamination in maize from fields treated with atoxigenic aspergillus flavus (aflasafe ke01) in lower eastern Kenya*. University of Nairobi.

Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C., & Lemanceau, P. (2006). Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of Medicago truncatula Gaertn. cv. Jemalong line J5. *New Phytologist*, *170*(1), 165–175. https://doi.org/10.1111/j.1469-8137.2006.01650.x

Mukanga, M., Derera, J., Tongoona, P., & Laing, M. D. (2010). A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia. *International Journal of Food Microbiology*, *141*(3), 213–221. https://doi.org/10.1016/j.ijfoodmicro.2010.05.011

Munkvold, G. P., Hellmich, R. L., & Showers, W. B. (1997). Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. *Phytopathology*, *87*(10), 1071–1077. https://doi.org/10.1094/PHYTO.1997.87.10.1071

Munkvold, Gary P. (2003). Cultural and genetic approaches to managing mycotoxins in maize.

*Annual Review of Phytopathology*, *41*(1), 99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

Mutegi, C. K., Cotty, P. J., & Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960-to date). *World Mycotoxin Journal*, *11*(3), 341–357. https://doi.org/10.3920/WMJ2018.2362

Muthomi, J., Fulano, A. M., Wagacha, J. M., & Mwang'ombe, A. W. (2017). Management of snap bean insect pests and diseases by use of antagonistic fungi and plant extracts. *Sustainable Agriculture Research*, *6*(3), 52. https://doi.org/10.5539/sar.v6n3p52

Muthomi, J. W., Lengai, G. M. W., Wagacha, M. J., & Narla, R. D. (2017). In vitro activity of plant extracts against some important plant pathogenic fungi of tomato. *Australian Journal of Crop Science*, *11*(06), 683–689. https://doi.org/10.21475/ajcs.17.11.06.p399

Mutiga, S K, Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (2015). Assessment of aflatoxin and fumonisin contamination of maize in Western Kenya. *Phytopathology*, *105*(9), 1250–1261. https://doi.org/10.1094/PHYTO-10-14-0269-R

Mutiga, S K, Were, V., Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (2014). Extent and drivers of mycotoxin contamination: Inferences from a survey of Kenyan maize mills. *Phytopathology*, *104*(11), 1221–1231. https://doi.org/10.1094/PHYTO-01-14-0006-R

Mutiga, Samuel K., Morales, L., Angwenyi, S., Wainaina, J., Harvey, J., Das, B., & Nelson, R. J. (2017). Association between agronomic traits and aflatoxin accumulation in diverse maize lines grown under two soil nitrogen levels in Eastern Kenya. *Field Crops Research*, *205*, 124–134. https://doi.org/10.1016/j.fcr.2017.02.007

Ndemera, M., Nyanga, L. K., De Saeger, S., De Boevre, M., & Landschoot, S. (2018). Effect of agronomic practices and weather conditions on mycotoxins in maize: A case study of

subsistence farming households in Zimbabwe. *World Mycotoxin Journal*, *11*(3), 421–436. https://doi.org/10.3920/wmj2017.2227

Ndung'u, K. W., Okalebo, J. R., Othieno, C. O., Kifuko, M. N., Kipkoech, A. K., & Kimenye, L. N. (2006). Residual effectiveness of Minjingu phosphate rock and fallow biomass on crop yields and financial returns in western Kenya. *Experimental Agriculture*, *42*(3), 323–336. https://doi.org/10.1017/S0014479706003565

Nduti, N., Njeru, P., Mwaniki, M., & Reid, G. (2017). Aflatoxin variations in maize flour and grains collected from various regions of Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *17*(1), 11743–11756.

Negedu, A., Atawodi, S. E., Ameh, J. B., Umoh, V. J., & Tanko, H. Y. (2011). Economic and health perspectives of mycotoxins: A review. *Continental Journal of Biomedical Sciences*, *5*(1), 5–26.

Nelson, P. (1983). *Fusarium species : An illustrated manual for identification*. *Published in 1983 in University Park (Pa.) by Pennsylvania state university press*. Pennsylvania State University Press.

Nirenberg, H. I. (1981). A simplified method for identifying *Fusarium* spp . occurring on wheat. *Canadian Journal of Botany*, *59*(9), 1599–1609. https://doi.org/10.1139/b81-217

Njeru, N. K., Muthomi, J. W., Mutegi, C. K., & Wagacha, J. M. (2016). Effect of cropping systems on accumulation of *Fusarium* head blight of wheat inocula in crop residues and soils. *Journal of Plant Sciences*, *11*(1), 12–21. https://doi.org/10.3923/jps.2016.12.21

Njoki, L. M., Okoth, S. A., & Wachira, P. M. (2017). Effects of medicinal plant extracts and photosensitization on aflatoxin producing *Aspergillus flavus* (Raper and Fennell). *International Journal of Microbiology*, *2017*(2001). https://doi.org/10.1155/2017/5273893

Nyangi, C. (2016). Assessment of pre-harvest aflatoxin and fumonisin contamination of maize in Babati District, Tanzania. *African Journal of Food, Agriculture, Nutrition and Development*, *16*(03), 11039–11053. https://doi.org/10.18697/ajfand.75.ILRI06

O'Callaghan, J., Stapleton, P. C., & Dobson, A. D. W. (2006). Ochratoxin A biosynthetic genes in Aspergillus ochraceus are differentially regulated by pH and nutritional stimuli. *Fungal Genetics and Biology*, *43*(4), 213–221. https://doi.org/10.1016/j.fgb.2005.11.005

Odendo, M., Obare, G., & Salasya, B. (2009). Factors responsible for differences in uptake of integrated soil fertility management practices amongst smallholders in western Kenya. *African Journal of Agricultural Research*, *4*(11), 1303–1311.

Ogara, I. M., Zarafi, A. B., Alabi, O., Banwo, O., Ezekiel, C. N., Warth, B., … Krska, R. (2017). Mycotoxin patterns in ear rot infected maize: A comprehensive case study in Nigeria. *Food Control*, *73*, 1159–1168. https://doi.org/10.1016/j.foodcont.2016.10.034

Okalebo, J. R., Othieno, C. O., Woomer, P. L., Karanja, N. K., Semoka, J. R. M., Bekunda, M. A., … Mukhwana, E. J. (2007). Available technologies to replenish soil fertility in East Africa. In *Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities* (pp. 45–62). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-5760-1_3

Okumu, O. O., Muthomi, J. W., Ojiem, J., Narla, R., & Nderitu, J. H. (2019). Effect of legume extracts on germination, seedling health of beans (*Phaseolus vulgaris* L.) and soil microorganisms. *International Journal of Plant & Soil Science*, *28*(1), 1–13. https://doi.org/10.9734/ijpss/2019/v28i130097

Olga, A. (2009). Mycotoxins in grains harvested in 2008: Wheat. Retrieved January 21, 2019, from https://en.engormix.com/mycotoxins/articles/mycotoxins-in-grains-harvested-

t34463.htm

Ono, E. Y.S., Sasaki, E. Y., Hashimoto, E. H., Hara, L. N., Corrêa, B., Itano, E. N., … Hirooka, E. Y. (2002). Post-harvest storage of corn: Effect of beginning moisture content on mycoflora and fumonisin contamination. *Food Additives and Contaminants*, *19*(11), 1081–1090. https://doi.org/10.1080/02652030210146828

Ono, Elisabete Yurie Sataque, Biazon, L., Silva, M. da, Vizoni, É., Sugiura, Y., Ueno, Y., & Hirooka, E. Y. (2006). Fumonisins in corn: Correlation with *Fusarium* sp. count, damaged kernels, protein and lipid content. *Brazilian Archives of Biology and Technology*, *49*(1), 63–71. https://doi.org/10.1590/S1516-89132006000100008

Onyango, F. O., & Ochieng‘-Odero, J. P. R. (1994). Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata*, *73*(2), 139–144. https://doi.org/10.1111/j.1570-7458.1994.tb01848.x

Onyango, K., Bii, H., Odhiambo, N., Kinyumu, E., Kirimi, L., & Ayieko, M. (2018). Food situation assessment and crop prospects for 20 17 / 2018.

Opande, G. T., Dida, M., Onyango, P., & Wesonga, C. (2017). Incidences and severity of maize ear rot disease in Western Kenya. *Int. J. Biosci*, *11*(2), 136–143. https://doi.org/10.12692/ijb/11.2.136-143

Overholt, W. A., Maes, K. V. N., & Goebel, F. R. (2001). Fieild guide to the stemborer larvae of maize , sorghwm and sugarcane in Eastern arnd Southern Africa. *ICIPE Science Press, Nairobi*, 31.

Owuor, M. J., Midega, C. A. O., Obonyo, M., & Khan, Z. R. (2017). Distribution of *Aspergillus* and *Fusarium* ear rot causative fungi in soils under push - pull and maize monocropping system in Western Kenya. *African Journal of Microbiology Research*, *11*(37), 1411–1421.

https://doi.org/10.5897/AJMR2017.8685

Owuor, M. J., Midega, C. A. O., Obonyo, M., & Khan, Z. R. (2018). Impact of companion cropping on incidence and severity of maize ear rots and mycotoxins in Western Kenya. *African Journal of Agricultural Research*, *13*(41), 2224–2231. https://doi.org/10.5897/AJAR2018.13396

Page, A. L., Miller, R. H., & Kenney, D. R. (1982). *Methods of soil analysis,Part 2.* (Second Edi). Madison, Winconsin, USA, Winconsin, USA: American Society of Agronomy.

Palencia, E. R., MIitchell, T. R., Snook, M. E., Glenn, A. E., Gold, S., Hinton, D. M., … Bacon, C. W. (2014). Analyses of black *Aspergillus* species of peanut and maize for ochratoxins and fumonisins. *Journal of Food Protection*, *77*(5), 805–813. https://doi.org/10.4315/0362-028X.JFP-13-321

Pandey, S. N., Abid, M., & Abid Ali Khan, M. M. (2018). Diversity, functions, and stress responses of soil microorganisms, 1–19. https://doi.org/10.1007/978-981-10-5514-0_1

Paredes, P., de Melo-Abreu, J. P., Alves, I., & Pereira, L. S. (2014). Assessing the performance of the FAO AquaCrop model to estimate maize yields and water use under full and deficit irrigation with focus on model parameterization. *Agricultural Water Management*, *144*, 81–97. https://doi.org/10.1016/j.agwat.2014.06.002

Parker, N. S., Anderson, N. R., Richmond, D. S., Long, E. Y., Wise, K. A., & Krupke, C. H. (2017). Larval western bean cutworm feeding damage encourages the development of Gibberella ear rot on field corn. *Pest Management Science*, *73*(3), 546–553. https://doi.org/10.1002/ps.4313

Parry, D. W., McLeod, L., & Jenkinson, P. (1993). *Fusarium* ear blight ( scab ) in small grain cereals — a review. *Plant Pathology*, *44*, 207–238.

Parsons, M. W. (2008). Biotic and abiotic factors associated with *Fusarium* ear rot of maize caused by *Fusarium verticillioides*. *ProQuest Dissertations and Theses*, 170.

Parsons, M. W., & Munkvold, G. P. (2012). Effects of planting date and environmental factors on *Fusarium* ear rot symptoms and fumonisin B1 accumulation in maize grown in six North American locations. *Plant Pathology*, *61*(6), 1130–1142. https://doi.org/10.1111/j.1365-3059.2011.02590.x

Pasuquin, J. M., Pampolino, M. F., Witt, C., Dobermann, A., Oberthür, T., Fisher, M. J., & Inubushi, K. (2014). Closing yield gaps in maize production in Southeast Asia through site-specific nutrient management. *Field Crops Research*, *156*(2014), 219–230. https://doi.org/10.1016/j.fcr.2013.11.016

Paterson, R. R. M., & Lima, N. (2010). How will climate change affect mycotoxins in food? *Food Research International*, *43*(7), 1902–1914. https://doi.org/10.1016/j.foodres.2009.07.010

Petzinger, E., & Weidenbach, A. (2002). Mycotoxins in the food chain: The role of ochratoxins. *Livestock Production Science*, *76*(3), 245–250. https://doi.org/10.1016/S0301-6226(02)00124-0

Pioneer. (2019a). Common corn ear rots. Retrieved May 16, 2019, from https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/corn-ear-rots/

Pioneer. (2019b). Vegetative corn growth stages and scouting tips.

Pittet, A. (2005). Modern methods and trends in mycotoxin analysis. *Mitt. Lebensm. Hyg*.

Queiroz, V. A. V., de Oliveira Alves, G. L., da Conceição, R. R. P., Guimarães, L. J. M., Mendes, S. M., de Aquino Ribeiro, P. E., & da Costa, R. V. (2012). Occurrence of fumonisins and zearalenone in maize stored in family farm in Minas Gerais, Brazil. *Food Control*, *28*(1), 83–

86. https://doi.org/10.1016/J.FOODCONT.2012.04.039

Rajcan, I., & Swanton, C. J. (2001). Understanding maize-weed competition: Resource competition, light quality and the whole plant. *Field Crops Research*, *71*(2), 139–150. https://doi.org/10.1016/S0378-4290(01)00159-9

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, *1312*(1), 105–112. https://doi.org/10.1111/nyas.12396

Ren, X., Zhang, Q., Zhang, W., Mao, J., & Li, P. (2020). Control of aflatoxigenic molds by antagonistic microorganisms: Inhibitory behaviors, bioactive compounds, related mechanisms, and influencing factors. *Toxins*, *12*(1), 1–21. https://doi.org/doi:10.3390/toxins12010024

Samapundo, S., Devlieghere, F., De Meulenaer, B., & Debevere, J. M. (2005). Effect of water activity and temperature on growth and the relationship betweenfumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on Corn. *Journal of Food Protection*, *68*(5), 1054–1059. https://doi.org/10.17660/ActaHortic.2005.674.54

Samson, R. A., & Varga, J. (2009). What is a species in *Aspergillus*? *Medical Mycology*, *47*(SUPPL. 1). https://doi.org/10.1080/13693780802354011

Sanchis, V., & Magan, N. (2004). "Environmental profiles for growth and mycotoxin production. In N. Magan & M. Olsen (Eds.), *Mycotoxin in Food: Detection and Control* (pp. 174–189). Cambridge: Woodhead Publishing Ltd.

Sarmento, R. de A., Aguiar, R. W. de S., Aguiar, R. de A. S. de S., Vieira, S. M. J., Oliveira, H. G. de, & Holtz, A. M. (2002). Biology review, occurrence and control of *Spodoptera frugiperda* (Lepidoptera, Noctuidae) in corn in Brazil. *Bioscience Journal (Brazil)*.

Schmaile III, D. G., & Munkvold, G. P. (2009). Mycotoxins in crops: A threat to human and domestic animal health. *Plant Health Instructor*, Reviewed 2014. https://doi.org/10.1094/PHI-I-2009-0715-01

Schmale, D. G., & Munkvold, G. P. (2009). Mycotoxins in crops: A threat to human and domestic animal health. *Plant Health Instructor*, 715–721. https://doi.org/10.1094/PHI-I-2009-0715-01

Schoeman, A. (2012). Mycoctoxins- toxins produced by maize ear rot fungi. Retrieved November 30, 2018, from https://www.grainsa.co.za/mycotoxins---toxins-produced-by-maize-ear-rot-fungi

Sciencing. (2020). What Are the Disadvantages of HPLC? Retrieved January 13, 2020, from https://sciencing.com/list-7237518-disadvantages-hplc-.html

Scudamore, K. A., & Patel, S. (2000). Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. *Food Additives and Contaminants*, *17*(5), 407–416. https://doi.org/10.1080/026520300404824

Shaise, J. (2011). High performance thin-layer chromatograpphy (HPTLC). Kerala, India.

Shephard, G. S. (2009, November). Aflatoxin analysis at the beginning of the twenty-first century. *Analytical and Bioanalytical Chemistry*. https://doi.org/10.1007/s00216-009-2857-y

Sherma, J. (2006). Thin-Layer Chromatography. In *Encyclopedia of Analytical Chemistry*. Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470027318.a5918

Shrestha, A., Thapa, B., Devkota, M., & Subedi, R. (2018). Comparative efficiency of different weed management practices on yield and economic in summer maize in Dang. *Advances in Crop Science and Technology*, *06*(02), 1–4. https://doi.org/10.4172/2329-8863.1000354

Sirma, A. (2016). Aflatoxin B1 occurrence in millet, sorghum and maize from four agro-ecological

zones in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *16*(03), 10991–11003. https://doi.org/10.18697/ajfand.75.ILRI03

Smith, L. E., Stoltzfus, R. J., & Prendergast, A. (2012). Food chain mycotoxin exposure, gut health, and impaired growth: A conceptual framework. *Advances in Nutrition: An International Review Journal*, *3*(4), 526–531. https://doi.org/10.3945/an.112.002188

Smith, Laura E., Prendergast, A. J., Turner, P. C., Humphrey, J. H., & Stoltzfus, R. J. (2017). Aflatoxin exposure during pregnancy, maternal anemia, and adverse birth outcomes. *American Journal of Tropical Medicine and Hygiene*, *96*(4), 770–776. https://doi.org/10.4269/ajtmh.16-0730

Snijders, C., & Perkowski, J. (1990). Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of kernels. *Phytoparasitica*, *80*, 566–570.

Sobek, E. A., & Munkvold, G. P. (1999). European corn borer (Lepidoptera: Pyralidae) larvae as vectors of *Fusarium moniliforme*, causing kernel rot and symptomless infection of maize kernels. *Journal of Economic Entomology*, *92*(3), 503–509.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology*, *3*(3), 94–99. https://doi.org/10.2478/v10102-010-0019-x

Sofa, T., & Doss, C. (2011). *The role of women in agriculture*. *ESA Working Paper No.11-02* (Vol. 11). https://doi.org/10.1002/2014GB005021

Sopialena, S., Rosfiansyah, R., & Sila, S. (2017). The benefit of top soil and fertilizer mixture to improve the ex-coal mining land. *Nusantara Bioscience*, *9*(1), 36–43. https://doi.org/10.13057/nusbiosci/n090107

Steinkellner, S., Lendzemo, V., Langer, I., Schweiger, P., Khaosaad, T., Toussaint, J., &

Vierheilig, H. (2007). Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant-fungus interactions. *Molecules*, *12*, 1290–1306. https://doi.org/10.3390/12071290

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V, Breiman, R., Brune, M.-N., … Wilson, D. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives*, *114*(12), 1898–1903. https://doi.org/10.1289/ehp.9302

Su, Y.-Y., Qi, Y.-L., & Cai, L. (2012). Induction of sporulation in plant pathogenic fungi. *Mycology*, *3*(3), 195–200. https://doi.org/10.1080/21501203.2012.719042

Sudheeran, K. P., Rinat, O., Ortal, G., Itay, M., Noa, S., Dalia, M., … Alkan, N. (2019). Glycosylated flavonoids: fruit's concealed antifungal arsenal. *New Phytologist*, (October), 0–2. https://doi.org/10.1111/nph.16251

Sullia, S. B. (1973). Effect of root exudates and extracts on rhizosphere fungi. *Plant and Soil*, *39*(1), 197–200.

Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., … Wang, J.-S. (2007). Fumonisin $B_1$ contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Additives and Contaminants*, *24*(2), 181–185. https://doi.org/10.1080/02652030601013471

Tabassum, N., & Vidyasagar, G. M. (2013). Antifungal investigations on plant essential oils. A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(SUPPL. 2), 19–28.

Tamene, L., Mponela, P., Ndengu, G., & Kihara, J. (2016). Assessment of maize yield gap and major determinant factors between smallholder farmers in the Dedza district of Malawi.

*Nutrient Cycling in Agroecosystems*, *105*(3), 291–308. https://doi.org/10.1007/s10705-015-9692-7

Tanveer, M., Anjum, S. A., Hussain, S., Cerdà, A., & Ashraf, U. (2017). Relay cropping as a sustainable approach: Problems and opportunities for sustainable crop production. *Environmental Science and Pollution Research*, *24*(8), 6973–6988. https://doi.org/10.1007/s11356-017-8371-4

Tapas, A. R., Sakarkar, D. M., & Kakde, R. B. (2008). Flavonoids as nutraceuticals: A review. *Tropical Journal of Pharmaceutical Research*, *7*(3), 1089–1099.

Taylor, P. J. (2005). Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clinical Biochemistry*. Elsevier Inc. https://doi.org/10.1016/j.clinbiochem.2004.11.007

Tenberge, K. B. (1999). Biology and life strategy of the ergot fungi. In V. Kren & L. Cvak (Eds.), *Ergot: The genus Claviceps* (pp. 40–71). London: CRC Press. https://doi.org/10.1201/9780203304198-9

The Organic Farmer. (2019). Desmodium is a good protein source. Retrieved January 14, 2020, from http://www.theorganicfarmer.org/content/desmodium-good-protein-source

Thompson, M., Raizada, M., Thompson, M. E. H., & Raizada, M. N. (2018). Fungal pathogens of maize gaining free passage along the silk road. *Pathogens*, *7*(4), 81. https://doi.org/10.3390/pathogens7040081

Tirado, M. C., Clarke, R., Jaykus, L. A., McQuatters-Gollop, A., & Frank, J. M. (2010). Climate change and food safety: A review. *Food Research International*, *43*(7), 1745–1765. https://doi.org/10.1016/j.foodres.2010.07.003

Tittlemier, S. A., Cramer, B., Dall'Asta, C., Iha, M. H., Lattanzio, V. M. T., Malone, R. J., …

Stroka, J. (2019). Developments in mycotoxin analysis: an update for 2017-2018. *World Mycotoxin Journal*, *12*(1), 3–29. https://doi.org/10.3920/WMJ2018.2398

Tola, M., & Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food & Agriculture*, *2*(1), 1–12. https://doi.org/10.1080/23311932.2016.1191103

Tuzimski, T., & Sherma, J. (2019). Thin-Layer Chromatography. In *Encyclopedia of Analytical Chemistry* (pp. 1–26). Wiley. https://doi.org/10.1002/9780470027318.a5918.pub2

UNDP/FAO. (1989). Regional network inter-country cooperation on pre-harvest technology and quality control of food grains and the ASEAN grain post harvest programme. Retrieved January 14, 2018, from Retrieved from www.fao.org/docrep/X5036E/x5036E00.htm

USDA. (2000). Maize diseases. Retrieved October 8, 2019, from https://ag.purdue.edu/ipia/Documents/afghanistan/SPS Documents/Maize-Diseases-Handout-English.pdf

Vacher, C., Vile, D., Helion, E., Piou, D., & Desprez-Loustau, M. L. (2008). Distribution of parasitic fungal species richness: Influence of climate versus host species diversity. *Diversity and Distributions*, *14*(5), 786–798. https://doi.org/10.1111/j.1472-4642.2008.00479.x

Van Rensburg, J. B. J. (2001). Larval mortality and injury patterns of the African stalk borer, *Busseola fusca* (Fuller) on various plant parts of Bt-transgenic maize. *South African Journal of Plant and Soil*, *18*(2), 62–68. https://doi.org/10.1080/02571862.2001.10634405

Vanlauwe, B., & Giller, K. E. (2006). Popular myths around soil fertility management in sub-Saharan Africa. *Agriculture, Ecosystems and Environment*, *116*(1–2), 34–46. https://doi.org/10.1016/j.agee.2006.03.016

Vanlauwe, B., Kanampiu, F., Odhiambo, G. D., De Groote, H., Wadhams, L. J., & Khan, Z. R. (2008). Integrated management of *Striga hermonthica*, stemborers, and declining soil fertility

in western Kenya. *Field Crops Research*, *107*(2), 102–115. https://doi.org/10.1016/j.fcr.2008.01.002

Vanlauwe, B., Tittonell, P., & Mukalama, J. (2006). Within-farm soil fertility gradients affect response of maize to fertiliser application in western Kenya. *Nutrient Cycling in Agroecosystems*, *76*(2–3), 171–182. https://doi.org/10.1007/s10705-005-8314-1

Viegas, C., Meneses, M., & Viegas, S. (2016). Climate changes influence in occupational exposure to fungi and mycotoxins. In *Occupational Safety and Hygiene IV* (pp. 11–15). https://doi.org/10.1201/b21172-4

Wagacha, J. M., & Muthomi, J. W. (2008, May 10). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*. Elsevier. https://doi.org/10.1016/j.ijfoodmicro.2008.01.008

Wagacha, John Maina, Njeru, N. K., Okumu, O. O., Muthomi, J. W., & Mutegi, C. K. (2016). Occurrence of *Fusarium* head blight of wheat and associated mycotoxins in Narok and Nakuru Counties, Kenya. *World Journal of Agricultural Research*, *4*(4), 119–127. https://doi.org/10.1016/j.jssc.2007.04.023

Wagacha, John Maina, Steiner, U., Dehne, H. W., Zuehlke, S., Spiteller, M., Muthomi, J., & Oerke, E. C. (2010). Diversity in mycotoxins and fungal species infecting wheat in Nakuru district, Kenya. *Journal of Phytopathology*, *158*(7–8), 527–535. https://doi.org/10.1111/j.1439-0434.2009.01653.x

Wang, P., Marsh, E. L., Ainsworth, E. A., Leakey, A. D. B., Sheflin, A. M., & Schachtman, D. P. (2017). Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated $CO_2$ and $O_3$. *Scientific Reports*, *7*(1), 15019. https://doi.org/10.1038/s41598-017-14936-2

Warburton, M. L., & Williams, W. P. (2014). Aflatoxin resistance in maize: What have we learned lately? *Advances in Botany, 2014*, 10. https://doi.org/10.1155/2014/352831

Weisskopf, L., Akello, P., Milleret, R., Khan, Z. R., Gobat, J.-M., & Le Bayon, R. (2009). White lupin leads to increased maize yield through a soil fertility-independent mechanism: A new candidate for fighting *Striga hermonthica* infestation? *Plant and Soil*, *319*(1–2), 101–114. https://doi.org/10.1007/s11104-008-9853-4

WHO, (World Health Organization). (2018). Mycotoxins. Retrieved April 1, 2019, from https://www.who.int/news-room/fact-sheets/detail/mycotoxins

Williams, J., Aggarwal, D., Jolly, P., Phillips, T., & Wang, J.-S. (2005). *Connecting the dots: Logical and statistical connections between aflatoxin exposure and HIV/AIDS*. *Exposure*.

Wu, F, & Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin-reduction strategies in Africa: case studies in biocontrol and post-harvest interventions. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *27*(4), 496–509. https://doi.org/10.1080/19440040903437865

Wu, Felicia, Miller, J. D., & Casman, E. A. (2004). The economic impact of Bt corn resulting from mycotoxin reduction. *Journal of Toxicology - Toxin Reviews*, *23*(2–3), 397–424. https://doi.org/10.1081/TXR-200027872

Yang, X., Zhang, L., Shi, C., Shang, Y., Zhang, J., Han, J., & Dong, J. (2014). The extraction, isolation and identification of exudates from the roots of *Flaveria bidentis*. *Journal of Integrative Agriculture*, *13*(1), 105–114. https://doi.org/10.1016/S2095-3119(13)60495-5

Zhang, L., Dou, X. W., Zhang, C., Logrieco, A. F., & Yang, M. H. (2018). A review of current methods for analysis of mycotoxins in herbal medicines. *Toxins*, *10*(2). https://doi.org/10.3390/toxins10020065

Zhao, M., Sun, B., Wu, L., Gao, Q., Wang, F., Wen, C., … Yang, Y. (2016). Zonal soil type determines soil microbial responses to maize cropping and fertilization. *MSystems*, *1*(4), e00075-16. https://doi.org/10.1128/mSystems.00075-16

Zinedine, A., Soriano, J. M., Moltó, J. C., & Mañes, J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food and Chemical Toxicology*, *45*(1), 1–18. https://doi.org/10.1016/J.FCT.2006.07.030

**Appendix**

**Appendix 1: Survey Questionnaire**

**To establish the socio-economic and agronomic factors influencing mycotoxin contamination in maize in western Kenya**

**Section I: Background information**

Farmer ID.: --------- Date: -------/-----/2017 Sub-county: ---------- Village ----------

Agro-ecological Zone: ---------- Latitude: ---------- Longitude: ----------

Elevation (m): ----------

Tel: ----------

**Section II: Farmer's social-economic profile**

a) Name of farmer: ---------- Age: -------- Gender: (M) (F)
b) Highest level of education: [1] no formal education [2] not completed primary [3] completed primary school [4] secondary [5] tertiary
c) Membership to a welfare group: (*Yes*) (*No*)
d) Years of maize production ----------
e) Source of funds to acquire farm inputs and cater for farm operations ----------
---
f) What is your average annual total income? (KES) [1] 20,000 – 35,000; [2] 36,000 – 55,000; [3] 56,000-75,000; [4] 76,000 – 100,000; [5] above 100,000

**Section III: Information on agronomic practices**

a) Total land size owned ----------
b) Area under maize production (acres): ----------
c) How do you utilize your farmland? -------% under crops ------% used for livestock ------% left fallow ---------- % others
d) Pre-season practices ----------
---
---
e) What method(s) of field preparation do you practice?

[1] Oxen ploughing [2] Jembe digging [3] Machete digging [4] burning [5] others (specify) ----------

f) Do you use any soil amendments in maize production? (*Yes*) (*No*)
g) If yes, which ones? ----------

h) Sources of seeds: a) Own b) Neighbor c) Market d) Agro-shop

i) Varieties of maize grown:

i) current season ----------

ii) previous season ----------

j) Other crops grown on the farm ----------

k) Do you intercrop maize crop with other crops? (*Yes*) (*No*) If yes, with what crop(s)?

---

l) Do you practice crop rotation in maize production? (*Yes*) (*No*) If yes, with what crop(s)?

---

m) Do you leave the land in fallow from time to time? (*Yes*) (*No*) If yes, for how many seasons?

---

n) What are the most common pests of maize in your maize field?

o) What method(s) of pest control do you employ?

| Pest | Control method | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| | | | | |
| | | | | |
| | | | | |

If chemical:

| Chemical | Source | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| | | | | |
| | | | | |
| | | | | |

p) What are the most common diseases of maize in your maize field?

q) What method(s) of disease control do you employ?

| Disease | Method | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| | | | | |
| | | | | |
| | | | | |

If chemical:

| Chemical | Source | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| | | | | |
| | | | | |
| | | | | |

Types of ear rots (show pictures of ear rots)

i) *Fusarium* ear rots

| | Low | Medium | Severe |
|---|---|---|---|
| Incidence | | | |
| Severity | | | |

Cause ----------

Management ----------

---

ii) *Giberella* ear rots

| | Low | Medium | Severe |
|---|---|---|---|
| Incidence | | | |
| Severity | | | |

Cause ----------

Management ----------

---

iii) *Diplodia* ear rots

| | Low | Medium | Severe |
|---|---|---|---|
| Incidence | | | |
| Severity | | | |

Cause ----------

Management ----------

---

iv) *Aspergillus* ear rots

| | Low | Medium | Severe |
|---|---|---|---|
| Incidence | | | |
| Severity | | | |

Cause ----------

Management ----------

---

v) Others

| | Low | Medium | Severe |
|---|---|---|---|
| Incidence | | | |
| Severity | | | |

Cause ----------

Management ----------

---

r) What method(s) do you use to control weeds?

| Weed | Method | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| | | | | |
| | | | | |
| | | | | |

s) What method of harvesting do you employ? [1] dehusking in the field, dry and thresh later [2] cutting stalks with ears, stood for further sun drying and dehusk and thresh later [3] mechanical [4] others (specify) ----------

---

t) Do you sort maize before threshing? (*Yes*) (*No*) If yes, why? ----------

---

u) What do you use the unwanted/rotten, broken? grains for? ----------

---

---

v) How do you handle the maize stalks after harvest? [1] harvest for hay [2] direct grazing of cattle [3] left to rot in the field and plough in during the next season [4] burning [5] as firewood [6] others (specify) ----------

w) Yield per harvest (bags/plot area)? ----------

x) What method(s) do you use to store maize after harvest?

| Storage method | Reason(s) | Effectiveness | | |
|---|---|---|---|---|
| | | None | Moderate | Effective |
| As unshelled ears in traditional granaries | | | | |
| Shelled grains in sacks in traditional granaries | | | | |
| Hanging ears | | | | |
| Sacks of maize grains stored in the house (raised) | | | | |
| Sacks of maize grains stored in the house (not raised) | | | | |
| Others (specify) | | | | |

y) Have you heard of push-pull technology? (*Yes*) (*No*) If yes, what do you think about it?

| Constraint | Effectiveness | | |
|---|---|---|---|
| | None | Moderately | Very |
| Stemborer | | | |
| Striga | | | |
| Soil health | | | |
| Crop yield | | | |
| Fodder | | | |

z) How do you compare ear rots in push-pull and non-push-pull plots? (for push-pull farmer)?

---

---

aa) Have you heard about aflatoxin? (*Yes*) (*No*) Fumonisin? (*Yes*) (*No*)

bb) What practices do you employ to manage them? (if any) ----------

---

cc) Have you had any training on aflatoxin? (*Yes*) (*No*) If yes, by who and when? ----------

---

---

dd) Do you have an off-farm employment? (*Yes*) (*No*) If yes, how many hours per week? -

---

**Other observations** ----------

---

---

---

---

**Vote of Thanks**

**Thank the farmer and explain what mycotoxins are**

**Appendix 2: Weather data for the three counties of western Kenya**

| Month | Maseno | | | | Ugunja | | | | Rongo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Avg. Temperature (°C) | Min. Temperature (°C) | Max. Temperature (°C) | Precipitation / Rainfall (mm) | Avg. Temperature (°C) | Min. Temperature (°C) | Max. Temperature (°C) | Precipitation / Rainfall (mm) | Avg. Temperature (°C) | Min. Temperature (°C) | Max. Temperature (°C) | Precipitation / Rainfall (mm) |
| Jan | 21.3 | 13.2 | 29.4 | 70 | 22.6 | 15.2 | 30.0 | 54 | 21.2 | 13.3 | 29.2 | 56 |
| Feb | 21.4 | 13.4 | 29.5 | 103 | 22.8 | 15.6 | 30.1 | 89 | 21.5 | 13.6 | 29.5 | 90 |
| Mar | 21.3 | 13.6 | 29.1 | 165 | 22.8 | 16.0 | 29.7 | 144 | 21.6 | 13.9 | 29.3 | 151 |
| Apr | 20.9 | 13.8 | 28.0 | 278 | 22.3 | 16.2 | 28.5 | 265 | 21.1 | 14.1 | 28.1 | 240 |
| May | 20.4 | 13.5 | 27.3 | 237 | 21.9 | 15.9 | 27.9 | 248 | 20.3 | 13.5 | 27.2 | 207 |
| June | 19.7 | 12.8 | 26.7 | 133 | 21.3 | 15.0 | 27.6 | 119 | 19.9 | 12.9 | 26.9 | 117 |
| July | 19.6 | 12.3 | 26.9 | 101 | 21.0 | 14.7 | 27.4 | 96 | 19.5 | 12.3 | 26.8 | 76 |
| Aug | 19.7 | 12.4 | 27.0 | 154 | 21.2 | 14.8 | 27.6 | 138 | 19.7 | 12.3 | 27.1 | 110 |
| Sep | 20.1 | 12.6 | 27.7 | 150 | 21.5 | 14.9 | 28.2 | 142 | 20.0 | 12.2 | 27.9 | 128 |
| Oct | 20.9 | 13.3 | 28.6 | 146 | 22.1 | 15.4 | 28.9 | 141 | 20.7 | 12.9 | 28.5 | 142 |
| Nov | 20.9 | 13.4 | 28.4 | 154 | 22.1 | 15.5 | 28.7 | 148 | 20.7 | 13.3 | 28.1 | 164 |
| Dec | 20.7 | 13.1 | 28.4 | 129 | 22.1 | 15.3 | 29.0 | 106 | 20.6 | 13.2 | 28.1 | 113 |

Source: https://en.climate-data.org/africa/kenya/migori/rongo-643444/
https://en.climate-data.org/africa/kenya/vihiga/maseno-103782/
https://en.climate-data.org/africa/kenya/siaya/ugunja-718029/