PHYLOGENETIC DIVERSITY AND TOXIGENIC POTENTIAL OF *FUSARIUM* SPECIES IN WHEAT AND LEVELS OF DEOXYNIVALENOL AND FUMONISINS IN MARKET WHEAT PRODUCTS, KENYA

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DECLARATION

The content of this thesis is my original Ph.D. work undertaken at the Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi and, at the University of Nairobi, Chiromo Campus. I have accordingly acknowledged all articles and text cited herein. Additionally, the work described in this thesis has not been presented in any institution of higher learning for a degree award or any other qualification.

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I do hereby dedicate this thesis to both my nuclear and extended family members for their immeasurable encouragement, all forms of support and the love they accorded me as I undertook my studies.

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ABSTRACT

In Kenya, wheat is the second most consumed cereal grain after maize and provides nutrition for about 50% of the world's population. However, production of the grain often faces setbacks occasioned by fungal infections and the related chemical contaminants. Unrelenting fungal disease control system, sufficient wheat seed system and observation of food safety measures to curb deficiency and alleviate ill health associated with consumption of mycotoxin contaminated wheat products is essential. This study assessed farmers' knowledge on fungal wheat diseases and, their practices in choices of wheat cultivars cropped in three ecological regions of Kenya. Prevalence and diversity of *Fusarium* species in the produce of farmer saved and certified seeds of wheat cultivars was also analyzed. In addition, the genetic ability of the isolated *Fusarium* spp. to produce mycotoxins was evaluated. Lastly, a survey to investigate occurances and levels of fumonisins and deoxynivalenol (DON) in selected market wheat products sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya was done. Two hundred and sixty wheat grain samples were collected from 123 farms in Narok, Uasin Gishu and Nakuru Counties between 2016 and 2017. Peptone Pentachloro-nitrobenze Agar was used for isolation of *Fusarium* spp. from the grains samples, while PDA, CLA and SNA media were used for cultural and morphological characterization. *Fusarium* species identity and diversity was determined using sequence analysis of the gene encoding translation elongation factor 1-alpha (tef1-alpha). The genetic ability of the *Fusarium* spp. to produce mycotoxins was determined using Tri13F/Tri13RDON and FUM1F/FUM1R primer pairs. ELISA Kit protocols were used for assessment of mycotoxin levels. ANOVA, the Tukey HSD test and microscopy were used for data analysis. Notably, barely 10% of wheat cultivars released into the Kenyan market were under cultivation in the targeted areas within the study period. In all the three regions, Njoro BWII wheat cultivar was the most preferred and the most frequently (48.8%) sampled wheat cultivar. Top on the list of agroeconomic factors that influenced the selection of wheat cultivars to plant were the weight of wheat grains at harvest and the resilience of wheat cultivars to wheat rust. Other than wheat rust, most farmers had limited knowledge about other fungal diseases while only 1.63% of them cited *Fusarium* head blight (FHB) as a problematic fungal disease in wheat production. Eight *Fusarium* spp. (*Fusarium poae, F. verticillioides, F. equiseti, F. heterosporum, F. tricinctum, Fusarium* sp. *F. oxysporum*, and *F. culmorum*) were identified. However, the species diversity in the study regions did not differ significantly. While certified commercial wheat seeds produced 33.75 percent of the recovered *Fusarium* spp., wheat grains from farmer-saved seeds produced 66.25%. Tri13DON gene was not detected in the isolated putative DON producers while FUM1 gene was detected in 60% of the isolated *Fusarium* species. Over 76% of the analyzed wheat grain samples had detectable levels of fumonisins. However, the highest level (9.6ppm) did not exceed the permissible levels of between 2000µg/kg and 4000µg/kg in whole grains according to EU guidelines. Over 75% of the sampled market wheat products contained levels of DON and fumonisins that were below the permissible maximum limits of 750µg/kg according to EU guidelines. Wheat flour contained the highest concentration of DON (5.6 µg/kg). The significant research finding is that a minimal percentage of wheat cultivars released into the market had been cultivated in the studied regions. Among other factors, farmers did not prioritize selection of cultivars based on their ecological growth requirements and they notably had minimal knowledge about other serious wheat fungal diseases such as FHB to the extend of referring to all diseases observed on the crop as wheat rust. *Fusarium* spp. with the ability to produce mycotoxins were prevalent in the wheat cultivars sampled in the three study areas.

This calls for intensified integrated control measures of the pathogens across the three regions. Finally, the quantities of DON and fumonisin found in the wheat products from the market samples were within acceptable limits. Hence, this result highlights the safety of the products for human consumption. However, frequent surveys are recommended to ascertain consistency in the levels of the toxins within the recommended measures. More extension services are also needed to educate wheat farmers on all-important qualities of wheat cultivars released in the market. Lastly, enhancement of awareness on the prevalence of other dangerous fungal diseases such as FHB, it's related health affecting mycotoxins and how farmers can participate in the control of such diseases.

Key Words: Pathogenic, *Fusarium* species, Wheat cultivars, Kenya, Translation elongation factor one alpha gene (tef1-alpha gene), Fumonisins, Deoxynivalenol, Wheat-Products.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
Bp	Base Pairs
PBS	Phosphate Buffer Saline
C	Centigrates
CAC	Codex Alimentarius Commission
CIMMYT	International Maize and Wheat Improvement Centre
CLA	Carnation Leaf Agar
CT	Culture
CTAB	Cetyltrimethyl Ammonium Bromide
DAP	Diammonium Phosphate
DON	Deoxynivalenol
DNA	Deoxyribonucleic Acid
Dntp	Deoxynucleotide Triphosphate
DON	Deoxynivalenol
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FB	Fumonisin B analogs
FDA	Food and Drug Administration
FHB	Fusarium Head Blight
FUM1	Fumonisin producing gene
FB1	Type B1 fumonisins
GPS	Geographical Positioning System
Ha	Hectares
IGS sequences	Intergenic Spacer Sequences
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KALRO	Kenya Agricultural and Livestock Research Organization
KARI	Kenya Agricultural Research Institute
KSC	Kenya Seed Company

LMT	Limit
LSD	Least Significant Difference
M	Millions
Min	Minutes
Mg	Milligrams
NaOCL	Sodium hypochlorite
NCPI	National Plant Breeding Station
NPBS	National Centre for Biotechnology Informatics
OEC	Observatory of Economic Complexity
OD	Optical Density
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PMTDI	Provisional Maximum Tolerable Daily Intake
PPA	Peptone Pentachloronitrobenzene Agar
ppb	Parts per billion
ppm	Parts Per Million
RNA	Ribonucleic acid
rRNA	ribosomal Ribonucleic acid
Sec	Seconds
SNA	Spezieller Nahrstoffarmer Agar
SPSS	Statistical Package for Statistical Sciences
TEF	Translation Elongation Factor
Tukey's HSD	Tukey Honest Significant Difference
TWA	Tap Water Agar
UoE	University of Eldoret
USDA	United States Department of Agriculture
UV	Ultra Violet rays
W:V	Weight per Volume
WFB	Wheat Flour Brand

WHO	World Health Organization
WFP	Wheat Flour Products
WG	Whole Grains
VAT	Value Added Tax
ZEN	Zearalenone

DEFINITION OF TERMS/LIST OF SYMBOLS AND NOMENCLATURE

α - alpha

°- Degree

©- Copy Right

$- Dollars

µ- Microgram

™- Trade Mark

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND INFORMATION

Wheat (*Triticum aestivum*), is among the major source of carbohydrates not only to man but also to some of the domesticated animals all over the world. Wheat is a grass that is cultivated for its grain, which is used globally to prepare various types of food such as muffins, nodules, Weetabix, crackers, chapatis, cakes biscuits and spaghetti among others. The genus *Triticum* is comprised of several species of wheat (Dvorak, 2013). However, wheat (*Triticum aestivum*), which is the most frequently cultivated, serves as the primary source of nutrition for about 40% of the world's population (Giraldo *et al*., 2019; Shewry & Hey, 2015). More than 200 million hectares of land are used for its cultivation globally and in terms of global trade, it exceeds all other crops put together (Giraldo *et al*., 2019; Shewry & Hey, 2015).

Kenya's population relies on the wheat crop as the second staple cereal crop after maize, in addition to being an important crop from an economic perspective even among the small-scale farmers (Kamwaga., *et al*., 2016). Kenya has the potential to be self-sufficient in wheat produce, if large-scale farmers embrace modern technology that makes their productivity at par with the international standards. It also has ideal climatic conditions and expertise to expand local production, which is mainly concentrated in the Rift Valley region. However, in the recent past years, the production of wheat has shifted from large and medium-sized commercial farms to production mostly by small-scale farmers as a result of the high costs of agricultural inputs and land subdivisions (Alessandro *et al*., 2015). From an ecological perspective, the Southern and upper Rift Valley Counties (Nakuru, Narok, and Uasin Gishu) as well as Meru, in the Eastern

portion of Kenya, are where the wheat crop is grown. However, one major challenge encountered by wheat farmers is *Fusarium* spp. infestation in the crop and contamination of the crop's produce with fungal metabolites. As a result, the quality of the harvest of the crop is downgraded and the yields lowered (Gong *et al*., 2015, Alisaac & Mahlein, 2023)). Mycotoxins released by some of the *Fusarium* spp. infecting the crop are harmful compounds linked to numerous illnesses in both humans and farm animals (Gong *et al*., 2015).

Control of some of the pathogenic fungi in the wheat crop in Kenya is based mostly on application of synthetic fungicides. The fungicides majorly target wheat rust (Beatrice *et al*., 2016; Wanyera & Kilonzo, 2010; Wanyera *et al*., 2016) and other foliar diseases. Unfortunately, the cost implications for synthetic fungicides does not only increase crop production costs but also limit the access and application of the fungicides by all wheat farmers. The later furthermore creates room for some farms to act as reservoirs for the targeted pathogens. Over-reliance on synthetic fungicides is not only eco-friendly but also induces production of mycotoxins like DON and, does not offer a sustainable solution to other persistent fungal diseases. Included in such categories of diseases are wheat head blights such as *Fusarium* Head Blight (FHB), a devastating disease of wheat all over the world caused by a complex of *Fusariun* species (Alisaac & Mahlein, 2023). Therefore, an all-inclusive approach in the control and management of such fungal pathogens is becoming central in the science of crop protection. Genetic modification is being employed in the development and use of wheat cultivars with good qualities such as resistance to fungal pathogens and other pests. The government of Kenya through Kenya Agricultural and Livestock Research Organization (KALRO) has taken the lead and devoted most of its research and resources on improving wheat production in Kenya (Kamwaga *et al*., 2016). The main mandate is to carry out focused research, promote and facilitate production of high yielding, better quality certified

seed and to enhance food self-sufficiency and quality living standards for sustainable economic development in Kenya and beyond. For more than 80 years, one hundred and eighty (180) wheat cultivars have been released by KALRO, Kenya Seed Company (KSC) and the University of Eldoret (UoE) to farmers for use in various wheat producing ecological zones (Kamwaga *et al.*, 2016; Macharia & Ngina, 2017; Njau *et al*., 2006). Programmes in Kenya aimed at breeding wheat have a global and regional scope. Breeding goals include creating wheat cultivars with traits like resistance to biotic and abiotic stressors (acid soils, drought, sprouting pre-harvest diseases like stem and yellow rust), and high and steady yields, among other attributes. The value of wheat crop's position in the food chain has also made it necessary to modify the wheat for diversified usage, which includes making bread and animal feed.

Mycotoxin accumulation in diverse crop produce depends on the natural occurrences of the etiological agents such as *Fusarium* spp. including the non-pathogenic species. Population dynamic studies on the diversity and prevalence of the diseases in sensitive crops are crucial for determining the best agro-economic solutions. Therefore, research into the *Fusarium* spp. and mycotoxins linked to FHB is essential for Kenya to produce wheat efficiently and sustainably. Research studies have shown that different *Fusarium* toxins have different distribution depending on both crop and geographical distribution and, even *Fusarium* species (Karlsson *et al*., 2022).

The aim of the study was to carry out a survey in three major wheat-producing Counties (Narok, Uasin Gishu and Nakuru) in Kenya on four areas. First, farmers' knowledge on fungal wheat diseases and, their practices in selection and choice of wheat cultivars. Secondly, determination of the prevalence and phylogenetic diversity of the *Fusarium* spp. isolated from the grains of the improved wheat cultivars sampled at harvest. Thirdly, to assess the mycotoxigenic potential of the fungal isolates based on occurrence of genes encoding the production of deoxynivalenol and

fumonisins in the isolated *Fusarium* spp. populations and occurance of the respective toxins in wheat grains at harvest. Finally, yet importantly, the study surveyed incidences and levels of deoxynivalenol and fumonisins in randomly selected market wheat based products in two of the studied Counties (Narok town and Nakuru city) and Nairobi, the capital city of Kenya where most of the wheat flour and wheat based processing industries are located.

1.2 STATEMENT OF THE PROBLEM

Various approaches have been employed worldwide in control of *Fusarium* spp. infection and mycotoxin contamination in wheat. Among such approaches are chemical and biological approaches while others are physical in nature. Yet none of them can completely eradicate the problem and hence there is desperate need for continuous systematic research (Prasad *et al*., 2016; Shah *et al*., 2018; Wan *et al*., 2020) that encompasses routine analysis of the mycotoxigenic ability of the predominant disease causative agents and the levels of their respective secondary metabolites contaminating wheat-based products.

Pathogenic *Fusarium* spp. affects crops of economic importance such as wheat (*T. aestivum*), causing great economic loses and raising concerns from agricultural, health and environmental point of view. Annually about 25% of harvested crops such as wheat crop are occasionally contaminated by harmful secondary metabolites such as mycotoxins, resulting into economic losses to agricultural and industrial commodities (Brites *et al*., 2018; Kamle *et al*., 2019; Marin *et al*., 2013). Use of fungicides increases wheat production cost. *Fusarium* head blight (FHB) causes wheat heads to become accumulated with detrimental secondary metabolites produced by pathogenic species. For example, mycotoxins released by some of pathogenic *Fusarium* spp. have been linked to numerous illnesses in both humans and farm animals (Arcella *et al*., 2017; Gong *et al*., 2015; Mielniczuk & Skwaryło-Bednarz, 2020; Sliwi, 2021). Some trichothecenes such as DON

are water soluble hence easily spread to kernels and spikes of cereals reducing germination, root and shoot growth in wheat (Perincherry *et al*., 2019).

Lack of wheat cultivars that are resistant to pathogenic fungi implies persistence of such related problems in wheat food chain value and, prolonged continuous use of fungicides whose long-term effects on both biotic and the abiotic environment are negative. Limited awareness of farmers about other fungal diseases in wheat and the recommended newly developed wheat cultivars suitable to certain abiotic factors defeats the objectives of meeting sufficient wheat production in Kenya (Gichangi *et al*., 2022). Kenya produces only 20% of the national wheat demand; therefore, it relies heavily on imported wheat (Monroy and Mulinge, 2013). Yet, the increased urban populations and their preference for wheat-based food products for both human and domesticated animals especially in the rural setups, means more demand for wheat and its products. Consequently, more importation of wheat and wheat-based products. There is also a general concern on the uncertainty of the safety of the commodity due to varied transportation and storage conditions of wheat grains along the supply chain. Hence, there could be possible exposure of many lives to mycotoxins through consumption of contaminated wheat-based food products.

Many reports on occurrence and prevalence of *Fusarium* spp. exist (Hafez *et al*., 2022). However, population composition and structure keep changing over time and the influential factors for this state are diverse and still largely unknown (Zhang *et al*., 2016). Additionally, unpredictable climatic conditions and cropping system have also been reported to have consequential effects on the population dynamics of fungi (Yang *et al.*, 2018). Genetic drift is also possible in populations of *Fusarium* spp. due to colonization of new environments or changes in the environment, exposure to antifungicides and, recombination during sexual reproduction (Silva *et al*., 2023). In Kenya, research has revealed low numbers in the population of *F. graminearum*, the main

causative agent of FHB and one of the major producers of DON (Muthomi *et al*., 2008, 2012; Wagacha *et al.*, 2010, 2016). Yet FHB incidences continue to be reported. Since the presence of the causative agents may not necessarily translate into a diseases condition, there is need for continuous research work to ascertain any arising phylogenetic diversity in *Fusarium* spp. and its implications in FHB control strategies.

1.3 JUSTIFICATION OF THE STUDY

A number of difficulties hampers a sustainable food system. Among them are finding the ideal balance between food production and nutritional security, preserving the environment and responding to climate change. Wheat production capacity in Kenya has been far below the increasing demand driven by the ever-increasing human population, urbanization, a shift to consumption of wheat products and the use of wheat in production of fodder. It is therefore necessary to determine whether farmers have embraced cultivation of wheat cultivars improved by KALRO, KSC and UoE to boost wheat production in the country, hence reduce the gap between wheat production level and the increasing demand. It is important to assess farmers' knowledge on types of wheat fungal diseases and, their practices in selection and choice of wheat cultivars planted. Accurate and timely information on the most cultivated wheat cultivars, commonly observed fungal diseases in wheat fields and, the knowledge level of wheat farmers on such diseases is vital in spearheading improvement and innovation research in the existing fungal control measures in the wheat crop. Furthermore, the constantly shifting climate necessitates ongoing research to determine the species identity, diversity, and occurrence of the pathogenic *Fusarium* spp. in Kenya's main wheat-producing regions. Background information on the overall frequency of genes involved in the mycotoxin biosynthetic pathway in the pathogenic *Fusarium*

spp. complex and the quantities of associated toxins is crucial for enhancing or strengthening disease-resistant wheat cultivars.

Fungal infection in wheat crop is more often associated with production and accumulation of dangerous secondary metabolites in the wheat produce. Further research findings on the prevalence of *Fusarium* species diversity in improved wheat cultivars in Kenya will raise awareness of the improved wheat cultivars' susceptibility to the disease-causing agents, offer insights on potential areas for improvement, and pave the way for the creation of more disease-resistant wheat cultivars. More frequently than not, a fungal infection in the wheat crop is accompanied by the generation and buildup of harmful secondary metabolites in the crop's tissues. To be able to assess the severity of the issue and the safety of wheat-based food products, it is crucial to identify the frequency of mycotoxin-encoding genes in the *Fusarium* spp. populations that are common in the wheat cultivars improved for various uses and for cultivation in certain ecological regions. Assessment of the mycotoxin occurrences, diversity and determination of the levels in the wheat grains at harvest provides important precautionary health information that emphasizes the need to improve the current pre- and post-harvest pathogen control techniques. To determine the safety of common wheat-based food products in the Kenyan market, the levels of deoxynivalenol and fumonisin were assessed in market wheat products sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya. As a capital city, Nairobi hosts diverse human communities and many factories for production of both wheat flour and wheat based products. Hence, has a wider variety of both locally manufactured and imported wheat products that are distributed to other parts of the country.

1.4 GENERAL OBJECTIVE

To determine the prevalence, phylogenetic diversity and mycotoxigenic potential of *Fusarium* pp. prevalent in seeds of wheat cultivars, factors determining farmers' choice of a cultivar and, levels of deoxynivalenol and fumonisins in market wheat products in Kenya.

1.4.1 Specific objectives

i. To investigate cultivars of wheat planted, occurrence of fungal infections and factors influencing farmers' wheat seed choices in Narok, Uasin Gishu, and Nakuru Counties, Kenya.

ii. To determine prevalence and diversity of *Fusarium* spp. in cultivars of wheat seeds cropped in three major wheat-producing Counties in the Kenyan Rift Valley.

iii. To verify mycotoxigenic potential of *Fusarium* spp. isolated from freshly harvested grains of the sampled wheat cultivars.

iv. To analyse levels of deoxynivalenol and fumonisins in market wheat products sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya.

1.5 RESEARCH QUESTIONS

i. What are the cultivars of the categories of wheat seeds planted factors that determine farmers' choice of wheat cultivar and field wheat fungal diseases in Narok, Uasin Gishu and Nakuru Counties?

ii. What differences exist in prevalence and diversity of *Fusarium* spp. infecting wheat cultivars planted in three major wheat-producing Counties in the Kenyan Rift Valley?

iii. What proportion of *Fusarium* spp. prevalent in grains of improved wheat cultivars have the cluster gene encoding production of deoxynivalenol (DON) and fumonisins?

iv. What are the levels of DON and fumonisins in market wheat products in Narok town, Nakuru and Nairobi, the capital city of Kenya?

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN, HISTORY AND USES OF WHEAT

The origin and cultivation of wheat is traced back to almost 10,000 years ago, in South East Turkey (Carver, 2009; White, 2010). Research findings have shown that cereals such as wheat (*Triticum* spp.), rice (Oryza sativa) and maize (*Zea mays*) are the main crops relied upon by all humans and are among the top resources that have contributed to the emergence of human civilization (Awika, 2011; Zohary *et al*., 2012). Cereals like maize and wheat have been documented for their possible continuity to serve a critical role in ensuring adequate and affordable intake of proteins and calories in diets in Asia and Africa (Braimoh *et al*., 2021) and other human population (Laskowski *et al*., 2019). The wheat kernel is a form of indehiscent dry fruit according to botanical classification. Genetically, the plant is a diploid organism with two sets of chromosomes. It falls in the genus *Triticum* and, numerous wheat species do exist. Wheat makes up to approximately between 29% and 30% of the world's total cereals' production (Carver, 2009). Quantitatively, over two thirds of wheat produce all over the world is used for food, providing 19% of the proteins and 20% of the calories consumed while a fifth is consumed by livestock (Braimoh *et al*., 2021).

Wheat is more widely and mostly cultivated for its seed, the cereal grain. In some countries such as Kenya, after grain harvest the straws are gathered and stored for use as fodder. Alternatively,

domesticated animals like cows among others feed on wheat remains on farms following harvest. Based on uses, wheat is categorized into two types: bread wheat and durum wheat. Durum wheat, for instance, is used to make pasta, whereas bread wheat has additional properties that are tailored to the tastes of leavened flat breads (Braimoh *et al*., 2021). Dry wheat grains can be milled to create

several brands of wheat flour, which is mostly used in crackers, breads, muffins, noodles, pastas (spaghetti), biscuits, cakes, cookies, and pastries.

Both Asia and Africa experience more consumption of wheat than production, with the two continents leading the world in net imports of the commodity (Braimoh *et al*., 2021). The place of staple food crops such as wheat in feeding the growing global population should be given the significance required for food sufficiency and safety. Improved food security depends on sustainability and management of resources including natural staple food crops. For many years, farmers selected from their fields cultivars of wheat crops that showed favorable phenotypes such as easy to harvest, high yield among others and, consequently new wheat types started to dominate (Mefleh *et al*., 2019; Thapa *et al*., 2009). Such natural genetic development has been reported to be highly successful. However, it takes many years hence Biotechnology is now exploring the various possible ways that genetic management can be achieved faster and more efficiently with high number of targeted genetic engineering resulting into diversity in wheat genotypes (Mefleh *et al*., 2019; Thapa *et al*., 2009). This has essentially led to changes over time in genetics, agronomy, end-product quality technologies in addition to changes in wheat cultivars from ancient to old, and finally modern durum wheat (Mefleh *et al*., 2019). Original parental wheat lines and advanced lines developed by breeders are usually evaluated for different quality aspects to identify those with the desired requirement for milling, processing the end use and nutritional values in different target regions (Guzmán *et al*., 2019). The other targeted areas of concern include effective control and management of phytopathogens such as resistance to pathogenic fungi.

2.2 FUSARIUM SPP. INFESTATION IN WHEAT

Fusarium is a cosmopolitan genus of fungi. It is of primary interest because numerous species cause plant diseases (Nelson *et al*., 1994). The genus has a global distribution, containing not less

than three hundred phylogenetically different species complex (O'Donnell *et al*., 2015). In this genus are some of the most economically destructive plant pathogens in the world, capable of infecting majority of plants of great economic importance and resulting in global annual damages worth billions of dollars in agriculture (Aoki *et al*., 2014). In addition, certain species in the genus due to both genetic and environmental factors, have the ability to produce toxic secondary metabolites such as zearalenones, fumonisins and trichothecenes hence, posing serious threats to food safety and the health of both man and domesticated animals fed on wheat food products (Arcella *et al*., 2017; Mielniczuk & Skwaryło-Bednarz, 2020; Sliwi, 2021; WHO, 2023). For example, in cereals such as wheat, pathogenic *Fusarium* spp. has been shown not only to annually cause high economic loss in small grain and maize but also produce and consequently cause accumulation of mycotoxins especially trichothecenes and fumonisins (Bottalico, 1998; Ferrigo *et al*., 2016). Additionally, it has been demonstrated that mycotoxins such zearalenones and fumonisins have an immediate impact on the microbiota and fermentation in ruminants as well as on the chewing behavior and overall health of dairy cows fed on moderate grains (Hartinger *et al*., 2022; Zain, 2011).

Major fungal diseases in cereals can be classified into four groups: ear and kernel rots, leaf blights and stalk rots (Mielniczuk & Skwaryło-Bednarz, 2020). The most important ear rot is *Fusarium* ear rot (Mielniczuk & Skwaryło-Bednarz, 2020). According to research results, *F. graminearum* and *F. culmorum* are mostly responsible for red ear rot, while *F. verticilloide*s, *F. proliferatum*, and *F. subglutinans* are responsible for pink ear rot (Czembor *et al*., 2019; Dweba *et al*., 2017). Unfavourable climatic circumstances and agronomy-related factors are to blame for *Fusarium* spp. infection and persistence in wheat crops globally (Bernhoft *et al*., 2012; Dweba *et al*., 2017; Mielniczuk & Skwaryo-Bednarz, 2020; Wagacha *et al*., 2010; Wenda-Piesik *et al*., 2017). Usually,

one of the key factors affecting the dispersion of fungal species is the climate and the host crop (Qu *et al*., 2008). For instance, in Asia, temperature has commonly been cited as the reason why *F. graminearum* predominates in the north while *F. asiaticum* does so in the South (Qu *et al*., 2008). In a different study, it was found that there was a high correlation between crops and pathogenic species, with *F. asiaticum* dominating in rice-wheat rotation areas and *F. graminearum* associated with maize-wheat rotations (Zhang *et al*., 2012, 2016). Thus, other factors that have been found to affect species diversity and spread include host choice and cropping system (Yang *et al*., 2018; Zhang *et al*., 2012). It has also been noted that maize and ear rot have a strong connection with *Fusarium* booth. However, it has only seldom been discovered in wheat in tiny populations in Kenya and Ethiopia (O'Donnell *et al*., 2008; Wagacha *et al*., 2010) among other places in the world.

Fusarium Head Blight and *Fusarium* foot rot are important and cause severe yield loss that can reach 50% damage for small grain, a phenomenon that hinders secondary grain storage (Mielniczuk & Skwaryło-Bednarz, 2020; Stanciu *et al*., 2015). *Fusarium* spp. can be identified from newly harvested cereals and some of them dominate in causing diseases in susceptible crops depending on the type of the host crop and the agro climatic conditions (Ferrigo *et al*., 2016). Although many reports on occurrence and prevalence of *Fusarium* spp. exist (Lee *et al*., 2015), population composition and structure keep changing over time and the influential factors for this state are still largely unknown (Zhang *et al*., 2016).

Fusarium spp. are among the main causative agents of economically significant diseases in cereals such as wheat in most regions of Kenya (Kamwaga., 2016 *et al*; Njau *et al*., 2006). Despite the fact that fungal disease control approaches such as proper land preparation, allowing disease infected plant debris to decompose and the production of wheat cultivars suited for specific

ecological regions of certified wheat seeds, many wheat fields still experience disease infestations. There are also fungicides available to control FHB in tiny grains to a certain level. However, when vulnerable wheat cultivars are cultivated and the environment is conducive to FHB epidemic, there may not be enough control over the disease. Prediction of the diversity and the most prevalent *Fusarium* spp. in wheat cultivars released into the market and subsequently those preferred by majority of farmers would help forecast resulting mycotoxigenic contamination and the possible related wheat food-safety risks for enhancement of the existing disease control strategies.

2.3 *FUSARIUM* SPP. AND MYCOTOXIN PRODUCTION IN CEREALS

Annually 25% of harvested crops are contaminated by mycotoxins, causing huge economic losses to agricultural and industrial commodities (Eskola *et al*., 2020). These mycotoxins are stable in nature and are rarely eliminated during food processing, cooking, baking, roasting and pasteurization (Alshannaq & Yu, 2017; Bullerman & Bianchini, 2007; WHO, 2023). Although several hundred different mycotoxins have been identified, aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone, and nivalenol/deoxynivalenol are the most frequently detected mycotoxins that pose a risk to livestock and human health (WHO, 2023). The meagre agricultural, as well as a post-harvest practices like inappropriate drying techniques, food handling procedures, packaging materials and methods, storage and transport conditions are additionally responsible for the increased risk of fungal growth and mycotoxin contamination (Marin *et al*., 2013). Fungal species can adopt to a wide range of habitats including the tropical and temperate areas. Their toxins are often overproduced because of external stress (Marin *et al*., 2013; Perincherry *et al*., 2019). Environmental factors such as pH, temperature, water activity, fungicides and secondary metabolites originating from plants (Daou *et al*., 2021; Reverberi *et al*., 2010) are other contributive factors that induce production of the toxins. Mycotoxins such as DON have been

reported to have some functions during plant infection. For example in a certain study, fungal mutants with disrupted TRI (Trichothecenes biosynthetic gene cluster) displayed remarkable reduction in infection of wheat due to their inability to produce deoxynivalenol (Proctor *et al*., 1995). Deoxynivalenol and Zearalenone (ZEN) may also be synthesized by certain producing *Fusarium* spp. to make competing microorganisms weak during saprophytic growth phase (Perincherry *et al*., 2019).

Mycotoxins produced by *Fusarium* spp. include trichothecenes, fumonisins and zearalenone (Bakker *et al*., 2018). *Fusarium* species such as *F. graminearum* and *F. culmorum* are devastating pathogens of small grain cereals like wheat and, are also the main synthesizers of type B trichothecenes reported to be wide spread all over the world in cereal growing areas (Pasquali *et al*., 2016). Trichothecenes have toxicity to both plant and animals (Ismaiel & Papenbrock, 2015; Perincherry *et al*., 2019) and their biosynthesis and variation is due to a TRI cluster of genes. Most important type I trichothecenes are T-2 toxin, Nivalenol (NIV), Fusaranon X and Verrucarin A, while most important type II trichothecenes include DON, crotocin and verracarol and, they are considered to be toxic to animals (Bhat *et al*., 2010; Egbuta *et al*., 2017). Some trichothecenes such as DON are water soluble hence easily spread to kernels and spikes of cereals reducing germination, root and shoot growth in wheat (Perincherry *et al*., 2019).

Fumonisins are another category of mycotoxins derived from polyketides produced by *F. verticillioides, F. subglutinan*s and *F. fugikuroi* among others. Biosynthesis pathway for fumonisins is regulated by fumonisins (FUM) encoding gene clusters (Proctor *et al*., 2003). Effects of this group of mycotoxins include equine leukoencephalomacesia, porcine pulmonary edema and human esophageal cancer (Lumsangkul *et al*., 2019). They are also phytotoxic (Abbas *et al*., 2005, 2013; Ismaiel & Papenbrock, 2015).

Zearalenone (ZEN) and F2 (fusarins) toxin are common in sorghum, rice, oats, barley and wheat (Rai *et al*., 2018). They are produced by *F. culmorum, F. fujikuroi, F. graminearum, F. oxysporum, F. poae, F. sporotrichoides* and *F. venaceam* (Pusztahelyi *et al*., 2015; Ropejko & Twarużek, 2021). Their effects include both estrogenic and phytotoxic activities (Rogowska *et al*., 2019; Ropejko & Twarużek, 2021). Fusarins are polyketide compounds occurring in different types (A, B, C and D) and some like fusarin C is reported to be mutagenic with a role in human oesophageal cancer (Ji *et al*., 2019; Munkvold, 2017; Savard & Miller, 1992; Yang *et al*., 2021). Moniliformins are produced by *F. avenaceum, F. proliferatum, F. subglutinans, F. oxysporum, F. chlamydosporum, F. authophillum* and are less toxic than T-2 toxins and reduces plant growth (Ismaiel & Papenbrock, 2015).

2.4 FUNGAL PATHOGENS IN WHEAT CROP IN KENYA AND PRODUCTION OF MYCOTOXINS

According to Cereal Growers Association (CGA) and by Kamwaga *et al* (2016) wheat is the second most important cereal crop in Kenya after maize. Large and medium-scale farmers produce the bulk of domestic wheat (Mwangi *et al*., 2021). However, fungal pathogens recovered from wheat kernels, spikelets, stems, and leaves provide evidence that the crop may be contaminated by a diverse range of mycotoxins (Wagacha *et al*., 2010). A survey conducted in three wheat-growing districts of Kenya during the 2008 cropping season to determine the incidence of *Fusarium* head blight (FHB) and T-2 toxin contamination in grain revealed high FHB incidences of up to 88% and further reported that *Fusarium* spp. was the most common fungal pathogens in stem heads and soil (Muthomi *et al*., 2012). In Nakuru County (Kenya), diverse fungal pathogens (*Fusarium, Alternaria, Penicillium, Aspergillus* and *Epicoccum*) were isolated from different parts of the wheat at either anthensis or harvest and contained varying spectrum and concentration of

mycotoxins (Muthomi *et al*., 2012; Wagacha *et al*., 2016). In a study conducted in Nakuru County seeking to unravel occurrence of FHB associated mycotoxins, level of susceptibility of wheat germplasm to FHB and DON contamination in four different agro-ecological zones positively correlated to FHB incidence and severity (Otieno & Njeru, 2014). Analysis of wheat samples from different farms in this study revealed incidences of contamination with different amounts of T-2 toxin. Another study that sort to understand occurrence of FHB of wheat and associated mycotoxins in Nakuru and Narok County in 5 different agro-ecological zones showed that a complex of fungal pathogens can infect wheat and hence result in production of different concentrations and types of mycotoxins (Wagacha *et al*., 2016). The findings revealed that the fungal pathogens majorly associated with wheat ears and kernels in Narok region included *Alternaria* spp., *Chaetomium* spp, and *Epicoccum* spp. while *Fusarium* spp. *Alternaria* spp. and *Epicoccum* spp. were the major fungal pathogens isolated from similar samples from Nakuru region (Muthomi *et al*., 2012; Wagacha *et al*., 2016). The entire wheat kernels sampled from Narok County were contaminated with T-2 toxin while 5.9% were contaminated with DON (Muthomi *et al*., 2008, 2012; Wagacha *et al*., 2010, 2016). The levels of DON in wheat kernels from Nakuru County varied from below the limit of detection-to-detection concentration (Wagacha *et al*., 2016). However, there is little data if any about incidences and levels of fomonisins in the improved wheat cultivars.

2.5 HEALTH AND ECONOMIC IMPORTANCE OF FUMONISINS AND DEOXYNIVALENOL

Deoxynivalenol (DON) is a mycotoxin that is mostly found in cereal grains and is mostly produced by *Fusarium* fungi. Research findings by scientists have indicated that DON can directly damage DNA and hence might be classified as a genotoxic causing elements with the ability to induce

apoptotic cell death (Kamle *et al*., 2021). In man DON has been associated with gastroenteritis (Mishra *et al*., 2019; Xu *et al*., 2022). However, its long term effects are not yet well unknown (Pestka, 2010; Warth *et al*., 2012). DON also directly suppresses protein synthesis, but it also has an indirect effect on DNA and RNA synthesis, inflammatory stress responses, and neurological functioning (Mishra *et al*., 2019; Pestka, 2010). In another study, it was again shown to inhibit protein synthesis, impair nutrient intake, affect hematopoiesis, induce neuroendocrine effect, and affect growth, reproduction, and immune function after chronic exposure (Payros *et al*., 2016). In horses, most farmed fish species, ruminants, chickens, rabbits, dogs and cats adverse effects are not anticipated based on projected mean dietary amounts. However, there is a chance that elevated amounts of food could result in immediate negative effects in cats and farmed mink, as well as persistent negative effects in fish and pigs (Mishra *et al*., 2019; Xu *et al*., 2022). In plants some trichothecenes such as DON are water soluble hence easily spread to kernels and spikes of cereals reducing germination, root and shoot growth in wheat (Perincherry *et al*., 2019). Hence, it's possible that the mycotoxins produced during plant infection cause or perhaps contribute to plant illnesses and DON is considered to be extremely phytotoxic and causes growth retardation, suppression of seedling, and regeneration of green plants (Bakker *et al*., 2018; Blandino *et al*., 2017).

Another group of toxigenic chemicals synthesized by other members of the genus *Fusarium* (*F. proliferatum, F. oxysporum* and *F. verticillioides*) is fumonisins. These toxic chemicals have been reported to be the causative agents of different animal diseases in rats, pigs and man. For example, in human beings, they have been linked to esophageal cancer (Bucci & Howard, 1996; WHO, 2000). Effects of this group of mycotoxins include equine leukoencephalomacesia, porcine pulmonary edema and human esophageal cancer (Lumsangkul *et al*., 2019). They are also

phytotoxic (Abbas *et al*., 2005, 2013; Ismaiel & Papenbrock, 2015). Moniliformins are produced by *F. avenaceum*, *F. proliferatum*, *F. subglutinans*, *F. oxysporum*, *F. chlamydosporum*, *F. authophillum* and reduces plant growth (Ismaiel & Papenbrock, 2015).

Frequently, the most significant worry about fungal infection in wheat and the related mycotoxins are the financial losses brought about by quality degradation such as decreased grain test and weight. When DON concentrations are higher than 1ppm or 2ppm, there may be price reductions, rejection, or additional expenses such as those used in cleaning supplies (Ensley & Radke, 2019). Many wheat classes contain a variety that is moderately resistant to FHB. Hence, the most crucial strategy for lowering losses from FHB and the related mycotoxins such as DON and fumonsins is to plant wheat cultivars that are moderately resistant to the infections (Mielniczuk & Skwaryło-Bednarz, 2020).

2.6 WHEAT CULTIVARS DEVELOPED IN KENYA AND THEIR QUALITIES

Wheat production and maintenance of quality seed with focus on genetic quality, physiological adaptations, physical quality and health quality has been of major concern in wheat production in Kenya. The government of Kenya through KALRO and other institutions (Kenya Seed Company-KSC and the University of Eldoret (UoE) have devoted time and research on improving wheat production in Kenya (Kamwaga *et al*., 2016; Macharia & Ngina, 2017; Njau *et al*., 2006). Some of the efforts put in place include development of new wheat cultivars in the fight against fungal diseases such as wheat rust and for improvement of nutritional values. Production and maintenance of quality wheat, wheat crop management (weed control, soil sampling, land preparation, fertilizer and fertilization), management of fungal diseases in wheat (symptoms and control measures), post-harvest wheat management, wheat processing and utilization and, social economic consideration in wheat production are other roles. Wheat cultivars developed include cultivars of wheat suited

for bread production, and wheat cultivars for various stresses and quality among other traits (Table 2.1) and high yield production at specific altitudes (Table 2.2). For over eighty years, one hundred and eighty (180) wheat cultivars have been released into the market for farmers to cultivate while the goal of wheat breeding programs is vast covering both regional and global frontiers. The objectives for wheat breeding include high and stable yields, tolerance to biotic and abiotic stresses such as drought and acidic soils, pre-harvest diseases such as stem and yellow rust. The government has also looked into the role of social economic factors such as cost, welfare gain, implication for the market and industry and implication on food security in wheat production.

Table 2-1. Examples of improved wheat cultivars in Kenya and their qualities.

S/N	Quality trait	Wheat cultivar
1	High yield	Kenya W- 8.5ton/h Kenya Korongo- 8.5ton/h Robin- 8.1 ton/h Kenya Hawk and Njoro BWII - 8ton/h Kenya Tai, Kenya Sunbird, Eagle 10, Kingbird, Kwale and Duma- 6-7.5 ton/h
2	Abiotic stresses	Kenya Wren- acidic tolerant soils Njoro BWII-logging, pre-harvest sprouting
3	Maturity period & moisture	Robin- early maturity, adapted to many zones Duma- Drought resistant Eagle10- early maturity, short rains Kenya Kingbird
4	Disease resistance-Rust	Kenya Wren, Kenya Tai, Kenya Sunbird, Eagle10, Kenya Kingbird,
5	Good Baking Qualities	Duma, Kwale, K. Wren. Kingbird, Kenya Hawk12, Kenya Sunbird, Robin (good for industrial and home baking) and Kenya Korongo that has outstanding baking properties.

Note: h: Hectare; BW: Bwana; K: Kenya. Source: (Kamwaga *et al.*, 2016)

Table 2-2. Examples of improved wheat cultivars in Kenya, their qualities and suitable ecological areas for cultivation.

S/N	Variety	Altitude (m (m) Above sea level.	Yield per bag 90kg bag/acre)	Maturity period (days)	Special attributes
1	KS Mwamba	1800- 2400	22-25	125	High yielding. Tolerant to field stress conditions. Widely adapted to East African conditions (especially in Trans– Nzoia, Uasin Gishu, Laikipia, Narok and Mt. Kenya areas.
2	KS Farasi	1800- 2400	16- 30	119 (+/-5)	Tolerant to most foliar diseases Good Baking Quality. High Yielding Hard red wheat highly recommended for Mount Kenya, Samburu, Laikipia West, Narok, Subukia, Rongai, Nyandarua, Nakuru, Trans-Nzoia, Kericho, Bomet and Uasin Gishu areas.
3	KS Chui	1800- 2400	37-75	119 (+/-5)	Good Tolerance to foliar diseases Adapted to high potential and marginal environments. High tillering ability, high yields. Hard red wheat
4	KS Simba	1800- 2400	17- 32	116-120	Good baking qualities. Good tillering ability. Hard red wheat is highly recommended for Mount Kenya, Samburu, Laikipia West, Narok, Subukia, Rongai, Nyandarua, Nakuru, Trans-Nzoia, Kericho, Bomet and Uasin Gishu areas.
5	KS Ndume	1800- 2400	37- 75	100- 110	High yielding. Good tolerance to foliar diseases. Resistant to sprouting and lodging. High tillering ability. Good baking qualities.
6	KS Nyota	1800- 2400	30-75	120-130	High yielding. Newly released with moderate resistance to stem rust Ug99 Good tillering ability. Bred for sprouting tolerance. Makes a very stable dough.
7	KS Kanga	1800- 2400	53-89	12-130	High yielding. Product of KSC and CIMMYT collaboration. Newly released with moderate resistance to stem rust Ug99 Good tillering ability. Good baking and milling qualities. Tolerant to most foliar diseases. Newly released with adult plant resistance to stem rust Ug99 (slow rusting).

Source: Kenya Seed- KS, 6TH March, 2018 Release.

2.7 SOURCE MARKETS FOR KENYA'S WHEAT IMPORTS

Domestic wheat production in Kenya meets less than a third of the existing need, creating a deficit for importation. Yet, the need for wheat and wheat products in the country has increased over time as a result of a growing economy and rapid urbanization (Nzuma & Kirui, 2021). Due to seed recycling, illnesses like wheat rust (UG99), and short-term land leasing practices, Kenya's wheat yields have historically been low. This is because farmers are discouraged from investing in soil fertility because they do not gain long-term benefits for enhancing soil conditions (USDA, 2021). The considerable growth of the home baking businesses, which complement the commercial ones, further escalates the need. Consequently, Kenya has become a net importer of wheat, with an annual consumption of approximately 900,000 tons, against the local production of about 350 tons (USDA, 2021. Over time, the majority of Kenya's wheat imports come from various regions of the world (Townsend & Gitonga, 2019; USDA, 2021), some of which are included in Table 2.2. In 2020, Kenya imported 400M tons in wheat, becoming the 34th largest importer of wheat in the world while at the same time, wheat was the sixth most imported product in the country (Kenya-OEC, 2022; USDA, 2021). Currently Kenya imports wheat primarily from: Russia ($127M), Argentina ($117M), Germany ($43.8M), Poland ($22M), and Canada ($20M). In 2018, the average tariff for Kenya in wheat importation was 13.7 % (Kenya-OEC, 2020).

Table 2-3. Top wheat exporters to Kenya for the year ending June 2020.

Source	Unit	Year				
		2016	2017	2018	2019	2020
EU External Trade	TON	-	-	389,964	164,579	681, 702
Russia	TON	454,152	439,462	838,276	579,365	579,887
Argentina	TON	0	394,376	371,322	234, 484	458,679
Ukraine	TON	150,061	212,628	191,156	246,160	300,675
Canada	TON	90,999	160,257	140,330	172,932	110,246
Australia	TON	-	-	43,766	44, 467	46,550
United States	TON	-	-	83,546	139,093	-
Latvia		210,570	0	131,240	-	-

Note: Data obtained from [1](Gitonga, 2019); [2](USDA, 2021a); TON- Tonnes

2.8 TYPES AND SOURCES OF WHEAT PRODUCTS ON THE KENYAN MARKET

Various wheat products exist in the Kenyan market for human consumption. Key among them are wheat flour and wheat flour products. Wheat flour brands (WFB) in the Kenyan supermarkets and stores within the period the samples were collected included the following brands: WFB1 that comprised of two sub- categories such as wheat flour fortified with vitamins and minerals and, home baking flour, WFB2, WFB3 (packed for various uses including the following: Chapati fortified wheat flour, All-purpose fortified wheat flour, Self-raising fortified wheat flour, Unga mandazi flour and Atta Mark fortified with vitamins and minerals). Additional brands were WFB4, WFB5, WFB6, WFB7 (Atta Mark Red, Karibu All-purpose fortified baking flour and Atta Mark flour), WFB8, WFB9 and WFB10.

Wheat product sales in Kenya consists of leavened bread, pastries, cakes, and flat breads such as chapatis (Scott, 2022). In urban areas such as Nairobi leavened bread constitutes the largest share of formal retail sales, however, a sizeable informal market exists throughout Kenya where flat breads are popular and other common market wheat flour products such as spaghetti, indomie, Weetabix, biscuits, muffins, noodles, pasta, cookies, cereal bars and mandazi. Most of the wheat flour products are locally produced using either whole or refined wheat flour. However, some of them such as biscuits and spaghetti are imported.

CHAPTER THREE

A SURVEY ON CULTIVARS OF WHEAT SEEDS CULTIVATED, FACTORS INFLUENCING CHOICES AND FUNGAL DISEASES OBSERVED ON THE CROP IN NAROK, UASIN GISHU AND NAKURU COUNTIES, KENYA

3.1 INTRODUCTION

Wheat production and maintenance of quality seed with focus on genetic quality, physiological adaptations, physical and health quality has been of major concern in wheat research in Kenya (Kamwaga *et al*., 2016). Wheat seeds arise from adapted cultivars and known recommended sources. Such sources include plant breeders at agricultural research centers that produce seeds with high varietal purity and germinability. Wheat production also includes seed processing that is capital intensive and uses sophisticated equipment (Kamwaga *et al*., 2016). The government has also factored in the role of social economic factors such as expenses incurred by wheat farmers, implication on food security, implication for the market and industry and lastly profit margins and gains. Production of the crop in Kenya has not yet attained the threshold for the national requirement. This situation is attributed to variable challenges and constraints such as diseases, poor soils and water management practices faced by farmers and other players. It is worth noting that even gender has been cited to affect the choice of improved wheat cultivars by wheat farmers in Kenya (Gichangi *et al*., 2018).

The high population growth, increased urbanization and related changing trends in food consumption patterns are among other factors that have scaled up national demand for wheat and wheat products hence widening the deficit gap. Challenges encountered by certain stake holders such as farmers along the wheat value chain includes deficient seed systems, low level of

knowledge and or lack of information about the new and improved wheat cultivars (Gichangi *et al*., 2022; Macharia & Ngina, 2017). Consequently, these have negatively influenced sufficient wheat production. In a recent study, results obtained from model estimates showed that farmers' adoption of improved wheat cultivars was possible as a product of education accessibility, availability of content appertaining to wheat farming, off-farm wheat income, exposure to extension advice services, access to credits and distance to inputs and produce markets (Gichangi *et al*., 2022; Macharia & Ngina, 2017). Public and private sectors may enable acquisition of advisory services to farmers to promote the distribution and circulation and hence, use of certified wheat seeds by farmers through training, workshops, and seminars (Gichangi *et al*., 2022).

Some of the essential conditions vital for thriving of the wheat crop is a well-prepared fine planting bed to enhance uniform germination. In this respect, every farmer is encouraged to observe certain basic requirements for land preparation such as thorough tilling to get rid of weeds and, harrowing at least 4 weeks before planting. Additionally, fresh plant or any other form of fresh compost should not exist on the land during planting. Among other physical factors, soil analysis to determine the soil type and hence the amount of fertilizer to apply during planting is recommended. One of the commonly used fertilizers is Diamonium Phosphate (DAP). The amount required ranges between 200kgs to 250kgs per hectare and, depending on certain conditions such as the stage of the crop. In order to make up for the nitrogen that is so crucial for stem elongation, ear growth and tilling, fertilizer is added. Farmers are also encouraged to plant wheat cultivars that are suited to the ecological and climatic conditions prevailing in their geographical localities. For example, Kenya Wren wheat cultivar is suited for planting in acidic soils.

In this study, wheat farms in neighboring Counties within the Kenyan Rift Valley were investigated to see whether improved wheat cultivars had been adopted to increase the output of

the crop in the nation. Data on common fungal infections that farmers detected on the crop in wheat fields and factors influencing the selection of the wheat type to cultivate were also gathered. Consequently, analysis to assess the infection of the crop by *Fusarium* species was done using both symptomatic and asymptomatic mature dry wheat grains sampled at harvest.

3.2 METHODOLOGY

3.2.1 Materials and methods

3.2.1.1 *Study Area*

The study was carried out in Narok, Uasin Gishu and Nakuru, within the Kenyan Rift Valley (Figures 3.1, 3.2 and 3.3 respectively). They are among the major zones for commercial wheat production in Kenya. The locations of the respective zones and wheat grain sampling sites were mapped using the Geographical Positioning System (GPS) (Appendix II). Annually, rainfall received within the zones ranges approximately between 800mm and 2000mm. However, occasionally the amounts rise up to 2,500mm in higher altitudes. During the study period, the average rainfall in the three specified regions were within the following ranges: 200mm to 400mm; 600 to 800mm and 400mm to 600mm in Narok, Uasin Gishu and Nakuru respectively. Average temperature ranges were as follows: Narok - 18°C to 22°C, Uasin Gishu less than or equal to 18°C and, Nakuru- 18°C - 22°C during the study period. Other than wheat cultivation, large-scale farms in the three areas are also used for growing of other crops such as maize and barley. Subsistence farming of legumes, cultivar of vegetables and Irish potatoes is a common practice on small-scale farms. Farms in these areas are under continuous cultivation, barely staying fallow beyond one or two cultivation seasons. Nakuru County is noticeably unique in this study because this is where the National Plant Breeding Station (NPBS) is based and therefore research on smallholder wheat technologies is prevalent.

3.2.1.2 *Field Sampling of Wheat grains*

Between September 2016 and October 2017, the sampling was conducted as follows: Narok region: from July 28 to early September 2016, Uasin Gishu region: from November 8 to early December 2016, and Nakuru region: from February 2017 to October 2017. Majority of the sampling in Nakuru region was done in Njoro, which is the key wheat-producing region and home to the wheat research Centre. The timing of sampling days was coordinated to coincide with wheat harvesting. On randomly chosen wheat farms, cross-sectional and deliberate sampling was conducted. Utilizing the spear-sampling approach, laboratory and field samples were taken from large quantities of freshly harvested wheat grains (El-Shall & Moudgil, 2014; Freese *et al*., 2015). Based on farm sizes varied number of samples were collected from every farm. At least three samples were collected from every farm. However, from larger farms at most 21 samples were collected. For every cultivar of wheat, replicates of three samples were collected per sampling site on every farm. Approximately 500gm of grains for each wheat cultivar were packed in sterilized khaki paper bags. This was followed by labelling that included the details of farm location and other study information (name of wheat cultivar, source of wheat seed- Appendix I). Additional data gathered included names of fungal diseases by the farmer on the crop in wheat fields, factors taken into account by farmers when choosing the wheat cultivar to sow, and the utilization of certified and farmer-saved classes of wheat seeds. The co-ordinates (Appendix II), from the Global Positioning System (GPS) were used to generate maps for sampling locations.

Table 3-1. Number of collected wheat samples and the number of wheat farms sampled per County.

County	No. of sampled wheat farms.	Total No. of wheat samples
Narok (A)	50	89
Uasin Gishu (B)	39	101
Nakuru (C)	34	70
Totals	**123**	**260**

3.2.1.3 *Preparation of wheat grains for fungal isolation*

For each wheat cultivar sampled from the various farms, representative samples were derived from multiple samples following the procedures developed by El-Shall & Moudgil (2014) and Freese *et al*., (2015). This entailed manual mixing of multiple samples from various sites for every farm in a decontaminated plastic paper bag and under, sterile conditions to make a uniform composite representative sample. Each composite sample was then further sub- divided and given the labels "test sample" or "file sample." Each subdivision weighed about 300gm. In cases, where farms /sites had few and small sample sizes measuring about 300gm, such samples were used directly as test sample and the remaining amounts kept as file sample.

3.2.1.4 *Isolation and morphological characterization of Fusarium* species

Isolation and morphological characterization of fungi belonging to the genus *Fusarium* was achieved following the procedures described by Nelson *et al*., (1993) and Nirenberg, (1981). One hundred and thirty (130) kernels of each test sample for each wheat cultivar sampled in every farm were plated for isolation of the targeted fungi. The used grains were picked for plating randomly regardless of whether they exhibited symptoms of infection by molds or not. Before plating, the grains were sterilized by soaking in two percent (2%) Sodium hypochlorite (NaOCl) for a period of two (2min) minutes, followed by rinsing three consecutive times using sterile distilled water. Next, each set of the test wheat grains were dried in a laminar floor on a sterile muslin cloth. Thirty

(30) grains from each of the three replicates of each wheat cultivar were plated in three petri dishes, each containing ten (10) grains. For every wheat cultivar, at least ninety wheat grains were plated per sampling site. Fungal isolation was done using Peptone Pentachloronitrobenzene, a selective media suitable for culturing of *Fusarium* species. Optimal temperature for fungal growth was maintained at 25°C for a period of four to seven days before sub culturing the growing fungal colonies on tap water agar and, at the same temperature, to induce sporulation within seven to fourteen days. The resultant spores were subjected to microscopic examination to determine whether they had features characteristic of micro and macroconidia for *Fusarium* genus.

Purification of the obtained potential fungal isolates for *Fusarium* genus was achieved by subjecting the respective spores to a 10^{-5} fold serial dilution followed by culturing at 25°C on tap water agar for eighteen hours. Using hyphal tips, colonies emerging from single spores were sub cultured in triplicates. Using three different types of media [Synthetic Nutrient Agar (SNA), Potato Dextrose Agar (PDA), and Carnation Leaf Agar (CLA)] under two different temperatures (25°C and 30°C), with alternating 12-hour periods of darkness and 12-hour periods of fluorescent light, the sub-culturing was carried out to enhance distinctive growth for morphological and cultural characterization. Procedures developed by Burgess *et al*., (1994) and Leslie & Summerrell (2006) were adopted for characterization. The spores resulting from the purified *Fusarium* spp. isolates were then suspended in 15% glycerol and stored at negative eighty (-80°C) degrees Celsius for further research analysis described in chapters four and five.

3.2.1.5 ***Data analysis***

Descriptive statistical approaches were used to analyze types of the collected wheat samples, occurrence of fungal wheat diseases, and the presence of *Fusarium*-related fungal strains in the products of certified and farmer-saved wheat seeds. The first identification and analysis of

Fusarium spp. relied on microscopic and macroscopic morphological and cultural parameters using different growth conditions at 25°C and 30°C.

3.3 RESULTS

The maps (Figures: 3.1, 3.2 and 3.3) for the study sites were generated using GPS collected coordinates. This was done concurrently with the collection of wheat samples.

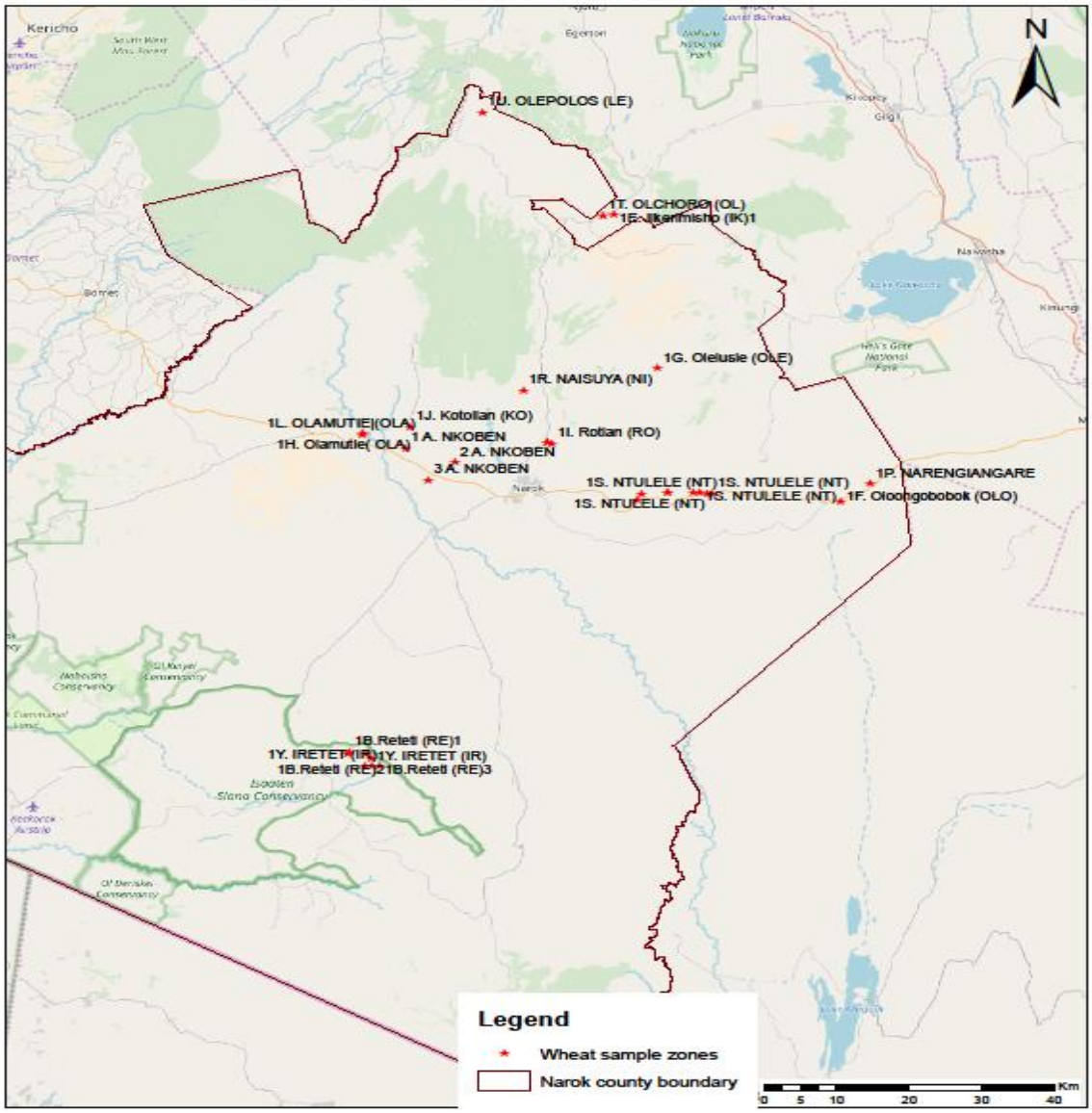


Figure 3-1. Map showing locations of wheat farms sampled in Narok County, Kenya. Maps generated using GPS coordinates picked during sampling.

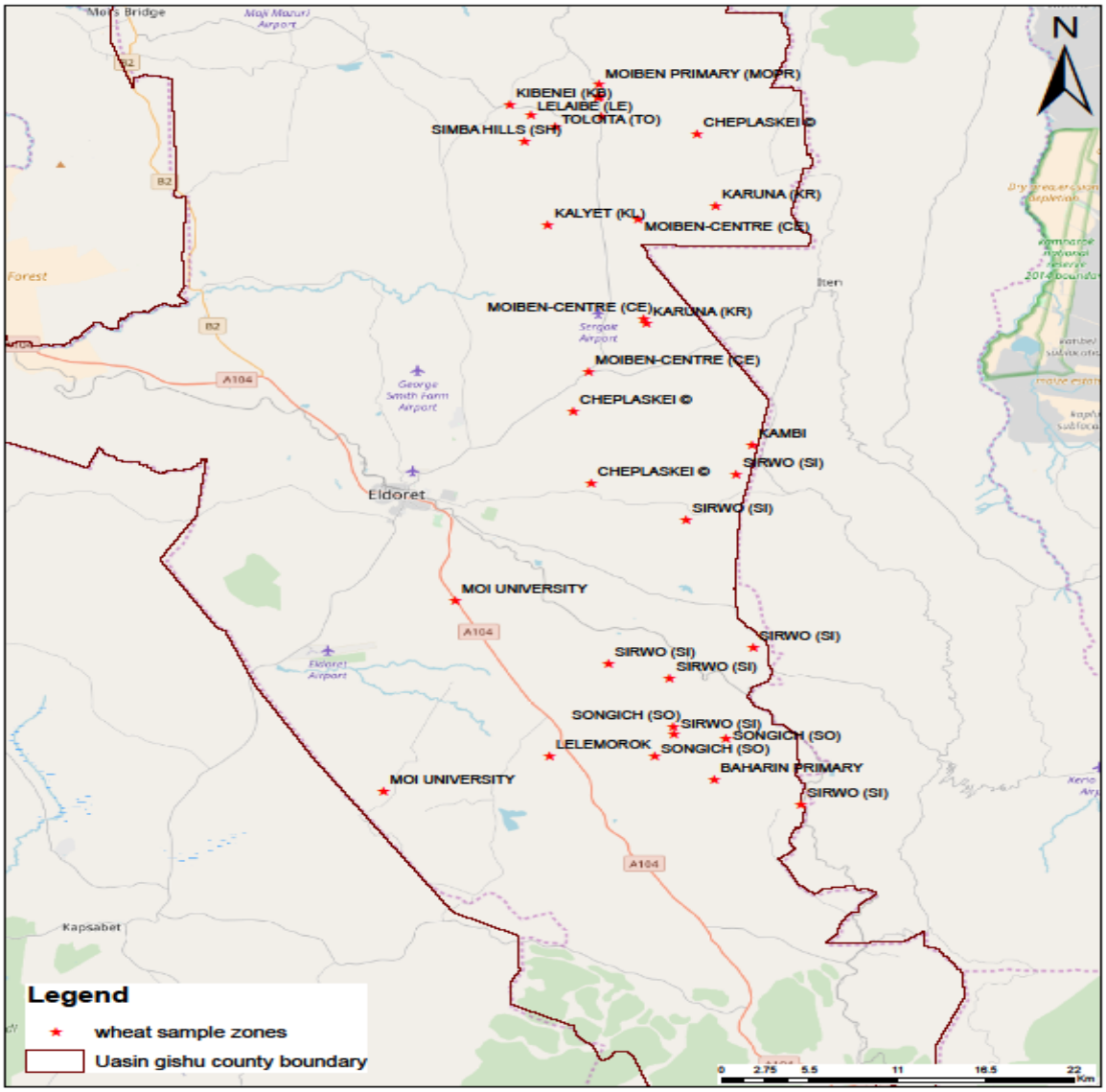


Figure 3-2. Map showing locations of wheat farms sampled in Uasin Gishu County, Kenya. Maps generated using GPS coordinates picked during sampling.

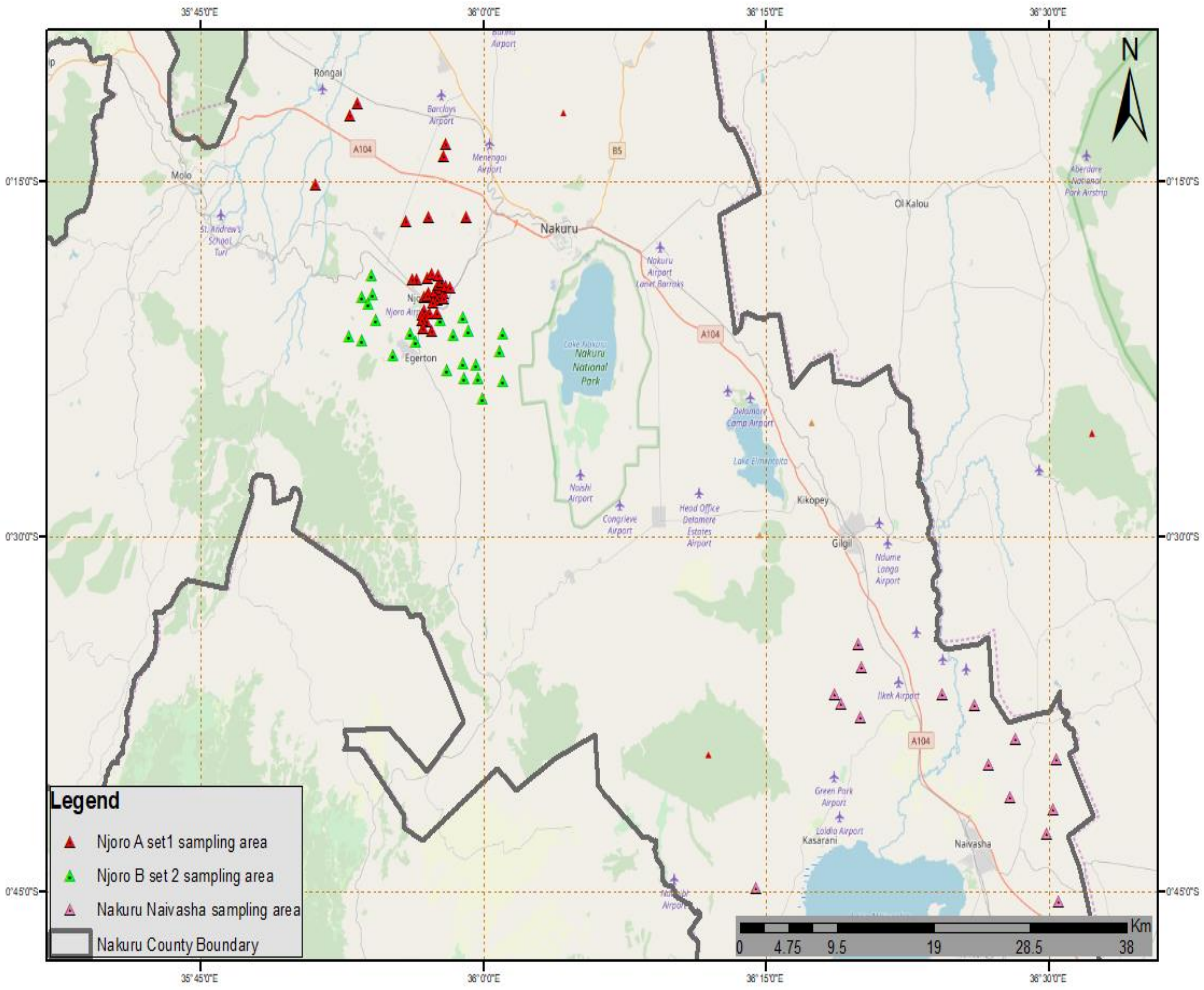


Figure 3-3. Map showing locations of wheat farms sampled in Nakuru County, Kenya. Maps generated using GPS coordinates picked during sampling.

3.3.1 Cultivated wheat cultivars, factors determining the choice of the cultivar and occurrence of fungal diseases in Narok, Uasin Gishu and Nakuru Counties

Results in this section included varieties of wheat cultivars grown, fungal diseases observed by farmers on the crop in wheat fields and factors that determine farmers' choice of wheat cultivars. Approximately two hundred and sixty samples of wheat grains were collected (Table 3.1). All the wheat grain samples were first classified into three categories: produce from certified commercial wheat seeds' produce from Farmer saved wheat seeds and produce from wheat seeds whose identity was not disclosed by farmers (Unknown state) (Figure 3.4). Wheat grains from farmer

saved wheat seeds constituted 31.2% of all the sampled grains while those from certified commercial seeds were 62.7%. However, since some farmers did not disclose the identity of the wheat cultivar they had planted, consequently another category (Undisclosed/Uknown state) of the sampled grains resulted. The samples with undisclosed identity made up 6.1% of all the sampled wheat grains.

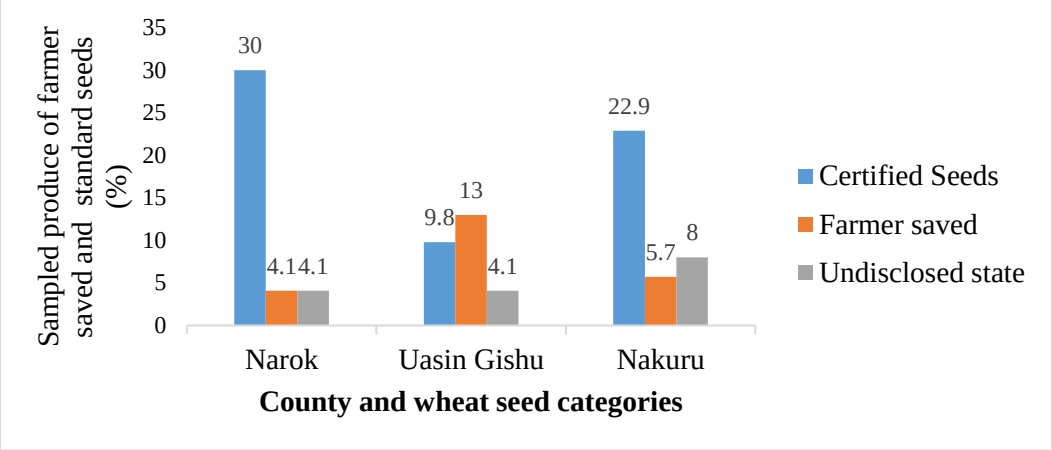


Figure 3-4. Graph showing the use of Certified and Farmer Saved wheat seeds in Narok, Uasin Gishu and Nakuru County between 2016 and 2018, Kenya.

Different wheat seed cultivars (Robin, Eagle 10, Ibis, Njoro BWII, Korongo, Farasi, Mwamba, K. Hawk, Ruiru, Simba, Ngamia, Duma, Kingbird, K. Tai, Yombi, K. Wren, Kwale and K. Sunbird) were sampled in one hundred and twenty-three (123) wheat fields (Table 3.2). The highest percentage (83.3%) of wheat cultivars were sampled in Nakuru County and they were as follows: Robin, Eagle 10, Ibis, Njoro BWII, Korongo, Mwamba, K. Hawk, Ngamia, Duma, Kingbird, K. Tai, Yombi, K. Wren, Kwale and K. Sunbird. Nakuru was followed by Narok County from which only nine wheat cultivars (Robin, Eagle 10, K. Ibis, Njoro BWII, Korongo, Farasi, Mwamba, K. Hawk and Ruiru) were sampled. The least number (four) of wheat cultivars (Robin, Eagle 10, Njoro BWII and Simba) was recorded in Uasin Gishu County. The type of wheat seed cultivars sampled in high frequency in the adjacent Counties of study were Njoro BWII - 48.8%, Robin 12.8, Eagle10

- 6.95% and 6.1% for samples whose identity was not disclosed. All the other remaining cultivars constituted 25.4% of the total wheat samples. The identity and percentage sampling frequency of each of the eighteen (18) types of wheat cultivars and, per County of study are shown in Table 3.2.

Table 3-2. Wheat cultivars sampled in the major wheat-producing Counties in the Kenyan Rift Valley region.

S/N	W. Cultivar	Sampling frequency per County (%)			% per W. cultivar
		Narok	Uasin Gishu	Nakuru	
1	Njoro BWII	11.5	31.5	5.8	48.8
2	Undisclosed	3.1	1.5	1.5	6.1
3	Eagle Ten	3.5	1.15	2.3	6.95
4	Robin	5.8	3.5	3.5	12.8
5	Mwamba	2.3	-	1.15	3.45
6	Ken. Korongo	2.3	-	1.15	3.45
7	Ruiru	2.3	-	-	2.3
8	Hawk	1.15	-	1.15	2.3
9	Ken. Ibis	1.15	-	1.15	2.3
10	Duma	-	-	1.15	1.15
11	Kwale	-	-	1.15	1.15
12	Fahari	1.15	-	-	1.15
13	King Bird	-	-	1.15	1.15
14	Ken. Tai	-	-	1.15	1.15
15	Ngamia	-	-	1.15	1.15
16	Ken. Wren	-	-	1.15	1.15
17	Simba	-	1.2	-	1.2
18	Sun Bird	-	-	1.15	1.15
19	Yombi	-	-	1.15	1.15
	Totals (%)	**34.25**	**38.85**	**26.9**	**100**

Farmers indicated that various factors ranging from social to agro economic factors determined their choices of the type of wheat cultivar to grow. However, the outstanding determinant for seed choice by most farmers was the potential of the seed cultivar to give high yields. Other factors taken into account included the wheat cultivar's resilience to wheat rust, the market's accessibility

of the enhanced wheat seed cultivar and, free wheat seeds that were on scheduled programs for either research or for production of certified standards wheat seeds for commercial purposes. The selling price of wheat seeds on the market and the resistance of wheat grains to pests during storage were further considerations.

Fusarium head blight (FHB) was mentioned as a significant issue influencing wheat crop productivity by only 1.6% of the farmers whose wheat grains were sampled. On the other hand, stem rust was identified by 100% of farmers whose wheat grains were analyzed as being the most serious fungal disease hindering sustainable wheat output. They attributed this to the massive destruction that wheat rust causes to the wheat crop and the costs incurred on application of fungicides for control of the same and other associated foliar diseases. However, the effects of FHB such as drying heads of wheat shoots before maturity, occurrence of small shrived boat shaped and chalky light wheat grains (Figure 3.5) were incidentally visible on some of the harvested grains and on the crop in some wheat fields that were yet to be harvested. None of the farmers acknowledged selection of the wheat cultivar grown or preferred for cultivation because of its suitability to the ecological conditions prevailing in their local regions. Incidentally, except for baking qualities of certain wheat cultivars farmers seemed not be aware of other motified properties of the wheat cultivar they selected for cultivation. In addition, worthy noting were occurances where certain wheat cultivars were planted on request by some wheat flour milling companies.

Figure 3-5. Effects of virulent *Fusarium* spp. on the wheat crop: A- *Fusarium* head blight (FHB) on wheat heads drying in the field before maturity; B- Boat shaped wheat kernel due to FHB infection; C- FHB diseased shriveled boat shaped chalky wheat seeds; D- Chalky granules from FHB diseased wheat seeds.

3.3.2 Occurrence of *Fusarium* genus isolates in grains of wheat cultivars and their morphological characteristics

Based on the above isolation procedures isolates with characteristic features for the genus *Fusarium* were identified (Tables 3.3, 3.4 and 3.5). Cultural characteristics such as the ability of the isolate to produce pigment in PDA during growth and its color, unique morphological phenotypes of mycelia and formation of distinctive conidial cells formed the basis for distinguishing isolates belonging to *Fusarium* genus. Optimal growth conditions on different media (CLA for enhancing sporulation, SNA for enhancing formation of chlamydospores and shapes of conidia and, PDA for vegetative growth, pigment formation and color) and at different temperatures (25°C and 30°C) were used to enhance phenotypic differences. The subsequent growth characteristics for some of the isolated *Fusarium* spp. were as follows: Isolate CT1 (*Fusarium* sp.) had beige pigment on PDA with dense, cream mycelium that filled the tube and turned brown with aging (Figure 3.6). Isolate CT2 (*F. verticillioides*) culture on PDA produced burgundy pigment and formed white-purple mycelium that turned brownish with age (Figure 3.7). Other growth characteristics included almost straight thin-walled macroconidia with notched basal cells. Chlamydospores were present while microconidia were not visible. Initial identity based on

these characteristics was *F. verticillioides.* Characteristics for isolate CT5 (*F. verticillioides*) included cream-beige mycelium that could hardly fill the plate by day 14 and, yellow-brown pigment in PDA media (Figure 3.8). Macroconidia were elongated, straight, slightly notched while the produced microconidia were many, small, round in shape and scattered. Another *Fusarium* spp., isolate CT10 (*F. heterosporum*) had the following phenotypes: red carmine mycelia, production of yellow orange pigment in and, some exudate on PDA media. Macroconidia were five septed and had a dorsal curvature. The basal cells were legged while apical cells had pointed but curved ends. Sporodochia, round microconidia and scattered chlamydospores formed on SNA media. Conidial cells were borne on monophialides. They quickly became morphologically distorted few days after formation.

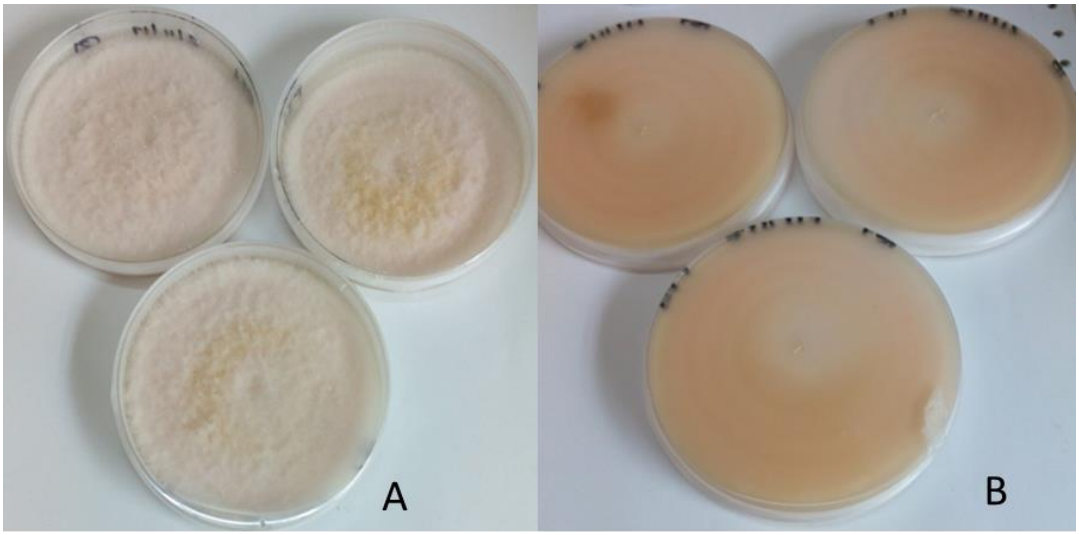


Figure 3-6. Colour of A- mycelia and, B- pigment of a 14 days old CT1 culture.

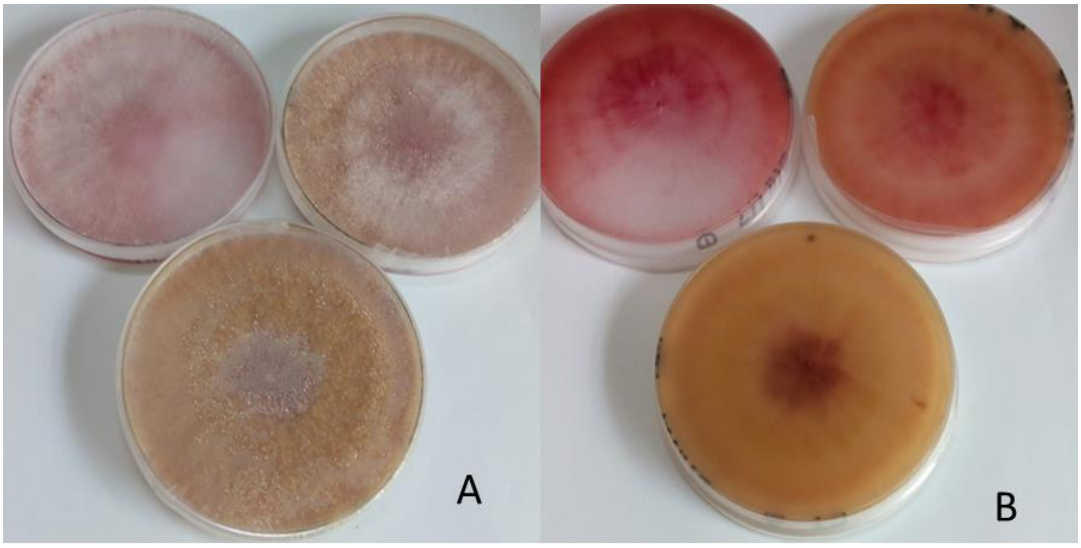


Figure 3-7. Colour of A- mycelia and, B- pigment of a 14 days old CT2 culture.

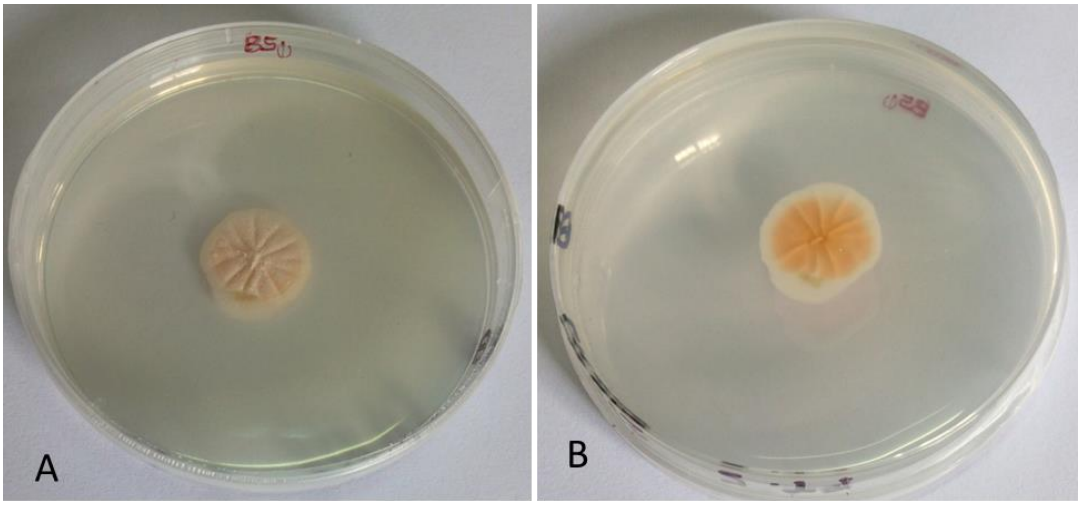


Figure 3-8. Colour of A- mycelia and, B- pigment of a 14 days old CT5 culture.

Isolate CT12 (*F. verticillioides*) culture had orange sporodochia insitu. The maximum diameter at 25°C degrees was 5.5cm while at 35°C it was 4.5cm on PDA media. Initially, the mycelia were cream white but turned purple with aging. Brown yellow pigment formed in the media. On CLA and SNA media, chlamydospores appeared in single, chained and clamped patterns. Sporodochia and false heads were present at 25°C. Formation of macroconidia on CLA arose from both monophialides and polyphialides. Two types of four-septed macroconidia (elongate spindle

shaped and short/squat) were formed with pointed apical cells and legged, almost notched basal cells. Macroconidia in some isolates had dorsal side more curved than ventral side. Mycelia formed a central pale brown mass by three weeks of age. Formation of false heads, chained chlamydospores, yellow brown sporodochia and yellow pigment formed on PDA media. Release of some exudate on PDA media also characterized the growth of isolate CT12.

Isolate CT14 and CT152 (*F. heterosporum*) cultured on PDA produced no pigment. However, formation of a lot exudate on PDA was very visible. On SNA media, the macroconidia for CT14 had 3 to 7 septa, straight needle like shape with blunt basal cell and a pointed apical cell. Other variations (straight, thin, curved, wavy) were observed on macroconidia, with basal notched cells and tapering apical cells. There were no chlamydospores formed. Isolate CT14 was isolated from wheat grains sampled in Uasin Gishu. Isolate CT19 (*F. verticilioides*) produced orange brown pigment, almost burgundy purple-whitish mycelium (Figure 3.9).

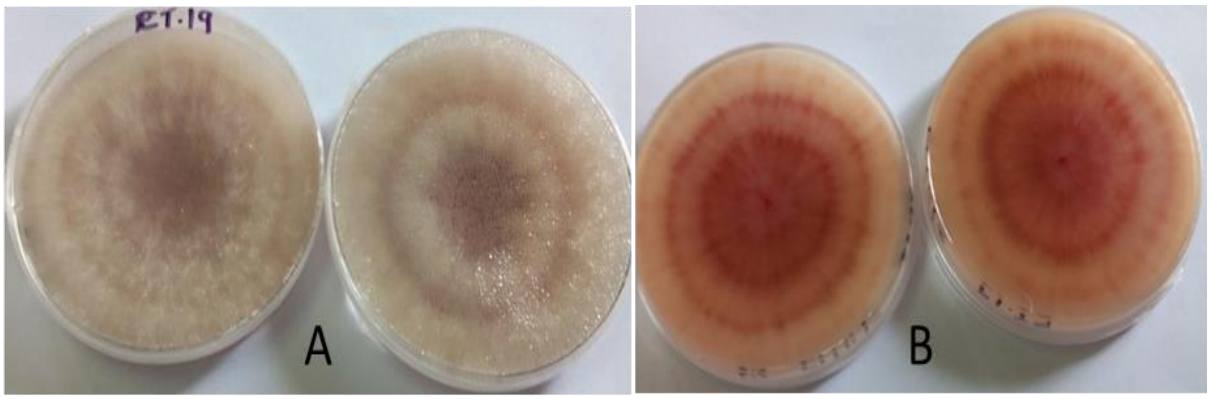


Figure 3-9. Colour of A- mycelia and, B- pigment of a 14 days old CT19/PT19 culture.

Isolate CT23 retrieved from grains sampled in Uasin Gishu County had cream white mycelia in the initial three to five days of growth that eventually turned purple. Yellow- brown pigment formed in PDA media. On SNA, chlamydospores were present either in single formations or in chains and clamps. By day14 of growth, sporodochia and false heads were present. Overall,

average growth in diameter by day 14 and at 25°C was 5.2cm. On CLA, formation of macroconidia was observed on both monophialides and polyphialides. Two different shapes (elongated spindle shaped and short/squat) of macroconidia cells were formed. Macroconidia were divided into four septa. Apical cells were pointed while basal cells were legged, almost notched. At three weeks old, the culture had a central pale brown mass. False heads, chained chlamydospores and yellow pigment formed in PDA media. Macroconidia in some isolates had dorsal side more curved than ventral side. Isolate CT20, isolated from wheat grains sampled in Nakuru had abundant chlamydospores on PDA, gray sporodochia insitu and conidia rising from monophialides. While microconidia were not visible, the observed macroconidia had six septa, tapering apical cells and legged basal cells. On SNA, chlamydospores like swollen cells in chains were observed. Molecular characterization later revealed that both CT20 and CT23 were *F. verticillioides* (Figure 3.10 and Figure 3.11). However, isolate CT20 was categorized as outgroup to node II and I in the phylogenetic tree. This confirmed the initially observed morphological and cultural differences between the isolates.

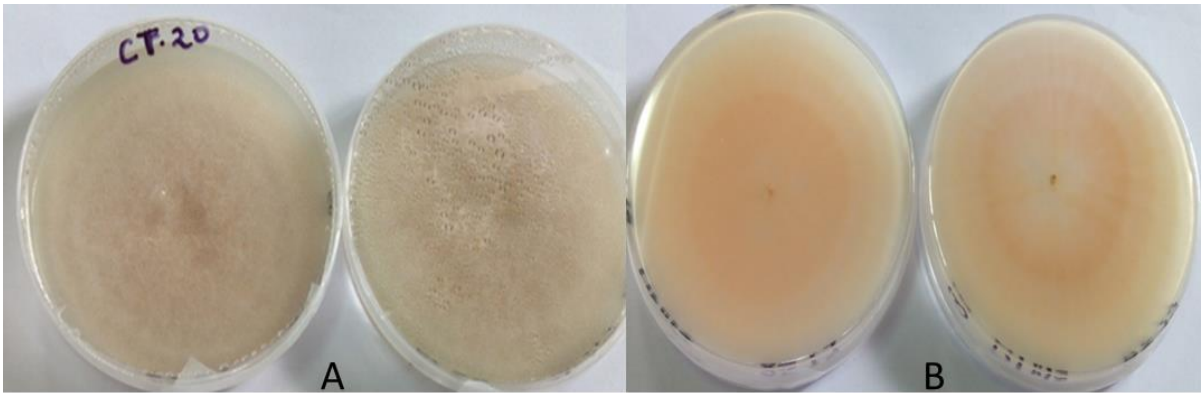


Figure 3-10. Colour of A- mycelia and, B- pigment of a 14 days old CT20 culture.

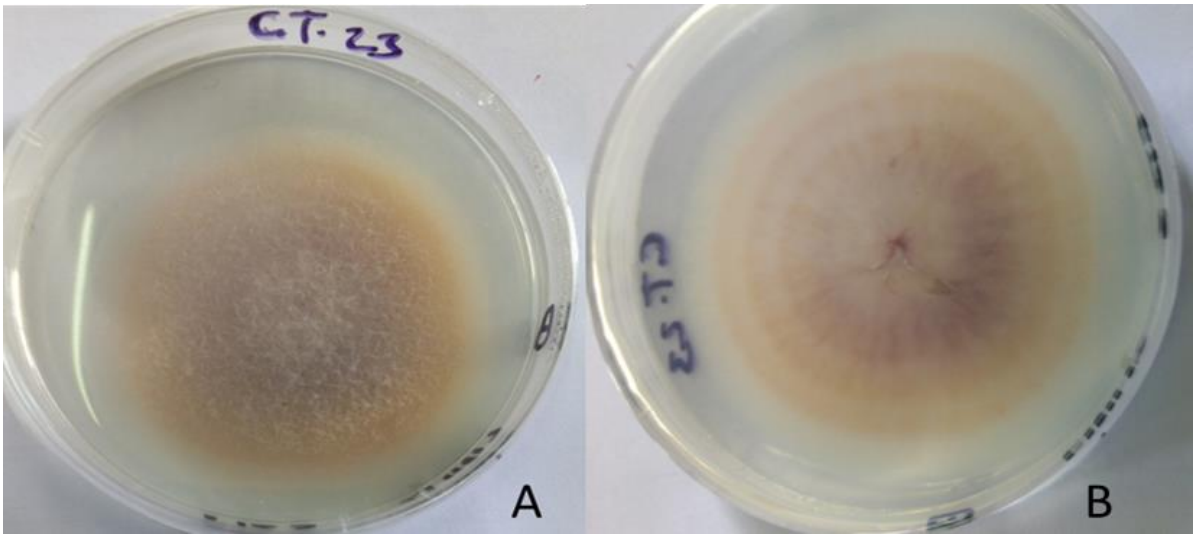


Figure 3-11. Colour of A- mycelia and, B- pigment of a 14 days old CT23 culture.

Isolate CT27 culture on PDA had yellow mycelium and red pink pigment. Other characteristics on CLA were production of small rod shaped microconidia, five- septed macroconidia with a dorsal curvature and notched basal cells. Growth characteristics of CT28 and CT31 included dense purple-white floccose mycelia that did not fill the tube and, formation of burgundy/ pink maroon pigment in PDA media (Figure 3.12 and 3.13). The average diameter of mycelium was 3cm at 25°C and 1.5cm at 30°C respectively. Napiform, globose, round shaped microconidia arising from short monophialides and polyphialides were visible. Features on CLA included microconidia in short chains, false heads and, short monophialides for both microconidia and macroconidia. On SNA, no chlamydospores formed. However, gray, round, scattered sporodochia that did not form from the centre were present. Growth characteristics of isolate CT34 (*F. verticillioides*) on PDA media were as follows: Gray-brown mycelium, single, double and chained chlamydospores and, yellow sporodochia. Characteristics on CLA initially included lack of macroconidia and abundant obovoid microconidia borne in heads. Characteristics on SNA included oval/rod shaped small microconidia borne on short monophialides. Additionally, chained, single, clumped

chlamydospores and few round yellow sporodochia were present. The characteristics were similar to those for isolate CT92.

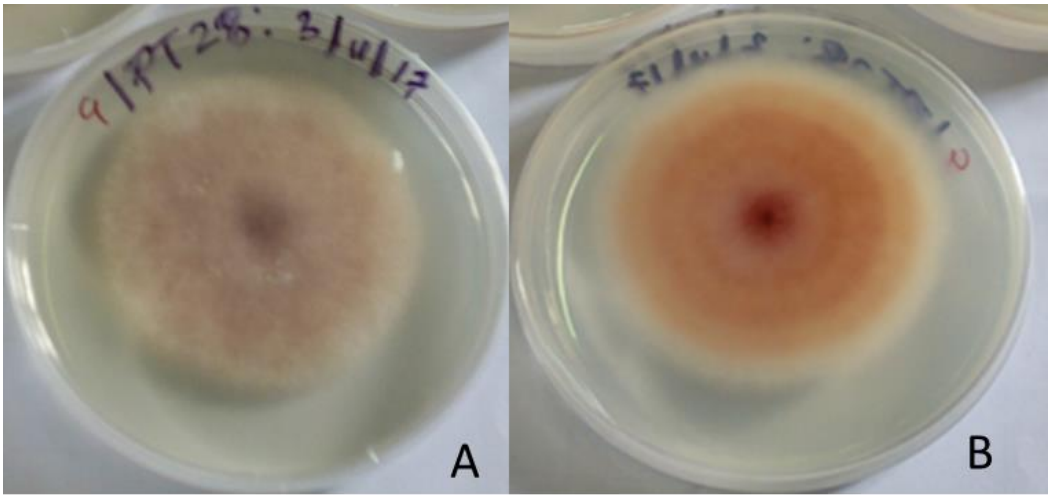


Figure 3-12. Colour of A- mycelia and, B- pigment of a 14 days old CT28/PT28 culture.

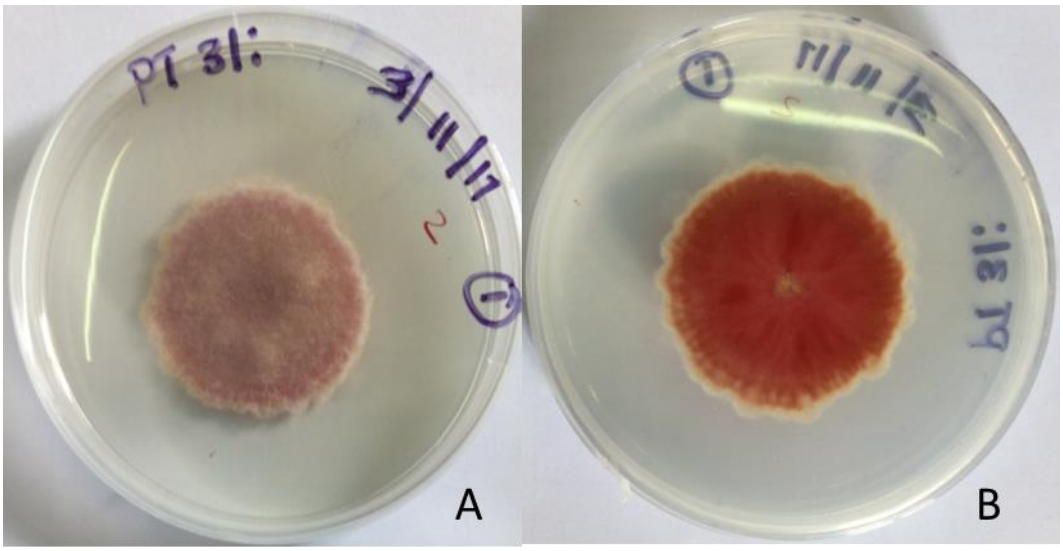


Figure 3-13. Colour of A- mycelia and, B- pigment of a 14 days old CT31/PT31 culture.

Isolate CT40 culture on PDA was characterized by white brown tuft of mycelium that filled the tube, turning colour to beige, cream pink (Figure 3.14) and turned slimy with age. Macroconidia were four septed while micro were single celled or septed into two cells. Short monophialides and

few gray sporodochia formed. Isolate CT42 culture (Figure 3.15) had almost similar growth characteristics on PDA media except for the irregular growth of its mycelium and production of yellow pigment. Isolate CT47 (*F. equiseti*) produced gray brown mycelia, turning to brick red with age. Chlamydospores occurred in different forms (chained or single). Macroconidia were five septed while microconidia had 1-3 septa. On CLA chained, single and clumped chlamydospores formed in the third week. On PDA- Cream/white, fluffy mycelium filled the tube, with uniform growth. Yellow orange pigment formed in media. The morphology of the mycelia showed degeneration by the onset of week three. Most macroconidia were six septed and two different shapes (slender short and slender long) were observed. Perithecia was also present. Isolates CT70 and CT72 exhibited almost similar characteristics as isolate CT47.

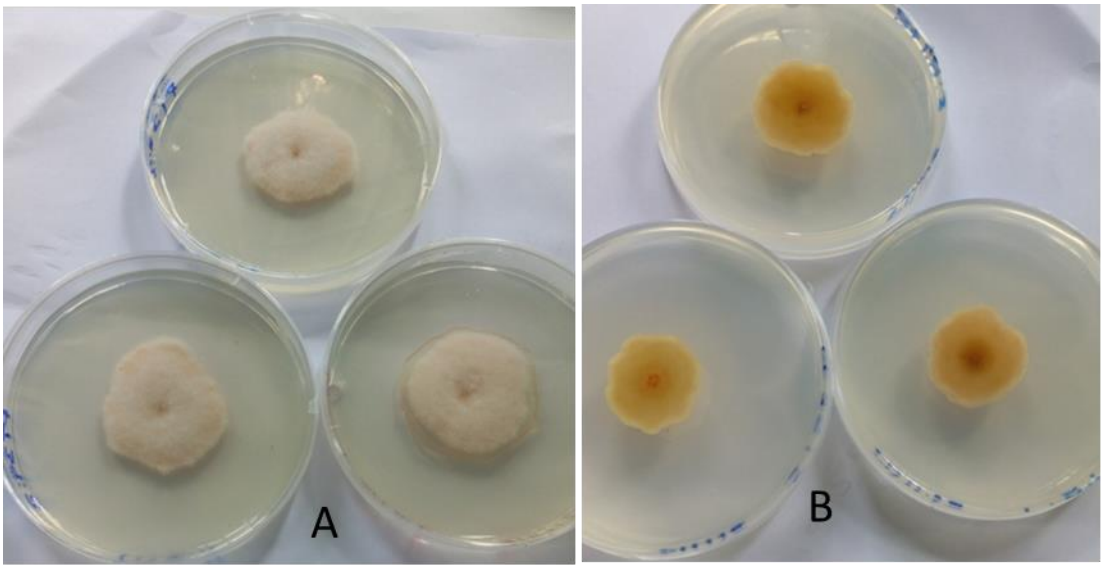


Figure 3-14. Colour of A- mycelia and, B- pigment of a 14 days old CT40 culture.

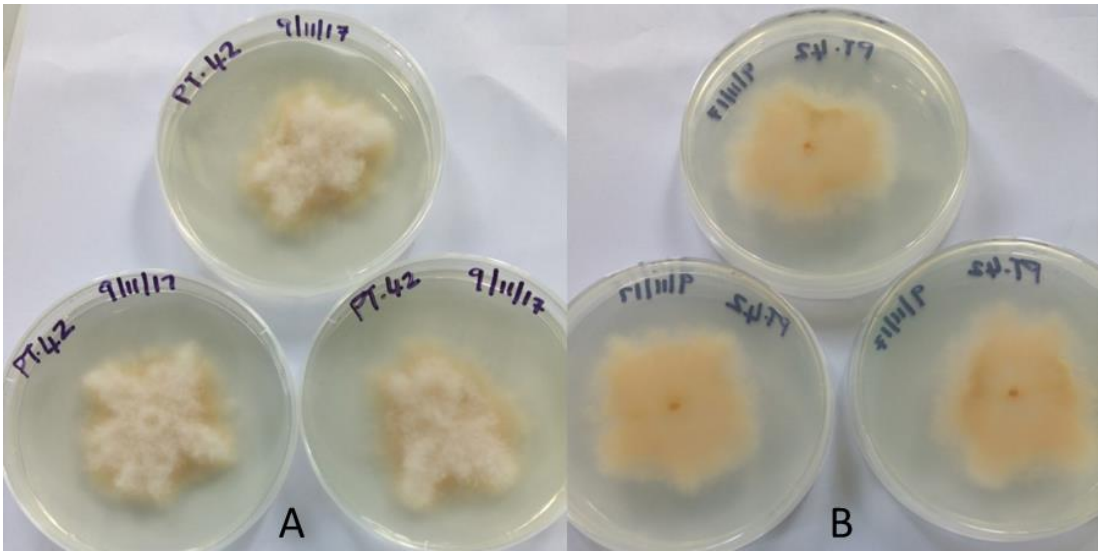


Figure 3-15. Colour of A- mycelia and, B- pigment of a 14 days old CT42/PT42 culture.

Isolate CT76 produced small rounded microconidia borne on monophialides and in chains. Polyphialides were few while sporodochia were present. Macroconidia were also absent. Yellow pigment formed in PDA media. Small rod shaped microconidia in chains, clumps and single scattered chlamydospores were formed on CLA. The culture of isolate CT80 on CLA produced sporodochia, 5-6 septed and slightly notched macroconidia with a swollen cell in the fourth septa. It formed tapering 2-septed apical cells. Characteristics of isolate CT18 (*F. verticillioides*) on CLA included the formation of very many oval, round, septed microconidia, short monophialides, few chlamydospores, single false heads and yellow sporodochia. Characteristics of isolate 193 on PDA included dark maroon red pigment in media, white mycelia that did not fill the tube. It produced many thick/dense, three different types of lemon like microconidia. It also produced short forms of monophialides and polyphialides. Very few, short, thick three septed macroconidia formed and majority were borne on monophialides. Sporodochia were present while chlamydospores were absent. Tables 3.3, 3.4, 3.5; Figures 3.16 and 3.17 highlights a summary of the characteristics of some of the randomly selected *Fusarium* spp. isolated from the grains of wheat cultivars sampled in the three regions of study.

Table 3-3. Characteristic features of *Fusarium* spp. isolated from grains of wheat cultivars sampled in main wheat producing Counties (Narok, Uasin Gishu and Nakuru) in Kenya.

Isolate	Characteristics on CLA						Characteristics on PDA			Species
	Microconidia formation (H, C &S) and Shape			Ph.	Cl.	Macroconidia:	Pigment colour	Colony diameter		
	+/-	H/ C/S	Shape	B	+/-	Size and Shape		25˚C	30˚C	
CT1	+	S	Pyriform & others	B	-	Elongate; 3	Beige	3.4	1.5	*Fusarium sp.*
CT2	+	S	Globose, lemon	M	-	Squat; 2	Burgundy	3.6	2.1	*F. verticillioides*
CT10	-	-	-	B	-	Not seen	Cream brown	2.3	1	*F. equiseti*
CT12	+	S	Obovoid	M	+	3; straight	Violet	3	3.5	*F. verticillioides*
CT14	+	S	Globose, lemon	M	-	Squat; 2	No pigment	3. 7	2.3	*F. hetrosporum*
CT192	-	-	-	B	-	Elongate; 3	Cream	3.2	1.7	*F. equiseti*
CT20	-	-	-	P	+	Straight	Yellow	3.0	1.5	*F. verticillioides*
CT21	-	-	-	B	+	Elongate; 2	No pigment	1.5	0.5	*F. heterosporum*

Note: Microconidia (H- Heads, C- Chains and S- singles); Ph- Phialides; Cl- Chlamydospores; Macroconidia (1- Straight, almost needlelike; 2- dorsal ventral curvature along all or a portion of the spore or 3- dorsal more curved than ventral); spp.- species, (+) - Present, (-) - Absent/not seen. B- Both monophialides and polyphialides present.

Table 3-4. Characteristic features of *Fusarium* spp. isolated from grains of wheat cultivars sampled in three main wheat producing Counties (Narok, Uasin Gishu and Nakuru) in Kenya.

Isolate	Characteristics on CLA						Colony Characteristics on PDA			Species
	Microconidia formation (H,C &S) and Shape			Ph.	Cl	Macroconidia	Pigment colour	Di (CM)		
	+/-	H/C/S	Shape	P/M/B	+/-	Size and Shape		25˚C	30˚C	
CT40	+		Round	P/M	+	Elongate	Yellow	2	2.6	*Fusarium sp.*
CT42	-	-	-	M	+	Elongate	Cream-yellow	1.5	1	*F. equiseti*
CT44	-	-	-	M	+	Straight, elongate	Orange brown	2.2	3.5	*F. equiseti*
CT25	+	Heads	Globose, lemon	B	+	Straight	Yellow brown	4.2	3.2	*F. verticillioides*
CT57	+	Heads	Obvoid	M	-	Straight	Yellow	2.0	1.9	*F. poae*
CT58	+	Heads	Round, obvoid	M	+	Straight, elongate	Orange brown	2.0	1.9	*F. poae*
CT59	+	Single	Lemon	B	+	Straight	Yellow brown	2.4	2.5	*F. verticillioides*

Note: Microconidia (H- Heads, C- Chains and S- singles); Ph- Phialides; Cl- Chlamydospores; Macroconidia (1- Straight, almost needlelike; 2- dorsal ventral curvature along all or a portion of the spore or 3- dorsal more curved than ventral); spp.- species, (+) - Present, (-) - Absent/not seen. B- Both monophialides and polyphialides present.

Table 3-5. Characteristic features of *Fusarium* spp. isolated from grains of wheat cultivars sampled in three main wheat producing Counties (Narok, Uasin Gishu and Nakuru) in Kenya.

Isolate	Characteristics on CLA						Colony Characteristics on PDA			Species
	Microconidia			Ph	Cl	Macroconidia	Pigment Colour	Di (CM)		
	+/-	H/C/S	Shape	P/M/B	+/-	Size and Shape		25˚C	30˚C	
CT60	+	-	Oval	B	-	Straight	Yellow	2.4	2.5	*F. poae*
CT61	+	Heads		B	-	Elongate	Brown	2.3	2.0	*F. equiseti*
CT62	-	-	-	P	+	Squat	Yellow	1.8	1.7	*F. equiseti*
CT63	-	-	-	P	+	Elongate, 2	Purple	1.5	0.5	*F. verticillioides*
CT64		-	-	B	+	Squat, 3	Cream	2.2	2.4	*F. equiseti*
CT71	+	Heads	Oval, round,	B	+	Squat	Pink to brown	1.9	1.0	*F. verticillioides*

Note: Microconidia (H- Heads, C- Chains and S- singles); Ph- Phialides; Cl- Chlamydospores; Macroconidia (1- Straight, almost needlelike; 2- dorsal ventral curvature along all or a portion of the spore or 3- dorsal more curved than ventral); spp.- species, (+) - Present, (-) - Absent/not seen. B- Both monophialides and polyphialides present.

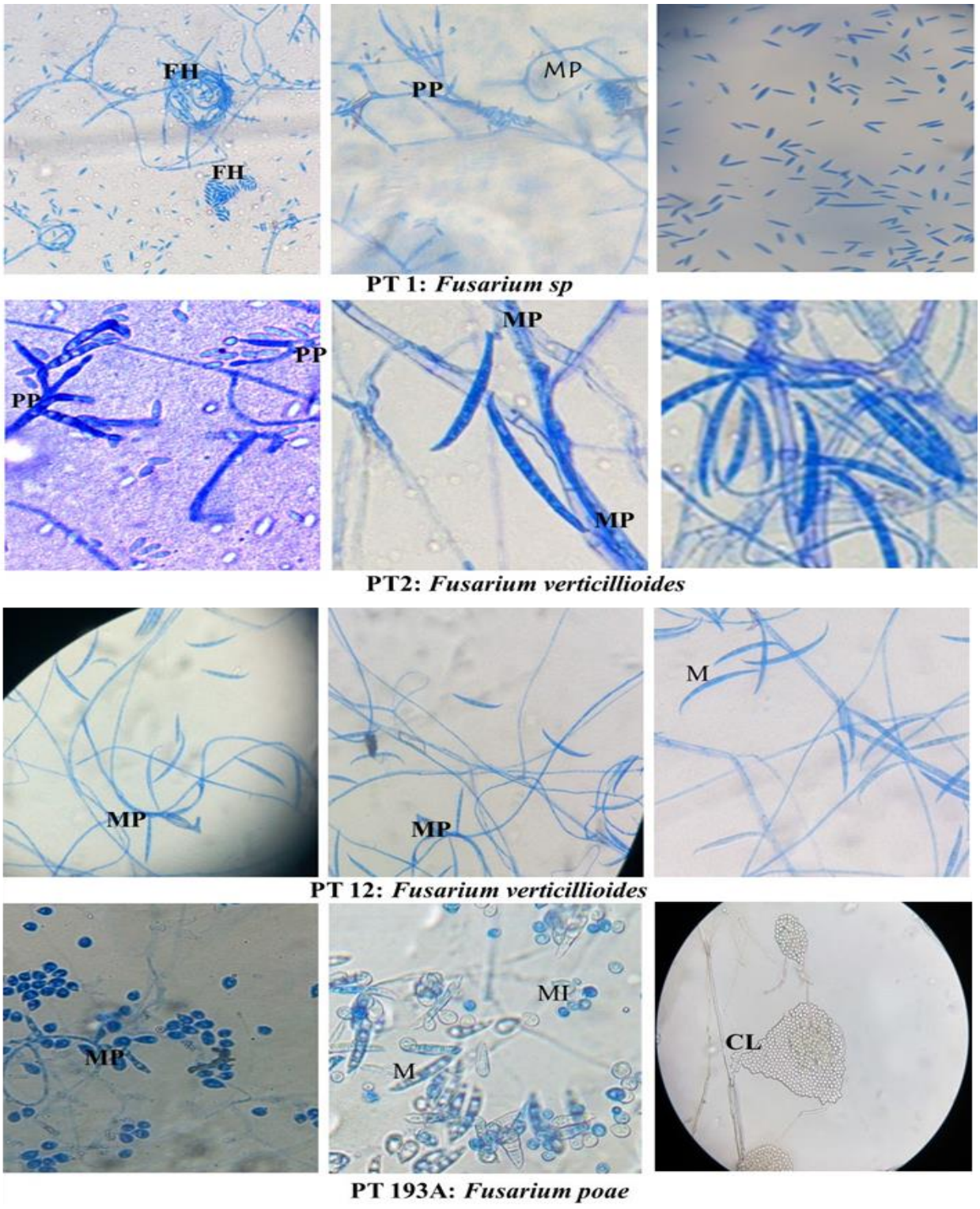


Figure 3-16. Formation of Microconidial (MI) and Macroconidial (M) cells from monophialides (MP), Polyphialides (PP), False Heads (FH) and Microconidial cells formed in clusters (CL) on carnation leaf agar (CLA). Mg. X400.

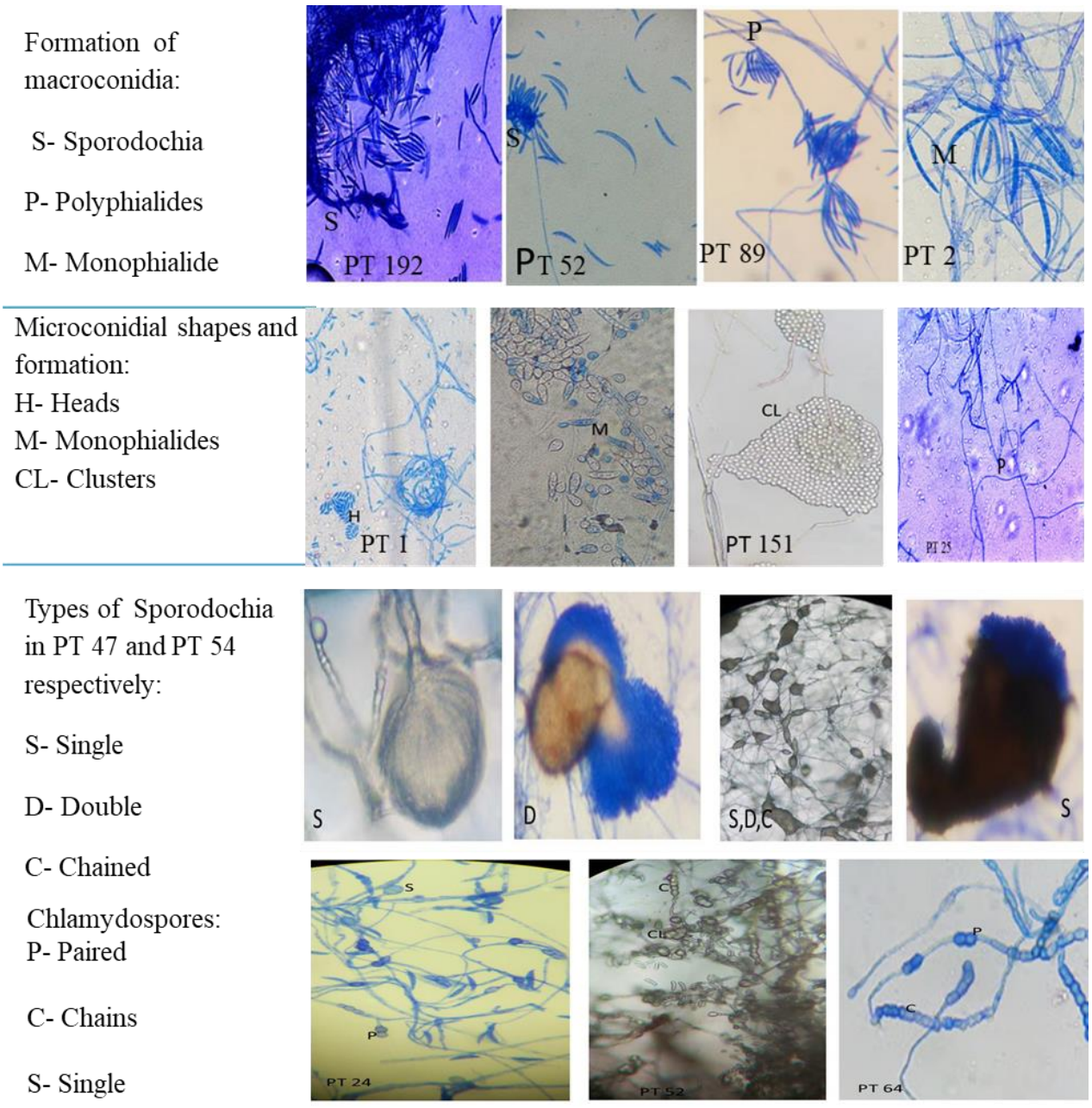


Figure 3-17. Other characteristic features of *Fusarium* spp. isolates: CT192- *F. equiseti*, CT52- *F. culmorum*. CT89- *F. equiseti.* CT2- *F. verticillioides*; CT1 *Fusarium sp.*, CT151- *F. poae*, CT23- *F. verticillioides*, CT47- *F. equiseti*, CT24- *F. verticillioides* and CT64- *F. equiseti*

3.4 DISCUSSION

Based on the results of the study, a minimal portion of the improved wheat cultivars had been cultivated by wheat farmers in Narok, Uasin Gishu and Nakuru within the study period. Moreover, the results also showed that ecological characteristics of the regions were not considered during the selection of the wheat cultivars to be planted. The two top-most determinants factors that influenced choice of wheat cultivars preferred most for cultivation were the amount/weight of wheat yield and, the resistance potential of the wheat cultivar to wheat rust. However, it was clear that other factors such as the ability of certain wheat cultivars to resist pests during storage, the cost and access to the wheat cultivar also influenced the selected cultivar remarkably. Finally, some famers opted for the cultivars of wheat seeds that were on offer either for research trials or for cultivation to produce commercial certified standard seeds, where they had no choice to make.

In spite of the varied reasons for choice of wheat cultivar to cultivate, noticeably three cultivars of wheat (Njoro BWII, Robin and Eagle 10) were predominantly cultivated in the three regions of study. Only four cultivars of wheat (Njoro BWII, Robin, Eagle 10, and Simba) were sampled in Uasin Gishu County. A possible indication of the minimal adoption by farmers of the vast cultivars of the improved wheat cultivation in the ecological region. The most frequently sampled wheat cultivar (Njoro BWII) was most collected from Uasin Gishu County. However, the use of standard commercial category of wheat seeds was not high among the farmers from Uasin Gishu County. The farmers considered the option of farmer saved wheat seeds as affordable and sustainable rather than purchasing the certified standard commercial wheat seeds that were costlier. In comparison to the other two regions of study, more wheat cultivars were sampled in Nakuru County. This provides possible evidence that a higher number of the developed wheat cultivars are cultivated in this region. However, it is worth noting that Nakuru County hosts the National Plant Breeding Station

(NPBS) and hence research on smallholder wheat technologies is prevalent in the area (Macharia & Ngina, 2017). The presence of NPBS may possibly have had an influence on the high number of wheat cultivars grown by farmers from this region. Narok County had the second highest number of wheat cultivars sampled. Farmers from this region relied a lot on the use of standard certified commercial wheat seeds. The farmers however, exhibited limited exposure to some of the important qualities of other wheat cultivars such suitability to the prevailing ecological conditions.

Fusarium head blight (FHB) was mentioned by only a small percentage of the farmers interviewed as one of the fungal diseases limiting sufficient wheat production. The rest had no idea of any other fungal infections in the wheat crop except wheat rust which was mentioned by all farmers as the most dangerous wheat infection of concern. However, it was observed that "wheat rust" was the name used by famers to describe any form of fungal infection/diseases affecting wheat. This included incidences where the disease symptoms on the wheat crop indicated infection by FHB. This observation provided evidence on either lack of knowledge or sufficient information among wheat farmers about important fungal infections prevalent in the wheat crop and their general subsequent consequences on wheat safety. Therefore, such farmers may as well be ignorant of the potentially harmful long-term effects of the secondary metabolites created by such phytopathogens on the health of domesticated animals that feed on farm's leftovers after harvest as well as humans who consume wheat products.

The isolated fungi showed distinct morphological characteristics that included among others; colony morphologies, pigment on PDA and colony diameter as shown in the results. The observed shapes of macroconidium, presence or absence of microconidia, shape and mode of formation of microconidia, nature of the conidiogenous cell bearing microconidia, presence or absence of chlamydospores, were the other unique features distinguished the isolates as members belonging to

Fusarium genus. In some isolates such as CT1 and CT14, there was no pigment production while in others there was pigment production. The former is a characteristic feature for species such as *F. heterosporum*. Most isolates produced greyish rose to burgundy and, yellow brown pigments while the rest produced other different pigments that were violet, cream, peach and yellow in colour. The colony diameter ranged from 1.5cm to 4.2cm at 25°C and 0.5cm to 3.5cm at 30°C. Other conspicuous characteristics included abundant microconidia with varied shapes, production of microconidia either from monophialides or polyphialides or from both types of conidiogenous cells. Macroconidia were septed differently some with pointed tempering apical cells and, either notched or legged basal cells. Nearly all the isolates formed chlamydospores in different forms- single, paired and in chains. However, sporodochia formation occurred in few of the fungal isolates. All the observed features were supported by diagnostic characteristics for *Fusarium* genus reported by Nelson *et al*., (1993), Burgess *et al*., (1994) and Nirenberg (1981). However, the diameters of the colonies of *Fusarium* isolated in this study were smaller and their most optimal growth was at 25°C. Also, worth noting was the disintegration of mycelia and macroconidia in isolates CT40 within 14 days of growth. Many differences existed in colour and growth pattern of mycelia necessitating the need for further molecular characterization to ascertain species identity.

The research findings show that only ten percent (10%) of the improved wheat cultivars suitable under some of the ecological conditions in the three Counties studied were grown during the study period. Additionally, factors found to influence farmers' wheat seed choices were to an extend skewed away from qualities and reasons for which the cultivars were developed to improve production. This is due to lack of sufficient knowledge about other various types of improved wheat cultivars and their superior qualities such as suitability to certain soil types or conditions. Other factors that benefited a small portion of participating farmers was the seeds provided on offer for

free to promote research or for production of certified wheat seeds. The research finding on factors determining cultivated wheat cultivars is supported by Gichangi *et al*., (2022) who also noted that there are diverse factors influencing the adoption of improved wheat cultivars that exists currently in Kenya. The findings also agrees with the report by Mburu *et al*., (2014) who reported that lack of capital, use of low quality farmer saved seeds, use of less than the optimal levels of fertilizers and technical inefficiencies (e.g. lack of farm equipment) limit optimal production of wheat farms.

The baseline information provided on the diversity of the factors and criteria used by farmers to determine the type of wheat cultivar to cultivate will help to inform on how to increase the adoption of the improved wheat cultivars while promoting crop protection, mycotoxin awareness and safety in wheat food chain. Based on the finding of this study, adoption of improved wheat cultivars suitable to specific ecological areas, awareness of mycotoxins producing fungi in the wheat crop, the production and accumulation of their toxins in wheat are critical gaps that if addressed would benefit farmers and consumers of wheat. The findings also provide additional information to wheat seed producing companies on the level of preferences of the improved wheat cultivars among wheat producing farmers. More efforts must be made to promote knowledge and understanding of the several types of recommended wheat cultivars available on the market and their characteristics, such as drought tolerance, disease resistance, and compatibility to the soil conditions in the areas of production. Therefore, there is a need to enhance constant communication between stakeholders involved in research and production of wheat seeds, agricultural extension officers and wheat farmers.

3.5 CONCLUSION

In conclusion, for the study period farmers in the three-targeted regions cultivated barely 10% of the improved wheat cultivars. Although both certified and farm saved wheat seeds were in use,

reliance or preference for farmer saved wheat seeds was a common practice among some of the farmers especially in Uasin Gishu and Narok Counties. The preference was attributed cutting down on wheat production costs. Farmers primarily took into account the amount of wheat grains to be produced in terms of weight and, a cultivar's capacity to resist wheat rust in choosing which cultivar to plant. Farmers' awareness about other types of improved wheat cultivars and, types of fungal diseases affecting wheat crop in the fields was scanty. Despite the fact that incidences of FHB were evident, as confirmed by presence of *Fusarium* genus isolates from the wheat grains sampled at harvest. All isolated members of the *Fusarium* genus exhibited diagnostic features that confirmed their identity. However, certain characteristics such as colony sizes, colour of mycelia and pigment on PDA media varied extensively hence species identity could only be ascertained using molecular tools. Factors that determined farmers' choice of wheat cultivar to plant varied yet not so much related to their ecological suitabilities among other factors.

CHAPTER FOUR

PREVALENCE AND PHYLOGENETIC DIVERSITY OF *FUSARIUM* SPECIES IN CATEGORIES OF SEEDS OF WHEAT CULTIVARS GROWN IN NAROK, UASIN GISHU AND NAKURU COUNTIES, KENYA

4.1 INTRODUCTION

Fusarium is one of the most important pathogenic fungal genera with wide distribution in the world. Members in this genus are filamentous soil fungi and hence associated with field disease in various crops. They have been reported to be ubiquitous around the globe. They are causative agents of seedling blights, cankers, wilts, and rots in vulnerable plants including food crops (Logrieco *et al*., 2007). Chemical contaminants of fungal origin pause a problem of great magnitude in the agricultural sector. The depth of the problem includes depreciation in the value and quantity of the agricultural produce due to destruction of whole or plant parts such as grains, production of plant chemicals or metabolites with potential to cause diverse diseases in farm animals and human (Gong *et al*., 2015b). Without a doubt, wheat is one of the principle sources of nourishment for about forty percent (40%) of all the human population in the world an it is also the top-most crop grown all over the globe, on not less than two hundred and eighteen million hectares of land (Giraldo *et al*., 2019). Moreover, it has greater world trade when compared to all crops put together (Giraldo *et al*., 2019; Shewry & Hey, 2015). Globally, however, fungi-derived phytopathogens pose a significant threat to agricultural productivity. The issue is rooted in a variety of causes, including unfavorable meteorological circumstances and agronomy-related issues (Bernhoft *et al*., 2012; Dweba *et al*., 2017; Scala *et al*., 2016; Wenda-Piesik *et al*., 2017). *Fusarium* Head blight (FHB), a disease caused by a complex of populations of *Fusarium* genera destroys wheat grains with consequential resultant accumulation of mycotoxins, very toxic metabolites.

Researchers are looking into a variety of control measures around the world in an effort to successfully lessen the disease's impacts. The protective steps and methods explored for use in control of FHB encompass the use of varied methods before crop harvest and those suitable for use when the grains have been harvested. Technical agricultural practices that restrict the primary infection source are among the pre-harvest control strategies being investigated for use in the disease's management. Physical methods such as proper harvesting with minimal or no damage to the grains, use of recommended quality seeds, rotation of wheat and other none *graminaceae* crops, timely sowing and fertilization (Mcmullen *et al*., 2008; Wegulo *et al*., 2015), education and training of famers to adopt acceptable agricultural standards are some of the post-harvest control measures in use. Despite reports that the efficacy of fungicides against FHB is not consistent for some reasons such as the short time framework required for application and, a low number of such existing fungicides (Wegulo *et al*., 2015), their place in control of FHB cannot be underestimated for integration in control strategies. Chemical control tends to have a significant impact on the severity of the disease in spikes, buildup of DON, and contamination in the wheat kernels (Bonfada *et al*., 2019). Hence, spraying of the right amount of such type of fungicides with significant efficacy and within the specific time of application (Angelo *et al*., 2014; Haidukowski *et al*., 2005; Yoshida *et al*., 2012) has been reported to be an effective practice for curbing FHB especially when the plant is in the flowering stage (Wegulo *et al*., 2015).

The use of disease resistance-inducing agents, such as biologically active components, endophytes, and non-chemical fungicides, bio-preparations, and antagonistic agents of microbial origin (Beyer *et al.*, 2006*;* Blandino *et al*., 2012, 2017; Shah *et al*., 2018) are also included in this category. Determination and adoption of wheat genotypes that exhibit remarkable resistance level against *Fusarium* spp. infestation (Blandino *et al*., 2017; Wegulo *et al*., 2015) and, also resistant to lodging

(Nakajima *et al*., 2008) are as well worth emphasizing. Utilization of more than one or a collection of such control strategies especially when the conditions are conducive for infestation by *Fusarium* spp. is equally a more effective FHB control approach (Alisaac & Mahlein, 2023; Janssen *et al*., 2019; Mcmullen *et al*., 2008). Such control approaches reduce the endurance of the pathogens in plant remains on wheat farms hence, limiting their existence in wheat fields and the intensity of infestation.

The biggest and recurring problems that prevent Kenya from producing as much wheat as possible each year are fungal infections and the associated losses before and after harvest. Damaging fungal diseases like FHB and wheat rust (Beatrice *et al*., 2016; Muthomi *et al*., 2008, 2012; Wagacha *et al*., 2016) have not only been reported to be common but also to negatively influence wheat production in Kenya. Hence, raising crop production costs, lowering crop yields and contaminating the produce with fungal toxins. In general, the major wheat protection regime carried out by most famers against all fungal foliar diseases in the crop is the application of fungicides in addition to the recommended proper farm management procedures. However, since foliar diseases affect shoots and consequently wheat spikes, the resultant effect is increased susceptibility of the sections to FHB. The application of fungicides thus emerges as a critical treatment to combat the effects of the illness on wheat. Unfortunately, the limited effectiveness and narrow spectrum of these fungicides have frequently made it difficult to effectively control diseases like FHB are due to resistant causative agents. The inconsistent farm and weather circumstances also need the employment of constant, effective FHB control strategies, such as the use of wheat cultivars that are highly resistant to the diseases causing *Fusarium* species.

Producing wheat cultivars that are disease resistant or tolerant has been and still is a compelling objective of the Kenya Agricultural and Livestock Resarch Organization (KALRO) Wheat

Research program (Kamwaga *et al*., 2016). However, fungal diseases remain a challenge in wheat production. Consequently, wheat cultivars with certain traits such as high stable yields, ability to withstand abiotic and biotic stresses, insect pests and pre-harvest sprouting diseases have been developed. Subsequently, farmers from different regions in the country where wheat is cultivated can choose the wheat cultivar best suited to their locality in addition to other important qualities aimed at increasing production. As such, there is need for continuous flow of current information regarding the prevalence of *Fusarium* spp. complex in the frequently planted wheat cultivars or their susceptibility to the pathogens. For progression in development of new wheat cultivars with greater disease resistance as well as for enhancement on quality of the existing wheat cultivars, related accurate current background knowledge is a crucial need. This study investigated the prevalence and diversity of *Fusarium* spp. infecting wheat cultivars developed and released for cultivation in some of the key Counties (Narok, Uasin Gishu and Nakuru) producing wheat in the Kenyan Rift Valley.

4.2 METHODOLOGY

4.2.1 Materials and methods

4.2.1.1 *DNA extraction*

The fungi strains isolated earlier from the respective samples of wheat cultivars and characterized into *Fusarium* genus were analysed further in this section to ascertain species identity, prevalence and diversity in Narok, Uasin Gishu and Nakuru Counties. Each *Fusarium* spp. isolate's spores were cultivated on PDA at 25^{o}C with 12 hours of fluorescent light and 12 hours of darkness alternated. Each isolate was cultured for a period of between seven to fourteen days to form good mycelia growth, both quantitatively and qualitatively for use in DNA extraction. A Fungal/Bacterial DNA Mini Prep Kit from Zymo Research (Epigenetics, Hatfield, South Africa) was used for DNA

extraction and, according to the manufacturer's procedures. The Genomic Lysis Buffer was diluted with beta-mercaptoethanol to a final concentration of 0.5% (v/v), of approximately 500µl per 100 ml, in order to obtain optimum performance. About 10mg to 20mg of fungal cells were first re-suspended in 200µl of isotonic Phosphate Buffer Saline (PBS) and then put in a ZR BashingBead™ Lysis Tube (0.1 & 0.5mm). Next, 750µl BashingBead™ buffer was added to the tube, capped tightly and then secured in a bead beater fitted with a 2ml tube holder assembly for processing at maximum speed for at least 5 (five) or more minutes. The time for processing varied depending on the sample input. Next, the preparation was centrifuged for 1 minute at 10,000-x g and the supernatant (400µl) transferred to the Zymo-Spin™ III-F Filter in a collection tube. This was followed by another centrifugation for 1min at 8,000-x g. The Zymo-Spin™ III-F Filter Column was then discarded while the filtrate in the collection tube was mixed with 1,200µl of Genomic Lysis Buffer. Next, 800µl of the resultant mixture was transferred to a Zymo-Spin™ IC Column 2 (two) in a collection tube followed by centrifugation for 1 minute at 10,000xg. The content of the collection tube was discarded and the previous step repeated. DNA Pre-Wash Buffer (200µl) was then added to the Zymo-Spin™ IC Column in a fresh collection tube and, centrifuged for one minute at 10,000xg. Next, 500µl g-DNA Wash Buffer was added to the Zymo Spin™ IC Column and centrifuged at 10,000xg for one minute. It was then transferred to a clean 1.5ml microcentrifuge tube. Next, twenty (20µl) DNA Elution Buffer was added directly to the column matrix and was incubated for one minute before the DNA was extracted by centrifuging at 10,000 x g for 30 seconds.

4.2.1.2 *Amplification of Fusarium spp. DNA using translation elongation factor 1-alpha (tef1) primer pairs*

A 700 bp DNA fragment was amplified using a set of primers that targeted the gene encoding translation elongation factor 1-alpha (tef1-): EF1 (forward primer; 5' -ATGGGTAAGGA (A/G)

GACAAGAC-3') and EF2 (reverse primer; 5' -GGA (G/A) GTACCAGT (G/C) ATCATGTT-3'). The PCR was performed in 25μl reactions which included 1μl of a 1:10 or 1:100 DNA dilution, 1URED Taq DNA polymerase (Sigma–Aldrich Company, Milan, Italy), 2μl REDTaq buffer supplemented with 1.7μl of 22mM MgCl2 for a final concentration of 3.0mM, 10Mm deoxyribonucleotide, and 1.0μlM of each primer; reverse and forward (ef1 and ef2). Reactions were run in a Mastercycler ep-gradient (Bio-Rad, California, USA) with a thermal profile of 4min at 94°C followed by 35 cycles of 60s at 94°C, 60s at 57°C, and 1min at 72°C followed by 72°C for 5min. The amplified DNA was electrophoresed in 1.5% (w/v) Tris-acetate Ethylene diamine tetraacetic acid (EDTA) agarose gels. Amplicons were visualized at 700bp to 710bp using a 1kb bp DNA ladder (bioline) as a size standard. This was done using 1% agarose gel concentration. The agarose was boiled at 100°C for 5 minutes to create the gel, and then allowed to cool to 55°C. After that, 0.3μl of ethidium bromide was added while the flask was being swirled to allow the gel and bromide to mix. With the combs on, the mixture was put into a gel tank and allowed to set. In one well, a molecular marker (2μl) was added, and in the other wells, 4μl of DNA and the loading dye were added. The arrangements were noted. The control was distilled water. The gel was operated at 80 volts for 45 minutes. Viewing was conducted using the USA and California-based Geldoc Bio-Rad Molecular Imager Gel Doc XR-CLASS Imaging System. Since the purpose of the DNA analysis was to determine the species identity of the isolates, a qPCR to determine the degree of infection for each specific *Fusarium* spp. per sample was not performed.

4.2.1.3 *Analysis of Tef1-nucleotide Sequences*

Using a QIAquick Gel Extraction Kit and following the manufacturer's instructions (Qiagen manual), the TEF-1 alpha gene amplification products were purified. Both strands of DNA for each isolate were sequenced using the ABI 3700 DNA Sequencer at Macrogen (Netherlands)

Sequencing Service Unit. Using Geneious version 11.1.5 software, the ef1-apha (tef1-) raw nucleotide sequences were put together, contigs were created and consensus sequences were extracted. By using the Basic Alignment Search Tool for Nucleotide Sequences (BLASTN), which can be found at https://blast.ncbi.nlm.nih.gov/Blast.cgito, it was possible to find sequences that had a high similarity index to those in the NCBI database. Using ClustalW, a matrix-based technique developed in Geneious version 11.1.5 software, the obtained sequences were aligned with the consensus sequences of the *Fusarium* spp. isolates. To determine how closely the 49 aligned sequences were linked, a phylogenetic tree was created using the Tamura-Nei model and Maximum Likelihood (ML) analysis. For ensuring the stability and robustness of each branch, 1,000 bootstrap replications were carried out (Kumar *et al*., 2018; Tamura *et al*., 2013). The tree with the highest log probability was displayed (-31270.16). The percentage of trees in which the associated taxa clustered together was shown next to the branches. By automatically applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances calculated using the Maximum Composite Likelihood (MCL) technique, and then choosing the topology with the best log likelihood value, the initial tree(s) for the heuristic search were created. The final dataset contained 4167 locations in total. MEGA X was used for evolutionary analysis (Kumar *et al*., 2018).

4.2.2 Data analysis

The gathered data were examined using SPSS version 23 one-way ANOVA function. This study sought to examine, at a significance level of 0.05, the prevalence of each isolated *Fusarium* spp. with respect to overall occurrence and the *Fusarium* spp. complex in each of the studied wheat cultivars grown in the three locations. In order to identify any variations in general occurrences between any of the two regions studied, numerous comparisons were made. Diversity indices were calculated using Simpson's and Shannon's diversity equations to measure species richness and

relative prevalence in each region (Appendices IV, V, and VI). Using descriptive statistics, the prevalence of *Fusarium* spp. in wheat seeds derived from farmer-saved wheat seeds and certified commercial wheat seeds was assessed.

Simpson's Diversity Index (D) is given by:

$D=1-\sum (pi)\ 2$

Shannon's Weiner Diversity Index (H) is given by:

$H=-\sum pilnpi$

"D" stands for Simpson's Diversity Index, "H" stands for Shannon's Diversity index, "∑" is sum of, "pi" is the relative prevalence of each species, and "ln" is the natural logarithm.

4.3 RESULTS

4.3.1 *Fusarium* species complex in grains of improved wheat cultivars at harvest time

Uniqueness in certain traits (mycelia, pigment formation and colour (Figure 4.1) and conidial cells' resulting from the spores of each of the isolates cultured on PDA reconfirmed the fungal genera under study.

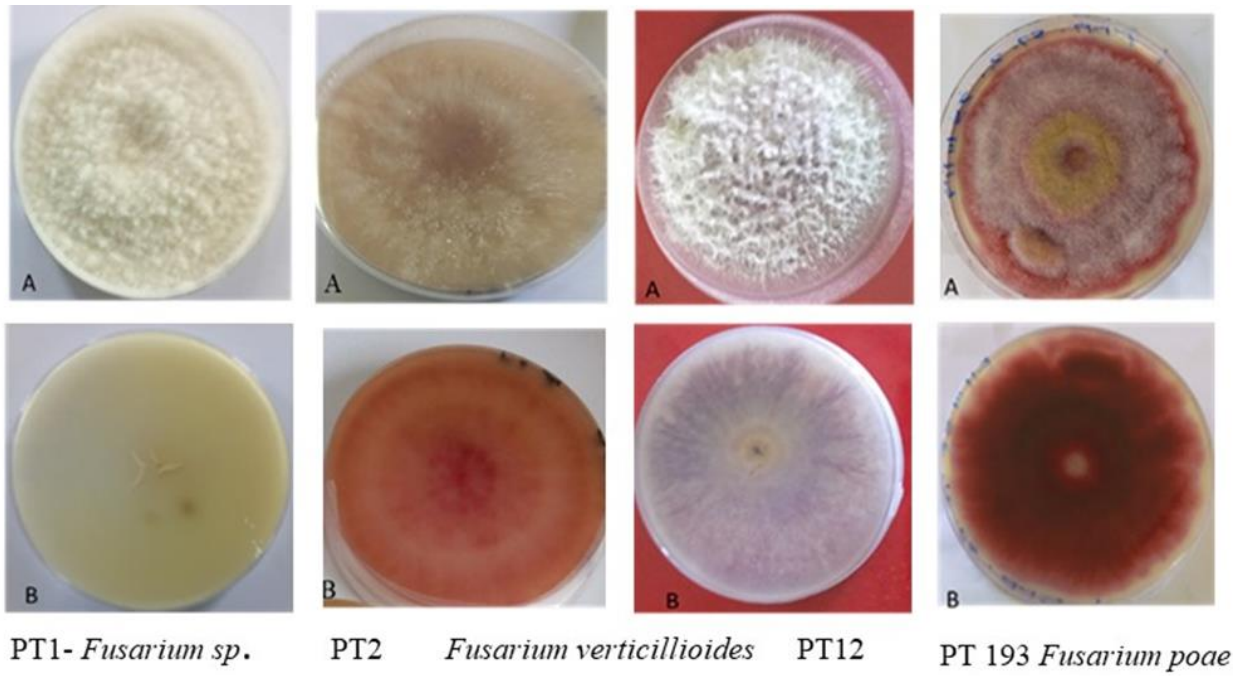


Figure 4-1: *Fusarium* spp. 14 days old cultures on PDA media. A- Color of mycelia and B- Pigmentation. Note: PT/CT- Code for fungal isolates.

Each of the isolates under examination formed a single, clearly amplified band with a fragment total size ranging from 700bp to 710bp (Figure 4.2).

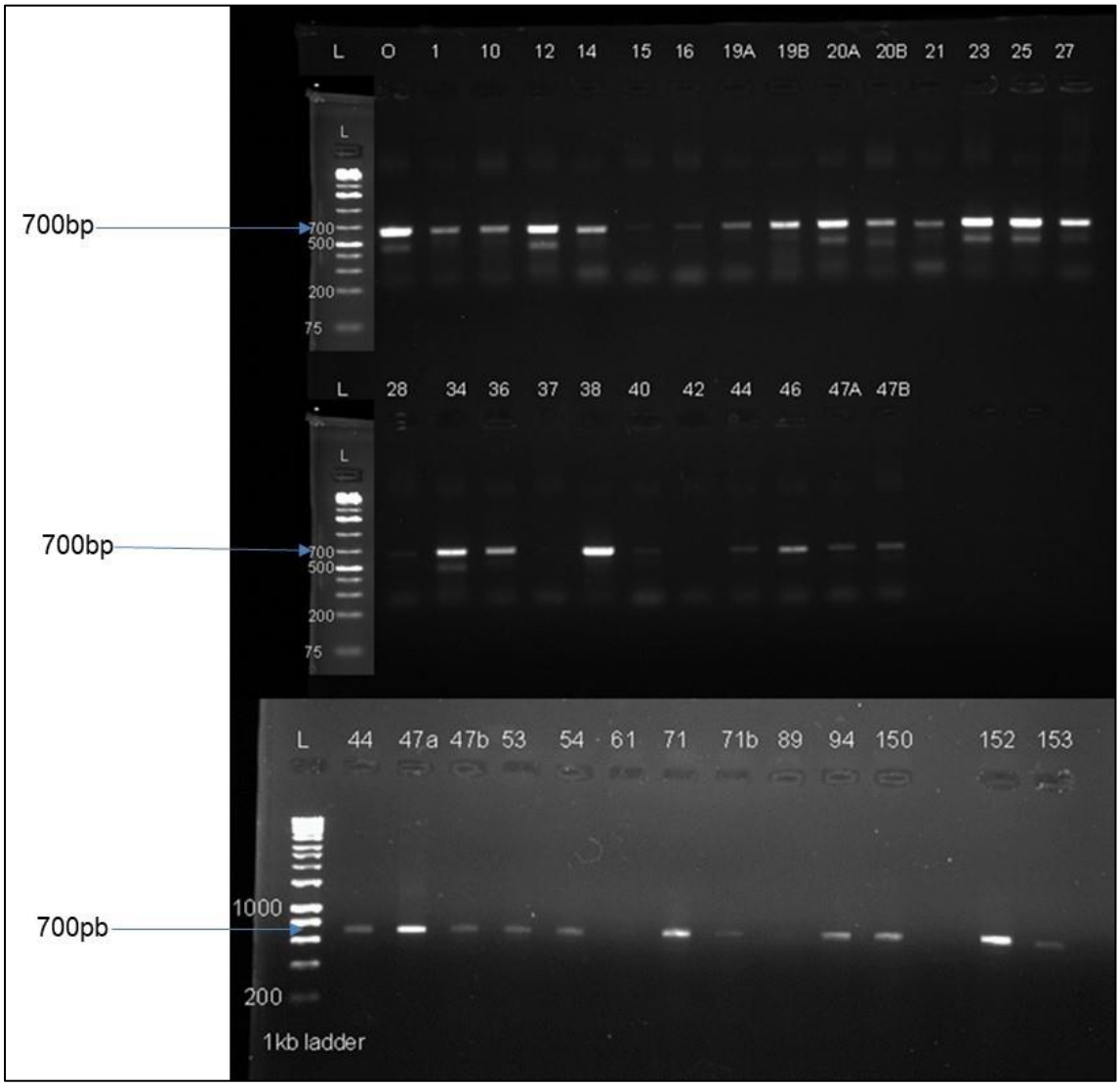


Figure 4-2. Bands for Agarose 1% Gel electrophoresis of PCR amplified elongation factor 1 alpha gene for identification of *Fusarium* spp. isolated from grains of wheat cultivars grown in Narok, Uasin Gishu and Nakuru Counties within the Kenyan Rift Valley.

Comparison of the resultant sequences of the amplified DNA of each isolate with sequences from the National Centre for Biotechnology Informatics (NCBI) database using Basic Alignment Search Tool for Nucleotide Sequences (BLASTN), https://blast.ncbi.nlm.nih.gov/Blast.cgito confirmed

the genus and consequently the species identity of each isolate. All the isolates were confirmed to belong to the genus *Fusarium* as indicated earlier in chapter three of this thesis. The characterized isolates were classified into eight different *Fusarium* spp. namely: *Fusarium poae*, *F. verticillioides*, *F. equiseti*, *F. heterosporum*, *F. tricinctum*, *Fusarium sp*. *F. oxysporum and F. culmorum* (Table 4.1).

Table 4-1. Identity of *Fusarium* spp. isolated from grains of wheat cultivars sampled from wheat farms in Narok, Uasin Gishu and Nakuru Counties in Kenya at harvest time.

S/N	Isolate Number	Species identification based on tef1-α gene sequence	Ref. Gene bank accession Number used in identification	Gene used in identification & study region
1.	CT1	*Fusarium sp.*	GQ505595	ef- Nakuru
2.	CT34	*F. verticillioides*	MH582324	ef- Nakuru
3.	CT25	*F. verticillioides*	MH582324	ef- Narok
4.	CT23	*F. verticillioides*	MH582324	ef- U. Gishu
5.	CT38	*F. verticillioides*	KU554687	ef- Narok
6.	CT54	*F. verticillioides*	MH582332	ef- Narok
7.	CT46	*F. verticillioides*	MH582332	ef- Narok
8.	CT36	*F. verticillioides*	MH582332	ef- U. Gishu
9.	CT20	*F. verticillioides*	MH582332	ef- Nakuru
10.	CT01	*F. verticillioides*	MH582332	ef- Nakuru
11.	CT189	*F. equiseti*	DQ465946	ef- Narok
12.	CT89	*F. equiseti*	DQ465946	ef- Nakuru
13.	CT10	*F. equiseti*	DQ465946	ef- U. Gishu
14.	CT61	*F. equiseti*	DQ465946	ef- Nakuru
15.	CT74	*F. oxysporum*	KU671036	ef- U. Gishu
16.	CT 53	*F. equiseti*	KT224312	elf- Narok
17.	CT 70	*F. equiseti*	KT224312	ef- U. Gishu
18.	CT15	*F. equiseti*	LS398491	ef- U. Gishu
19.	CT47	*F. equiseti*	LS398491	ef- Nakuru
20.	CT44	*F. equiseti*	LS398491	ef- Nakuru
21.	CT195	*F. equiseti*	MK168567	ef- U. Gishu
22.	CT192	*F. equiseti*	MK168567	ef- U. Gishu
23.	CT51	*F. equiseti*	MK168567	ef- Nakuru
24.	CT 42	*F. equiseti*	MK168567	ef- Narok
25.	CT40	*F. tricinctum*	MH464151	ef- Nakuru
26.	CT150	*F. verticillioides*	MH936002	ef- U. Gishu
27.	CT71	*F. verticillioides*	MH936002	ef- Narok
28.	CT68	*F. verticillioides*	MH936002	ef- U. Gishu
29.	CT12	*F. verticillioides*	MH936002	ef- Narok
30.	CT2	*F. verticillioides*	MH936002	ef- Nakuru
31.	CT191	*F. culmorum II*	LT548347	ef- Nakuru
32.	CT152	*F. heterosporum*	KR909339	ef- U. Gishu
33.	CT14	*F. heterosporum*	KR909339	ef- Nakuru
34.	CT21	*F. heterosporum*	KR909339	ef- Nakuru
35.	CT193A	*F. poae*	MK729605	ef- Nakuru

NOTE: PT/CT- Isolate's code; ef- Translation Elongation factor 1 alpha.

4.3.2 Prevalence of *Fusarium* spp. in the produce of farmer-saved and standard certified commercial seeds of the sampled wheat cultivars

In comparison to the products from certified wheat seeds, the levels and distribution of *Fusarium* spp. per wheat cultivar in the category of farmer saved wheat seeds was more and widespread (SD = 6.63). This suggests that the grains from farmer-saved seeds crop had a higher frequency of *Fusarium* spp. than wheat grains from the crop of certified commercial wheat seeds (Table 4.2). It is also important to note that, in certain cases, only a very small percentage of the fungi were isolated from the grains of some of the wheat cultivars. The highest occurrence frequency (24.5%) of the isolates per wheat cultivar occurred in the category of the produce of farmer-saved wheat seeds, with a mean of 7.4% and minimal isolation frequency of 2.45%. The occurance frequency *Fusarium* spp. in wheat grains resulting from crops planted using certified wheat seeds ranged from 1.3% to 4.78% with a mean of 2.4 percent (2.4%).

Table 4.2. Percentage occurrence of *Fusarium* spp. in the produce of Certified and Farmer-saved wheat seeds sampled at harvest in three major wheat producing Counties in Kenya.

S/N	W. Cultivar	Occurrence frequency of *Fusarium* spp. (%)	
		Produce of Certified seeds	Farmer saved seed produce
1	Njoro BWII	2.49	24.5
2	Undisclosed	-	6.25
3	Eagle Ten	3.74	7.4
4	Robin	1.3	4.7
5	Mwamba	2.49	-
6	Ken. Korongo	-	6.2
7	Ruiru	-	5
8	Hawk	1.3	3.7
9	Ken. Ibis	-	6.2
10	Duma	4.78	-
11	Kwale	3.74	-
12	Fahari	2.49	2.45
13	King Bird	1.25	-
14	Ken. Tai	1.3	-
15	Ngamia	2.49	-
16	Ken. Wren	-	-
17	Simba	2.49	-
18	Sun Bird	1.25	-
19	Yombi	2.49	-
Totals (%)		**33.6**	**66.4**

KEY: Ken- Kenya

4.3.3 Prevalence of *Fusarium* spp. in the grains of various wheat cultivars sampled at harvest time

The overall prevalence of *Fusarium* spp. in all wheat cultivars evaluated in each of the Counties studied did not differ significantly (all p= 0.05), (Figure 4.3 and Appendix VIII). Similar to this, there was no significant difference in the species diversity indices for *Fusarium* spp. isolated from the wheat grains collected from the three regions of study (Uasin Gishu had 1.23, Nakuru County - 2.01 and Narok - 1.460). Following LSD pairwise analysis, there were notable variations in the prevalence of of some of the *Fusarium* spp. (*F. oxysporum, F. tricinctum, Fusarium* sp. *F. culmorum*, and *F. verticillioides*) in the three regions. The differences between Nakuru and Uasin

Gishu for *F. culmorum* (p=1.00), Nakuru and Uasin Gishu for *F. verticillioides* (p=0.58), Narok and Uasin Gishu for *Fusarium* sp. (p=0.079) and Nakuru and Narok for *F. tricinctum* (p=1.00) were detected. However, some were not statistically different in prevalence.

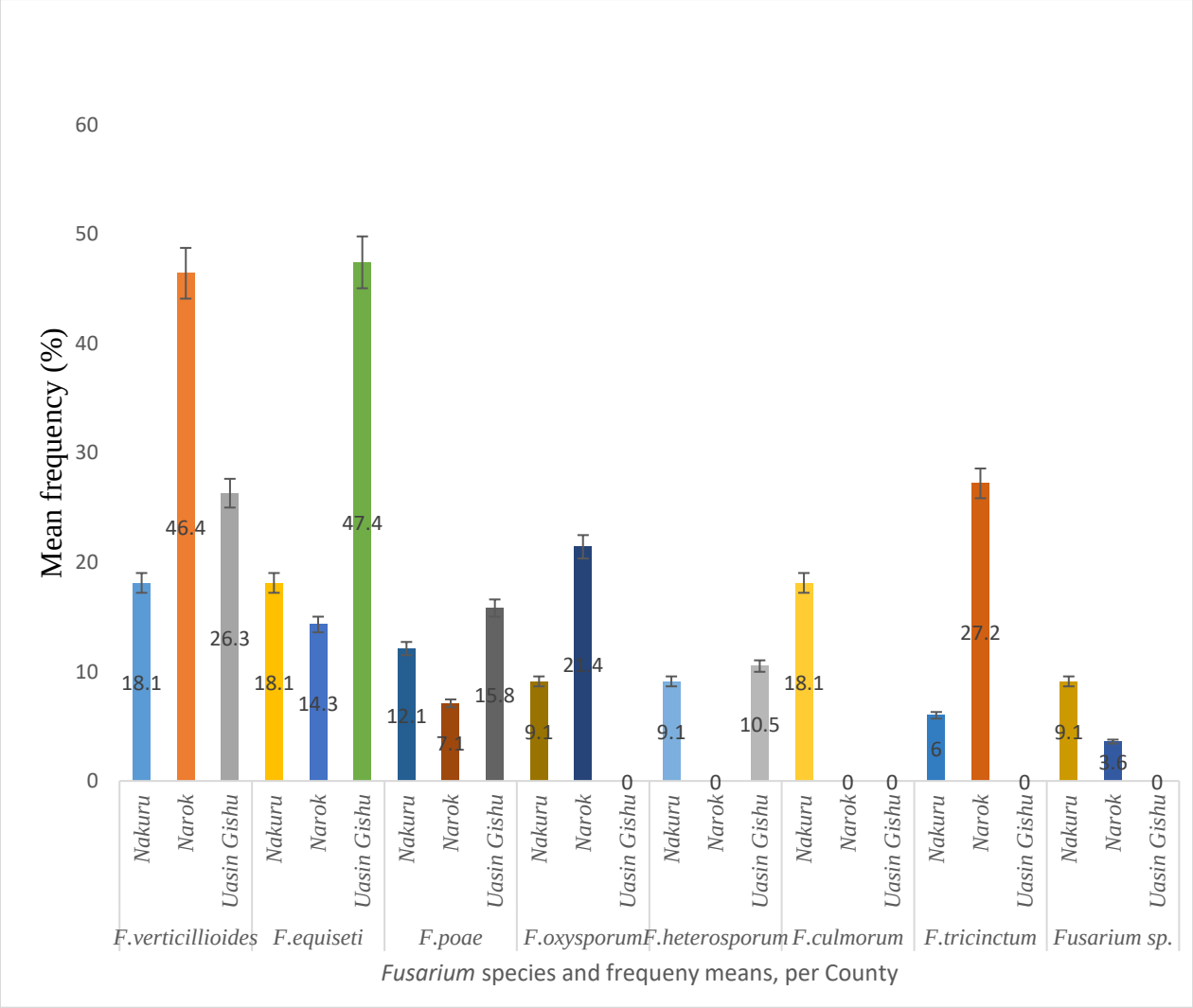


Figure 4-3. Prevalence of *Fusarium* spp. in the grains of wheat cultivars sampled at harvest time in three Counties in the Kenyan Rift Valley.

4.3.4 Phylogenetic diversity of *Fusarium* species isolated from grains of various wheat cultivars sampled at harvest time

A phylogenetic tree (Figure 4.4) was created to infer the relatedness of the aligned sequences based on bootstrap values of over 75%. The species identity of the isolated fungi and their similarity to the comparative reference sequences in the NCBI were determined using identity matrix values ranging from ninety-five (95%) to one hundred percent (100%) of the total. The evolutionary links between the different fungal isolates further supported the previous analyses on species identity. Additionally, it demonstrated some degree of species-level dissimilarity amongst individuals. Members of *F. equiseti* and *F. verticillioides* showed notable intra-species differences as shown on the phylogenetic tree, where isolates in the two species fell in more than one node. Other fungal isolates emerged as outgroups to nodes containing certain *Fusarium* species (*F. verticillioides*, *F. equiseti* and *F. heterosporum*). The phylogenetic tree (Figure 4.4) grouped the strains into four (I, II, III, and, IV) main nodes. The identity matrix values for the *Fusarium* isolates assigned to cluster one (I) ranged from 97.85% to 100%. The bootstrap value of 100% was used to support their identity. The species found in this cluster comprised *F. verticillioides* strain F28- GeneBank Acc. No. KU554687 and *Fusarium* sp. strain MCR2228-GeneBank Acc. No. MH582332. Five *Fusarium* spp. made up Group II. This included *F. verticillioides* strain CM-CNRG 455- GeneBank Accession Number MH936002, *F. tricinctum* strain PPRI20693- GeneBank Accession Number MH464151, *F. heterosporum*- GeneBank Accession Number DAOMC235644, *F. culmorum* strain E24- GeneBank Accession Number LT548347 and *Fusarium* sp Gene Bank Accession Number GQ505595. The highest identity matrix values in this cluster ranged from 99.85 to 100 percent, with bootstrap values of 100% supporting each species. Also included in the group were four *Fusarium* spp. isolates (CT20, CT21, CT53 and CT27). All thefour isolates were designated as

outgroups to nodes II and I. In comparison to the NCBI reference sequences utilised for identification, they showed low identity matrix values. *Fusarium* spp. (*F. poae* strain Montana II-MK729605, *F. oxysporum*- KU671036 and *F. equiseti* isolate LQ144- MK168567) in group III were divided into three subclusters. The bootstrap value for every species in this cluster was 100%, and the similarity matrices for all of them ranged from 97.70% to 100%. The identified strains of *Fusarium* spp. in group IV had a bootstrap value of one hundred percent. The group was made up of a single subcluster and an out-group isolate (CT61), whose reference accession number was *F. equiseti*. This isolate was most frequently isolated from grains sampled in Uasin Gishu County. Two subgroups made up the cluster and, both of them had bootstrap values of one hundred. *Fusarium equiseti,* with the identity reference GeneBank Acc. No. - DQ465946, was the species identity for isolates in subgroup one. *Fusarium verticillioides,* reference number MRC929 and genebank accession number MH582324 belonged to the other group.

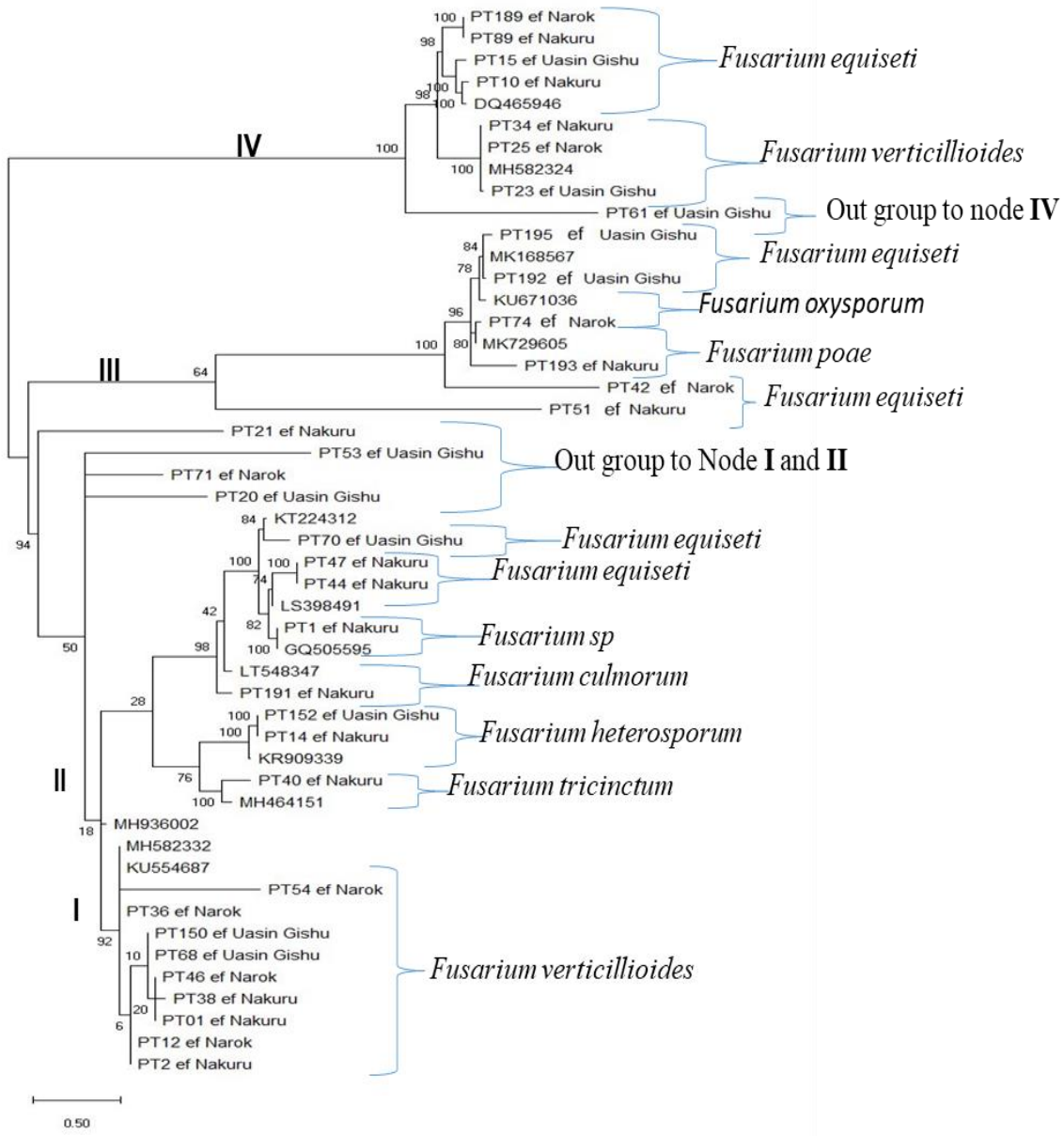


Figure 4-4: Analysis of the diversity of *Fusarium* species isolated from wheat grains in three Rift Valley counties in Kenya. Tef1- alpha gene sequences and the Maximum Likelihood technique with 1,000 bootstraps were used to create the phylogenetic tree.

4.3.5 Diversity of *Fusarium* spp. in the grains of each sampled wheat cultivar

The pathogenic *Fusarium* spp. complex diversity that was present in the grains of the sampled categories of wheat cultivars is shown by the phylogenetic analysis in Figure 4.4. They consisted of *F. verticillioides, F. equiseti, F. poae, F. oxysporum, F. tricinctum, Fusarium* sp. and *F. culmorum*

(Table 4.3). In the phylogenetic tree, *F. verticillioides* and *F. equiseti* isolates were found in more than one cluster and were isolated from more than 70% of the wheat cultivars examined. Hence, this result indicates a possible variation in the members of these species or a kind of polymorphism. Every individual evolves differently and interacts differently to environmental changes. Within species, based on the gene or DNA sequence targeted or amplified and sequenced, unless conserved, the slight variation up to around 60-100% flips members of the same species upto another clade. Hence, they may grow, colonize and sporulate at different times and rates.

When compared to the prevalence of the other isolated *Fusarium* spp., the percentage prevalence of *F. verticillioides*, *F. equiseti*, and *F. poae* was 94.7%, 78.9%, and 47.3%, respectively, in all the wheat seed cultivars. While Kenya Wren was the only wheat cultivar that was free of *Fusarium* species, all the isolated eight *Fusarium* spp. were more common in the grains of Njoro BWII and Eagle 10 types of improved wheat cultivars (Table 4.3) investigated. The following proportions of Njoro BWII, Robin and Eagle 10, wheat cultivars were sampled from the three regions under investigation: 48.8%, 12.8% and 6.95% respectively. Nine of the sampled wheat varieties and the undisclosed set were infected with over 50% (Table 4.3) of the isolated *Fusarium* species. *Fusarium graminearum* was not isolated from the grains of any of the sampled wheat cultivars.

Table 4.3. *Fusarium* spp. diversity in the grains of each sampled wheat cultivar within the study period (September 2016 and October 2017).

Fusarium species	Wheat cultivar and *Fusarium spp*. diversity in the grains at harvest time.																			
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**I**	**j**	**k**	**l**	**m**	**n**	**o**	**p**	**q**	**r**	**s**	**%**
1. F. verticillioides	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	94.7
2. F. equiseti	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	-	-	78.9
3. F. poae	✓	✓	-	✓	✓	-	✓	✓	-	-	✓	-	-	-	✓	-	✓	-	-	47.4
4. F. oxysporum	✓	✓	✓	✓	✓	✓	-	✓	-	-	-	-	-	✓	-	-	-	-	-	42
5. *F. tricinctum*	✓	✓	-	-	-	-	✓	✓	-	-	✓	-	-	✓	-	✓	-	-	-	36.8
6. F. heterosporum	✓	✓	-	-	-	-	✓	-	-	-	-	✓	-	-	-	-	-	-	-	21.1
7. Fusarium sp.	✓	✓	-	-	-	✓	-	-	✓	-	-	-	-	-	-	-	-	-	-	21.1
8. F. culmorum	✓	✓	-	-	✓	-	-	-		-	-	-	-	-	-	-	-	-	-	15.7
Occurrence (%)	100	100	38	50	50	50	63	63	38	25	50	38	25	50	25	38	38	6.5	0	

Key: a- Njoro BWII; b- Eagle Ten; c- Undisclosed wheat cultivar; d- Robin; e-Kenya Ibis; f- Hawk, g- Duma; h- Ruiru; i- Kenya Korongo; j- Kwale; k- Kingbird; l- Simba; m- Ngamia; N- Mwamba; o- Fahari; p- Yombi; q- Kenya Tai; r-Sunbird; s: Kenya Wren. "✓"- *Fusarium* spp. isolated; "-''- Not isolated.

4.4 DISCUSSION

As revealed in the above results the sampled wheat grains were at harvest time already significantly infected with *Fusarium* species. Additionally, some of the identified species in the fungal genus under study are potential pathogens to the wheat crop. Such pathogenic *Fusarium* spp. if not ousted out of the wheat seeds before planting may inherently influence the growth of the plant negatively from germination. Non-decontaminated wheat grains such as those infected with *Fusarium* spp. when used as seeds for the subsequent generation may result into wheat crops with poor vigor and poor stands (Inch & Gilbert, 2003; May *et al*., 2010). Seedling blights may also occur, substantially reducing optimal crop yield (Inch & Gilbert, 2003; May *et al*., 2010). Wheat grains colonized by pathogenic *Fusarium* spp. may be contaminated with harmful mycotoxins as revealed in the previous analysis in this study. Long-term effects of occurrences of such toxins may cause mycotoxicosis in both domesticated animals and human beings when wheat-based foodstuffs originating from such grains are consumed.

Grains harvested from crops of farmer-saved wheat seeds had an elevated prevalence of *Fusarium* spp. compared to grains harvested from crops of certified commercial wheat seeds. Despite making up only twenty-two percent (22.7%) of all the sampled wheat grains, the produce of farmer-saved wheat seeds had a greater occurrence frequency (66.4%) of *Fusarium* spp. than the produce of certified commercial wheat seeds. As a result, farms could be inoculated with FHB-causing agents when such grains are used directly as seeds without any form of processing to reduce the fungal load. Consequently, FHB disease is spread or propagated in this manner in the subsequent crop more so in cases of substandard post-harvest and pre-harvest farming practices. In this view, it is recommended for farmers to consistently use certified and decontaminated seeds of high quality to minimize the initial contamination of farms by fungi infected wheat seeds (Inch & Gilbert,

2003a; May *et al*., 2010) and the subsequent disease effects (Nganje *et al*., 2004). Decontaminating seeds has been demonstrated to increase the yield of grains while decreasing DON levels in wheat produce (Sooväli *et al*., 2017; Xue *et al*., 2017).

Fusarium spp., the species complex responsible for FHB disease were discovered in 94% of the types of wheat cultivars sampled for examination in this study. The elevated percentage in the occurrence of the pathogens is a pointer to the degree of susceptibility to these fungal agents by some of the developed wheat cultivars studied. Nevertheless, the prevalence and spread of *Fusarium* genus within the eighteen (18) wheat cultivars sampled was not evenly distributed. Wheat cultivars that were predominantly cultivated in all the three Counties were Robin, Njoro BWII and Eagle 10. The predominantly cultivated wheat cultivars in all the three Counties contained more than fifty percent (50%) of the *Fusarium* spp. identified in the investigation. Additionally, it is important to note that there was generally low prevalence of *Fusarium* spp. in certain wheat cultivars. Such cultivars included Kenya Sunbird, Kenya Wren, Kenya Kingbird, and Kenya Tai. All of them having been developed for resistance against stem rust and yellow rust. There was either less than fifty percent or zero overall occurrence of the identified *Fusarium* spp. in these cultivars.

The most preferred wheat cultivar (Njoro BWII) by farmers was infected by all the *Fusarium* species identified in the study and contributed to twenty-five percent of the isolated *Fusarium* spp. recovered from the produce of farmer saved wheat seeds. It is also important to note that 6.25% of the wheat grains sampled had undisclosed cultivar identity. Yet they were infected with three species of the isolated *Fusarium* spp. (*F. verticillioide*s, *F. equiseti* and *F. oxysporum*). Almost all the wheat cultivars sampled contained two or more of the isolated *Fusarium* spp. (*F. culmorum, F. equiseti, F. poae, F. verticillioides, Fusarium*. sp., and *F. tricinctum*) which is indicative of the

diversity and prevalence of *Fusarium* spp. in the wheat crop and the potential negative effects the phytopathogens may have on wheat production. Additionally, isolated in this study were the less common or occasional *Fusarium* spp. in cereals, *F. heterosporum* and *F. oxysporum* (Bottalico, 1998). Hence, their detection in the sampled wheat cultivars raises a concern for further research work to ascertain their role in FHB problem.

The absence of *F. graminearum*, one of the principal FHB-causing fungi, in the grains of any of the wheat cultivars studied was noteworthy. Contrarily, this conclusion conflicts with prior studies on occurrences of *F. graminearum* on wheat kernels in two of the Counties (Nakuru and Narok) addressed by this study. In the previously referenced studies, *F. graminearum* was identified, although in very low numbers (Muthomi *et al*., 2012; Wagacha *et al*., 2016). The extended drought that was recorded in Kenya from 2016 to 2017 (Schmidt *et al*., 2017) during the time when the analyzed crop produce was in season may be a contributing factor to why there was no *F. graminearum*. The dry season may have provided unsuitable conditions for the growth of the species. Additionally, the extremely low numbers of *F. graminearium* found in earlier research may also be a sign of a widespread drop in the species' population in the locations under investigation.

Overall, *F. verticillioides* was isolated in higher frequency in the grains of the sampled wheat cultivars. *Fusarium verticillioides* is one of the pathogenic fungi that caused pink ear rot in all the three Counties. As a result, its high prevalence throughout the study's research areas is consistent with earlier research reports indicating that the species has a widespread distribution around the planet (Burgess *et al*., 1994; Leslie & Summerrell, 2006). However, in the previous reports *F. verticillioiedes* was isolated from diseased maize (Munkvold & Desjardins, 1997) and not wheat. Because the species is a serious global pathogen of agriculture and cattle (Nagaraj, 2017), this high

prevalence detected in wheat is of significance since it produces harmful secondary metabolites (fumonisins). This is the first study to detect and report existence of *Fusarium* spp. in the wheat grains from Uasin Gishu County, since most of the existing published reports are for Nakuru and Narok Counties in Kenya.

The diversity and prevalence of the isolated *Fusarium* spp. was not different in spite of the study Counties being in agro-ecological zones that showed slight variations. Nevertheless, some of the elements that make the regions comparable are the similarity of unfavorable weather circumstances, farming methods, and agronomy-related issues. The data from the baseline survey also revealed that other contributing factors included the sharing of farm tillage and wheat harvesting equipment in the cultivation of succeeding crops like maize and barley without disinfection against fungal diseases, the use of unskilled labour, the use of low-quality farmer-saved wheat seeds, the application of less fertilizer, poor weed management and, continuous land use. Documented research reports in other countries around the world, including Kenya, have indicated that these agronomic conditions contribute to the spread and proliferation of FHB in wheat crops (Guo *et al*., 2010; Keller *et al*., 2011; Njeru *et al*., 2014; Njeru *et al*., 2016). According to the findings, maize, wheat, and barley cereal debris frequently served as the first inoculum for FHB on wheat fields. Consistent cultivation of succeeding cereal crops in a poorly managed farm creates avenues for invasion of fungal pathogens to the wheat crop.

Wheat grains collected from Nakuru County contained all of the identified *Fusarium* species. Two primary explanations for this phenomenon are possible: First, for research purposes, the County cultivates a large number of wheat cultivars, some of which may be susceptible to the studied fungi. Additionally, the region's warm and humid climate is conducive to FHB infection. The ability to produce high yields and its durability against wheat rust made Njoro BWII the wheat

grain that farmers preferred over other cultivars. However, based on the findings from this research, the Njoro BWII wheat cultivar harbored all the isolated *Fusarium* species and yet in most cases the grains used in isolation of the characterized *Fusarium* spp. were asymptomatic. There are possibilities that this cultivar indirectly acts as a carrier and hence propagator of the isolated fungal pathogens because it is widely traded among the farmers in the three regions for cultivation.

4.5 CONCLUSION

Molecular characterization of the fungal isolates confirmed that *Fusarium* spp. were prevalent and widespread in the grains of the improved wheat cultivars sampled and assessed. However, a small percentage of them were less susceptible to *Fusarium* spp. based on the evidence that their grains were not infected with the identified *Fusarium* species. A good example was Kenya Wren wheat cultivar. In contrast to certified commercial wheat seeds, the produce from Farmer saved wheat seeds had a higher percentage of *Fusarium* spp. in the grains they produced in general. Additionally, there were no differences in the diversity and prevalence of the identified fungal strains in the three ecological regions of study. A number of the isolated fungi were outgroups in the phylogenetic tree, indicating that either some members of *Fusarium* genus from the three regions have not yet been identified or an indication of possible ongoing changes in population of *Fusarium* species in the ecological regions.

CHAPTER FIVE

MYCOTOXIGENIC POTENTIAL OF *FUSARIUM* SPP. ISOLATED FROM GRAINS OF WHEAT CULTIVARS SAMPLED AT HARVEST TIME IN NAKURU, NAROK AND UASIN GISHU COUNTIES, KENYA

5.1 INTRODUCTION

Fusarium is a genus that exists in agriculture and, some of the life-threatening dangers it poses are generally brought on by the chemical compounds it produces (Zidan, 2020). The mycotoxigenic characteristics of these secondary metabolites are not only vast but also differ a lot in the activities they elicit in different organisms (Perincherry *et al*., 2019). Such activities range from zootoxic to phytotoxic reactions (Zidan, 2020; Perincherry *et al*., 2019) The long term accumulation of these chemicals in receptor cells, tissues and organs of plant crops such as cereals, vegetables and animals often results into multi-organ life-threatening chronic diseases (Zidan, 2020; Perincherry *et al*., 2019). For example, their high levels in certain target body parts may severely limit the functioning of the associated biological systems in exposed animals including human beings (Perincherry *et al*., 2019). These secondary metabolites constitute significantly the main contaminants released by certain fungal genera in crops such as maize, oats, wheat and wheat based products. Their formation in food crops vary depending on the fungal strain, the type of host crops, prevailing ecological and climatic conditions among other predisposing factors (Ferrigo *et al*., 2016; Perincherry *et al*., 2019).

In the genus *Fusarium*, the most commonly detected mycotoxin contaminants in wheat are fumonisins, Zearalenones and DON (Lemus-Minor *et al*., 2018; Nirenberg, 1981; Sadhasivam *et al*., 2017). Under favourable conditions for their growth, specific *Fusarium* spp. that infect cereal crops in fields and food storage facilities are able to emit deoxynivalenol (DON). This metabolite

belongs to the trichothecenes group of mycotoxins and it is mostly generated by *F. graminearum* (Kushiro, 2008). However, there are other potential DON producers such as *F. poae* and *F. culmorum* (Pasquali *et al*., 2016).

Reported DON induced reactions in animals and, specifically in man includes abdominal pain, diarrhea, acute temporary nausea, vomiting, fever, headache and dizziness (Sobrova *et al*., 2010). Health related risks in other animals have been presented by reduced weight gain due to feed refusal (Bergsjø *et al*., 1993; Goyarts & Dänicke, 2006). Another group of toxigenic chemicals synthesized by other members of the genus *Fusarium* (*F. proliferatum, F. oxysporum* and *F. verticillioides*) is fumonisins. These toxic chemicals have been reported to be the causative agents of different animal diseases in rats, pigs and man. For example, in human beings, they have been linked to esophageal cancer (Bucci & Howard, 1996; WHO, 2000; Lumsangkul *et al*., 2019). They are also phytotoxic (Abbas *et al*., 2005, 2013; Ismaiel & Papenbrock, 2015).

The management and control of pathogenic *Fusarium* spp. has not been easy to achieve because of their broad host spectrum and immense variability (Ploetz, 2015). Due to their high diversity in physiological characteristics and morphology, *Fusarium* spp. are adapted to a vast scope of different abiotic and biotic environmental conditions (Ploetz, 2015). Hence, their contribution to global economic losses is enormous. The Food and Agriculture Organization (FAO) reports estimate that approximately 25% of the world's food crops are contaminated with mycotoxins on yearly basis. Consequently, losses of close to a billion metric tons of food products occur (Boutrif &. Canet, 1998; Eskola *et al*., 2020). To curb mycotoxin-related health risks due to the consumption of contaminated cereals-based food and feeds, control measures have been developed to allow only recommended limits of specific type of mycotoxin in food for animal and human consumption. One body held with such a responsibility and that is globally recognized is Codex

Alimentarius Commission (CAC), which was established by the WHO and FAO in CAC/RCP, 5/2003 (Codex Alimentarius Commission, 2017; (Gmp), 2014). To maintain the safety of food, such bodies have established permissible upper limits for *Fusarium* toxins in grains.

Most mycotoxins including trichothecenes and fumonisins withstands high temperatures and cooking does not destroy or denature them. Therefore, prevention or reduction of their production in the field is among the best ways of avoiding health risks related with the consumption of wheat based food products (Perincherry *et al*., 2019). In order to implement effective strategic control in the target hosts, it is essential to establish the existence of such pathogenic populations and, evaluate their frequency of occurrence in sensitive host crops of vast economic importance such as wheat. Additionally, analyzing the occurrences and quantities of secondary metabolites they produce provides essential background data for food safety measures. Hence, the need for determining the frequency of the regulatory genes for the mycotoxin biosynthetic pathway in populations of potential producers of the toxins. Such information forms the basis for evaluation of the quality of the grain for use not only as food staffs and seeds but also for the enhancement of mycotoxin control approaches in the now unpredictable climatic conditions. It is also essential for determining the prevalence of obscure *Fusarium* species. Moreover, the regulatory genes involved in the expression of various phenotypes such as growth of the fungus and generation of mycotoxin appear to be influenced by interactions between abiotic stress inducing factors like temperature and water activities (Medina *et al*., 2015; Perincherry *et al*., 2019). Hence, the need to assess production of the related toxins in wheat cultivars improved for certain qualities and for ecological conditions.

Disease persistence have been one of the major challenging obstacles in wheat production in Kenya (Kamwaga *et al*., 2016). Most of these diseases, especially wheat rust are determined by

differences in geographical zones and the respective prevailing climatic conditions (Kamwaga *et al*., 2016). However, remarkable achievement in some of the improved wheat cultivars such as the Kenya Kingbird, a good parental line in breeding for rust diseases has been reported. The line has exhibited a level of resistance to both yellow rust and stem rust. Nevertheless, previous research work in Kenya has shown incidences of *Fusarium* spp. infestation in wheat crop (Muthomi *et al*., 2008, 2012; Wagacha *et al*., 2010, 2016). To attain the full food safety potential of the improved wheat cultivars in Kenya, it is appropriate that regular assessment of incidences of infection of such wheat cultivars by *Fusarium* spp. be carried out. This is because of the unstable changing climatic and other ecological conditions unique to specific fields. Determination of the occurrence of the potential fungal pathogens in improved wheat cultivars in specific geographical wheat growing locations in Kenya and, analysis of the frequency of genes determining mycotoxin synthesis in populations of *Fusarium* genus existing in different ecological regions is prerequisite knowledge for undertaking effective food-safety control measures in the interest of sufficiency and mycotoxin contaminations.

Various approaches have been employed worldwide in control of *Fusarium* spp. infection and mycotoxin contamination in wheat. Among such approaches are chemical and biological approaches while others are physical in nature (Prasad *et al*., 2016; Shah *et al*., 2018; Wan *et al*., 2020). Yet none of them can completely eradicate the problem. Hence, there is desperate need for continuous systematic research (Prasad *et al*., 2016; Shah *et al*., 2018; Wan *et al*., 2020) that encompasses routine analysis of the mycotoxigenic ability of the predominant disease causative agents and their respective amounts of secondary metabolites contaminating wheat-based products. Determination of the mycotoxigenic ability can be carried out in various ways. These approaches include toxicity confirmation tests and molecular biology procedures (PCR-

Polymerase Chain Reactions) based on particular primers for the relevant genes (Omori *et al*., 2018; Sadhasivam *et al*., 2017; Sampietro *et al*., 2010). The two approaches help to ascertain the presence of the gene cluster required in the biosynthesis of mycotoxins and, evaluates the occurrence and, amounts of the corresponding synthesized mycotoxins (Omori *et al*., 2018; Sadhasivam *et al*., 2017; Sampietro *et al*., 2010). Background information on the overall frequency of genes involved in the mycotoxin biosynthetic pathway in the pathogenic *Fusarium* spp. complex and the quantities of associated toxins is crucial for enhancing or strengthening disease-resistant wheat cultivars, which in turn reduces the buildup of associated toxins in the fields. This study assessed the levels of fumonisins in wheat grains at harvest time in as pointer to the mycotoxigenic potential in populations of *Fusarium* spp. isolated from grains of improved wheat cultivars. The frequency of genes that encode for the formation of fumonisins and DON in *Fusarium* spp. isolated from grains sampled in three separate wheat-growing geographical locations in Kenya (Nakuru, Narok, and Uasin Gishu Counties) was also analyzed.

5.2 METHODOLOGY

5.2.1 Materials and methods

5.2.2 Sampling of wheat grains, isolation and characterization of *Fusarium* species

Samples of wheat were taken at different wheat-producing locations on randomly chosen wheat farms that were in the process of being harvested in three of the main wheat-producing Counties of the Kenyan Rift Valley: Narok, Nakuru, and Uasin Gishu Counties. The wheat grains were collected in sterile khaki papers using spear-sampling technique (El-Shall & Moudgil, 2014; Freese *et al*., 2015). At least three samples were collected from every farm. However, from large sized farms more samples were collected, 21 samples at most. For every cultivar of wheat, replicates of three samples were collected per sampling site on every farm. Approximately 500gm of grains for each wheat cultivar were packed. Two hundred and sixty (260) samples from 123 farms were obtained during harvesting time. This was followed by labelling that included the details on farm location and information on the identity of each sampled wheat cultivar. A total of eighteen wheat cultivars (Robin, Eagle Ten, Ibis, Njoro BWII, Korongo, Farasi, Mwamba, Hawk, Ruiru, Simba, Ngamia, Duma, King Bird, K. Tai, Yombi, K. Wren, Kwale, K. Sun Bird) were collected. Another category of the sampled grains was classified as undisclosed since some of the farmers declined to disclose the identity of the cultivar of the wheat seeds they planted. Ninety randomly selected grains of each wheat cultivar were plated on selective media (Peptone Pentachloronitrobenzene) for isolation of *Fusarium* species followed by characterization of the isolates based on the procedures described by Nelson *et al*., (1993) and Nirenberg, (1981). Fungal isolates with characteristic features for *Fusarium* genus were purified and stored at -80°C for further analysis to ascertain species identity and for determination of their toxigenic potential. For

analysis of fumonisins, only 13 of the cultivars that were frequently sampled in at least two of the three Counties were used.

5.2.3 Determination of the frequency of genes encoding production DON and fumonisins in *Fusarium* spp. isolated from grains of wheat cultivars

Fusarium spp. isolated from grains of several categories of wheat cultivars were cultured on PDA media for approximately seven days before harvesting for the extraction of DNA that was used to assess the genetic identity and, the ability of each isolate to synthesis mycotoxins (DON and fumonisins). For each isolate, the growing culture mass was scraped into three Eppendorf tubes using a sterile spatula. To obtain a fine product, the mycelia was ground with 2mm wolfram beads using RetschMM 400 Mixer Mill-30Hz, for three minutes. The needed total fungal DNA was extracted using the ZR Fungal/ Bacterial DNA Miniprep extraction kit (ZYMO Research, USA) in accordance with the manufacturer's instructions. Aproximately 100mg wet weight of mycelium was used. The amount of DNA retrieved was measured using a NanoDrop spectrophotometer from Thermo Scientific in the United States. The identity of *Fusarium* spp. had to be established based on PCR amplification of the internal transcribed spacer (ITS) region of the rDNA using the universal primers ITS1 (5'-TCCGTAGGTGGAACCTGCGG-3') and ITS4 (5-TCCTCCGCTTATTGATATGC-3') in order to identify the isolates with the genetic capacity to produce mycotoxins (Walker *et al.*, 2012). The regions between the large nuclear 28S rDNA and the small nuclear 18S rDNA, as well as 5.8S rDNA, were the ones that were amplified. A Thermal Cycler (Applied Biosystems 9700) with reaction mixtures measuring 25.0µl and including the following elements: 60ng to 100ng of genomic DNA, 10mM of each ITS-1 and ITS-4 primer, and a premix of two x12.50µl portions, 9.50µl of water free of nucleases were used to amplify the DNA. The amplification cycle started with the denaturation process at 94ºC. Following a three-

minute step, there were 35 cycles at 94°C for 30 seconds, 56°C for 1 minute, 72°C for 2 minutes, and an 8-minute terminal extension at 72°C. In 1X TAE buffer at 70V for 50 minutes, amplified PCR products were separated on an agarose gel (1.5% w/v) by electrophoresis. Against a DNA ladder of 1 kb, the DNA amplicons for identity determination were defined at 500 bp to 550 bp. (Walker *et al.*, 2012).

The method outlined by Chandler *et al*., 2003 and Bluhm *et al*., 2004 was used to conduct analysis to determine the genetic capacity of the identified *Fusarium* spp. isolates to synthesize trichothecenes and fumonisins using Tri13F/Tri13DONR and FUM1F/FUM1R specific primer pairs respectfully (Table 5.1). Tri13DON and FUM1 genes, which encode for the formation of DON and fumonisin respectively, were the targeted mycotoxin-synthesis pathway genes in this study. The Veriti Thermal Cycler (Applied Biosystems, USA) was used to carry out the PCR amplification reaction in accordance with the predetermined time and temperature parameters. The amplification program went according to the following: 94 degrees for 3 minutes, 94 degrees for 45 seconds, 58 degrees for 45 seconds, 72 degrees for 1 minute, 35 cycles, and finally 72 degrees for 5 minutes of final extension (Lenart *et al*., 2013; Wolny-Koładka *et al*., 2015). By using 1TBE electrophoresis on an ethidium-bromide-stained 1% agarose gel, the PCR result was visualized.

Table 5-1. Primers used to determine prevalence of genes encoding the production of fumonisins and DON in *Fusarium* spp. isolated from grains of wheat cultivars.

Set of Primer references used	Base sequences (3'- 5'Sequences)	Length of expected Product (bp)	References
FUM1F	CCATCACAGTGGGACACAGT	183	Bluhm *et al*.,
FUM1R	CGTATCGTCAGCATGATGTA		2004
TRI13F	CATCATGAGACTTGTKCRAGTTTGGG	282	Chandler *et*
TRI13DONR	GCTAGATCGATTGTTGCATTGAG		*al*., 2003

5.2.4 Determination of levels of fumonisins in grains of different wheat cultivars sampled at harvest

5.2.4.1 *Organization of wheat samples for extraction of fumonisins*

The analysis included thirteen (13) different types of wheat cultivars. In three-replicate analyses, fifty-four (54) composite samples made up of all wheat grain gathered using the methods described in chapter were analyzed. Each representative sample was processed through a Romer Series II Mill to a fineness of 95% particle flour, which could pass through a screen with a mesh size of 0.84mm. Then, 100ml of a 70/30 (v/v) methanol/water extraction solution was added to twenty (20) grams of each sample of wheat flour that had been weighed in a clean container. The sealed jars were then vigorously shaken in an orbital shaker for three minutes at 250 revolutions per minute (rpm). The sample to extraction solution ratio used for extraction was 1:5 (w: v). The content of every sample was allowed to settle before the supernatant was filtered using Whatman 1 filter paper. For the last stage of total fumonisin detection, the filtrate was diluted using de-ionized water at a ratio of 1: 20.

5.2.4.2 *Analysis of fumonisin levels*

Using the AgraQuant® Total Fumonisins Assay 0.25/5.0 ELISA kit from Romer Labs Singapore Pte. Ltd. and following the manufacturer's instructions, fumonisins were detected and analysed. The green-bordered dilution strips were first arranged in the proper quantity in a micro-well strip holder. For the standards (i.e. 0ppm, 0.25ppm, 1.0ppm, 2.5ppm and 5.0ppm), five dilutions wells were used. A corresponding number of antibody-coated micro-well strips were placed in another micro-well strip holder. Into each of the green-bordered dilution wells, 200µl of the required amount of conjugate was put. Additionally, into each of the wells containing the same 200µl of the conjugate containing, 100µl of either each standard or test sample was added into each well using a single channel pipette. For every addition of either the standard or the test sample, a fresh pipette tip was used. An 8-channel pipette with fresh tips for each of the 8-well strips was used to pipette the contents of each well up and down three times in order to thoroughly mix the contents in each well. Immediately 100µl of the mixed content in each well was placed into the corresponding antibody-coated micro-well. Next, was the incubation of the preparations for 10minutes at room temperature. Contents of the micro-well strips were then emptied and each well washed five times by filling it with deionized water and then discarding the water from the micro-well strips for the five consecutive washes. To get rid of the residue completely, the micro-well strip was tapped on many layers of absorbent papers after every wash. After drying the bottom of the micro-wells, 100µl of the substrate was dispensed into every micro-well using an 8-channelled pipette. The analyzed stripes containing the contents were then incubated at room temperature for five minutes. Using an 8-channel pipette, the stop solution (100µl) was then pipetted into each micro-well strip. A micro-well reader with a 450nm filter and a 630nm differential filter was used

to read the strips. The optical density (OD) values for each micro-well were measured and then expressed as a percentage of the standards' optical density of zero (OD0).

Five standards with known levels of fumonisins were used to create a dose-response curve, and the amounts in the samples were calculated by extrapolating from the standard curve. The calibration curve's correlation coefficient (r) ranged from -0.99 to -1.00. The final reading was calculated using the Romer® log/logit spreadsheet, and the log/logit regression model was utilised to analyze the results. The quantification limit was 0.25ppm, while the lower limit for total fumonisins detection was 0.2ppm.

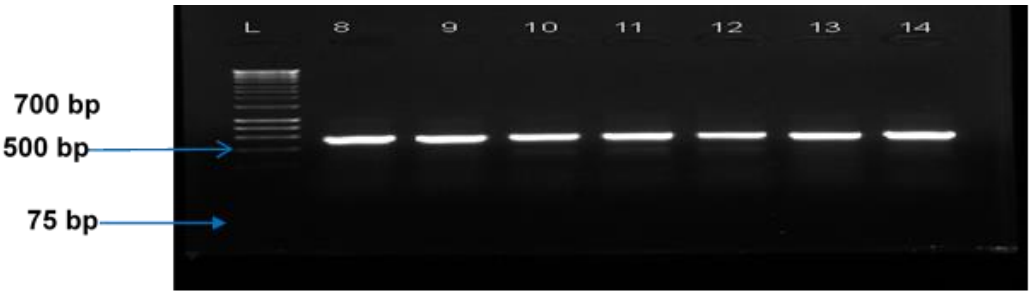
5.2.5 Data analysis

One Way ANOVA was used to test for any significant differences in the levels of fumonisins in grains of wheat cultivars in the three Counties. The Tukey HSD test was used for overall comparison of total fumonisin levels in the wheat cultivars. Njoro BWII wheat cultivar was sampled in each of the three Counties, whereas the other wheat genotypes (Eagle10, Robin, and Kwale) were only found in two of the Counties. Since Njoro BWII wheat cultivar was grown in all the three regions, the levels of fumonisin in samples from the three counties were compared. Hence, fumonisin levels for the Eagle10, Robin, and Kwale wheat cultivars were only compared within the Counties where they were sampled. Parametric test (Independent sample T-test) was used to assess whether there were any notable differences in fumonisin levels between the Counties for other analyzed cultivars.

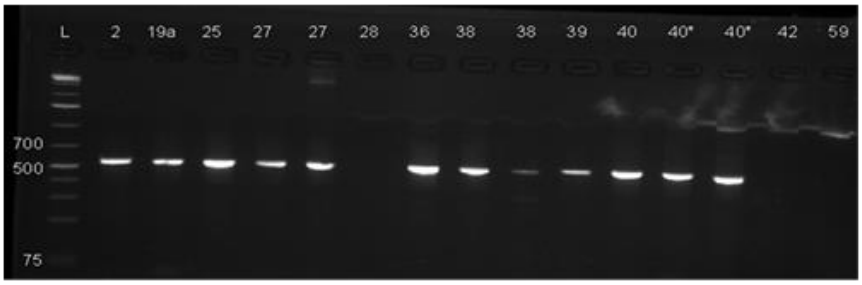
5.3 RESULTS

5.3.1 PCR confirmation of the genus and species identity of the fungal isolates

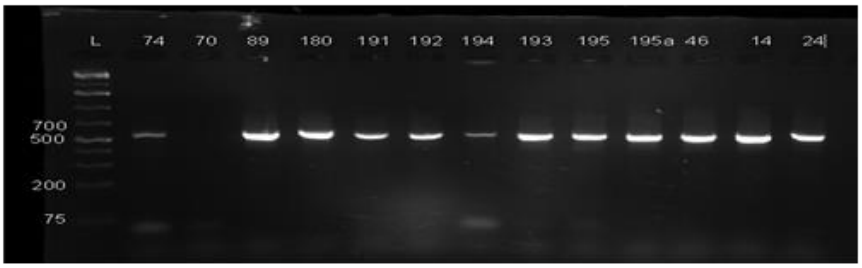
The isolates that had been morphologically identified were further confirmed as members of the genus *Fusarium* based on an amplified DNA band (Figure 5.1) that matched a fragment total size of between 500bp. and 550 base pairs. This result was based on the PCR amplification of the DNA of each of the isolates using the conserved ribosomal ITS (Internal Transcribed Spacer) regions, including the 5.8S rDNA gene. Since each species of the isolated *Fusarium* genera were to be analyzed for prevalence of genes encoding the production of fumonisins and DON their species identity was ascertained next using translation elongation factor 1- (tef1-) gene set of primers, Ef1 (forward primer; 5' -ATGGGTAAGGA (A/G) GACAAGAC-3') and EF2 (reverse primer; 5' -GGA (G/A) GTACCAGT (G/C) ATCATGTT-3'). The PCR results for each isolate were positive, with each fungal isolate producing a fragment size of 700 base pairs.



Isolates' Codes: 8- CT8, 9- CT9, 10- CT10, 11- CT11, 12- CT12, 13- CT53 and 14- CT44.



Isolates' Codes: CT2, CT19, CT25, CT27, CT28, CT36, CT38, CT39 and CT40.



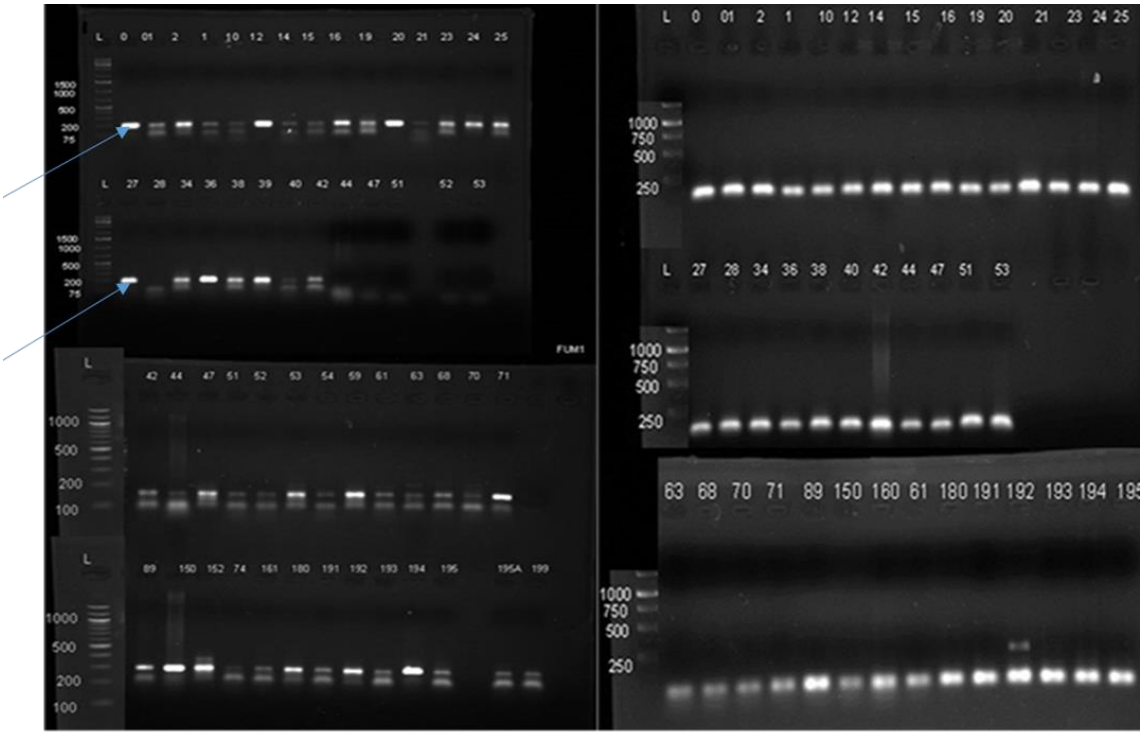
Isolates' Codes: CT74, CT70, CT89, CT180, CT191, CT192, CT194, CT193, CT195, CT195A, CT46, CT14 and CT24.

Figure 5-1. Agarose 1% Gels electrophoresis showing PCR products of the DNA for *Fusarium* spp. isolates obtained with the universal primers ITS1R and ITS4F.

5.3.2 Prevalence of genes encoding production of DON and fumonisins in *Fusarium* spp. isolated from grains of wheat cultivars

Based on the amplification of FUM1 gene, the mycotoxigenic potential of the recovered *Fusarium* spp. was confirmed with isolates derived from wheat types collected in Narok County containing 54% of the detected gene. The remaining percentages (25% and 21 %) was contained in *Fusarium* spp. isolated from wheat cultivars sampled in Uasin Gishu and Nakuru Counties respectively.

Tri13DON biosynthesis pathway gene was not detected in any of the assessed isolates (Figure 5.2), since the size (282bp) of the expected PCR amplified band for positive results was not realized.



Detection of the gene responsible for the synthesis of fumonisins

Detection of the gene responsible for the synthesis of DON

Figure 5-2. Agarose 1% Gels electrophoresis showing PCR results FUM1 and DON genes in *Fusarium* spp. isolates. Primer pairs used and expected positive results: FUM1F/FUM1R (183bp) and TRI13F/TRI13DONR (282bp).

5.3.3 Occurances and total fumonisins levels in the grains of wheat cultivars sampled at harvest in three of the major wheat producing Counties in Kenya

The first analysis done to determine the occurrence of fumonisins showed their presence in almost all the wheat grains sampled from each region (Figure 5.3). In general, the levels of fumonisins detected were low: Narok- 7.76ppm, Uasin Gishu- 10.36ppm and Nakuru- 3.36ppm. Significant differences were found when the fumonisin levels of all the wheat cultivars sampled in the three Counties were compared ($p<0.001$). Wheat grains from Uasin Gishu County had fumonisin levels that were noticeably higher than in those from Narok and Nakuru Counties (Figure 5.3).

Additionally, a pairwise analysis using the Tukey HSD test revealed that the fumonisin levels between Nakuru and Narok were significantly different (p=0.002). Similarly, fumonisin levels between Nakuru and Uasin Gishu ecological regions were different (p=0.011). On the contrary, the total fumonisin levels between Uasin Gishu and Narok Counties ware not significantly different (p=0.537).

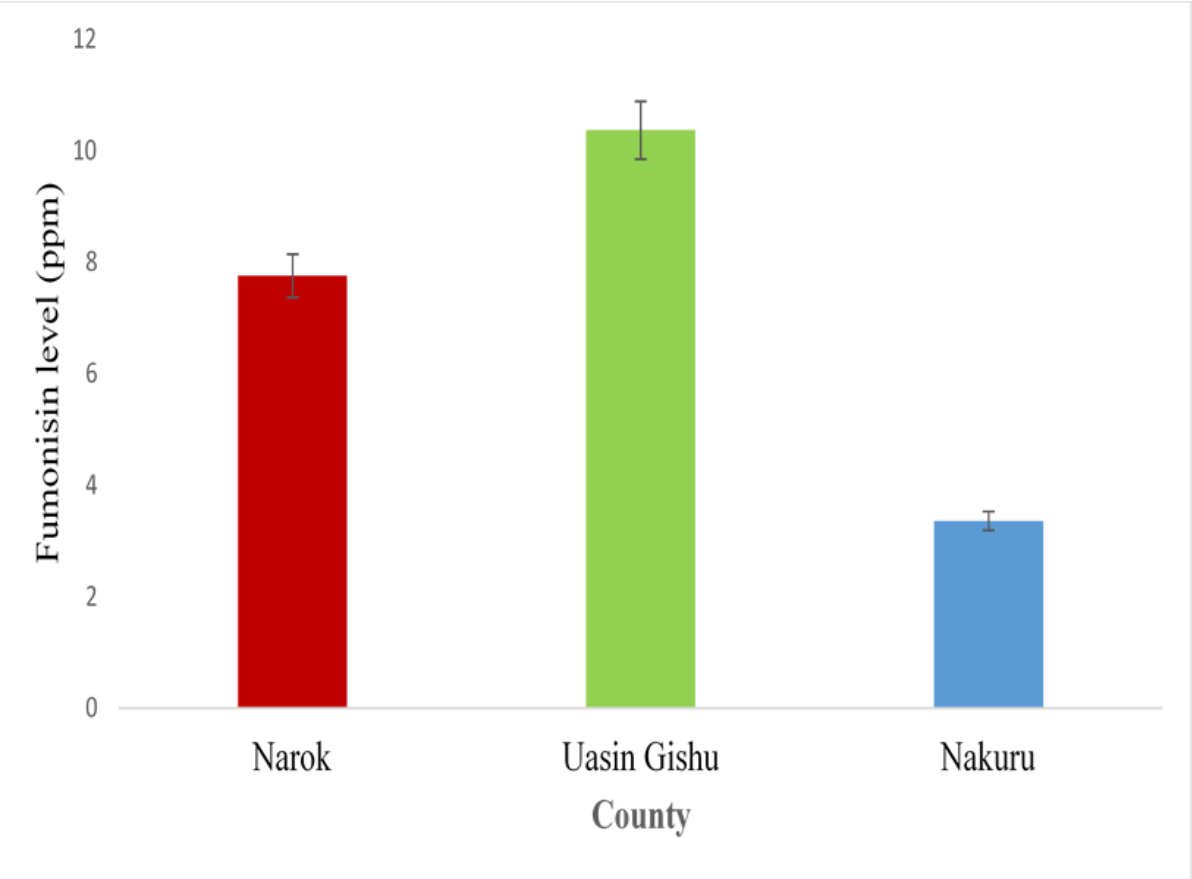


Figure 5-3: **Fumonisins levels in wheat grains sampled at harvest in Narok, Uasin Gishu and Nakuru Counties, Kenya.**

Comparing the amounts of fumonisins in the grains of each tested cultivar of wheat, it was discovered that 76% of them had detectable quantities, while the remaining 24% had undetectable levels. Robin and Njoro BWII wheat grains contained the highest (9.6ppm and 9.5ppm) levels of fumonisins. Differences in total fumonisin levels were observed between some of the assessed wheat cultivars (Figure 5.4). Njoro BWII and Kenya Ibis wheat grains showed significant

differences ($p<0.05$) in the detected fumonisin levels. However, the total fumonisin levels detected between Njoro BWII and other wheat cultivars such as Robin, Korongo and Eagle 10 were not significantly different, ($p>0.05$). Minimal levels of fumonisins were detected in grains belonging to Kenya Tai and King Bird wheat cultivars (Figure 5.5) while no fumonisins were detected in the grains of Kenya Wren cultivar.

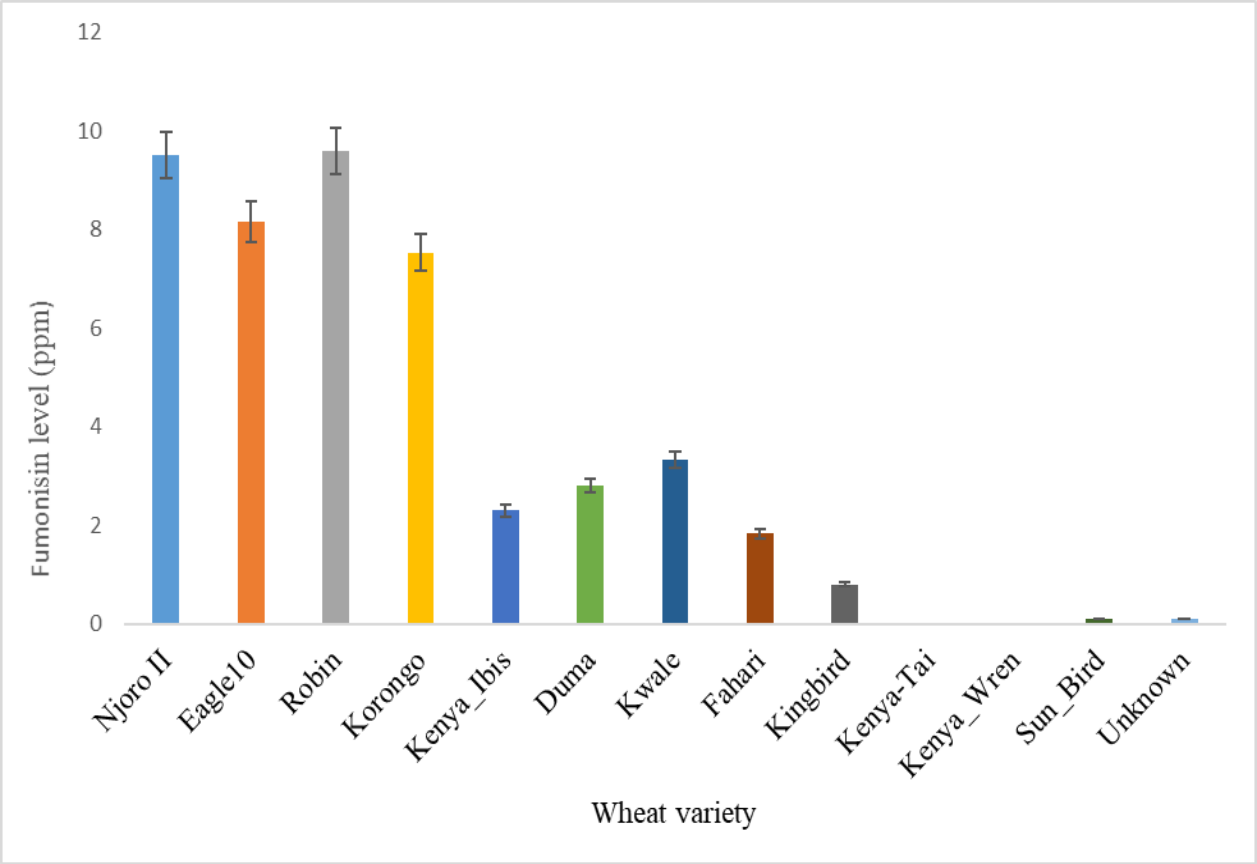


Figure 5-4: Comparison of fumonisins levels in grains of wheat cultivars at harvest from adjacent wheat producing Counties in the Kenyan Rift Valley.

The most sampled wheat cultivars i.e. Eagle 10, Njoro BWII, Kwale and Robin from the three regions were subjected to further analysis to determine differences in fumonisin levels among the Counties. Fumonisin levels in Njoro BWII wheat cultivar were significantly different ($p = 0.001$) among the three Counties (Figure 5.6). Fumonisin levels in the Njoro BWII wheat cultivar were

not significantly different between Uasin Gishu and Narok Counties, according to further analysis using the Tukey HSD test (p= 0.99). The Fumonisin levels detected in Eagle 10 wheat grains between Narok and Nakuru revealed significant differences ($p<0.05$). Likewise, the analysis of fumonisin levels in grains of Kwale wheat cultivar was also significantly different ($p<0.05$) between Nakuru and Narok Counties. However, there were no significant variations in fumonisin levels in the grains of Robin wheat cultivar across Nakuru and Narok Counties ($p > 0.05$). For Narok and Nakuru Counties, Kwale wheat cultivar had the least fumonisin levels, at 4.85ppm and 1.8ppm respectively.

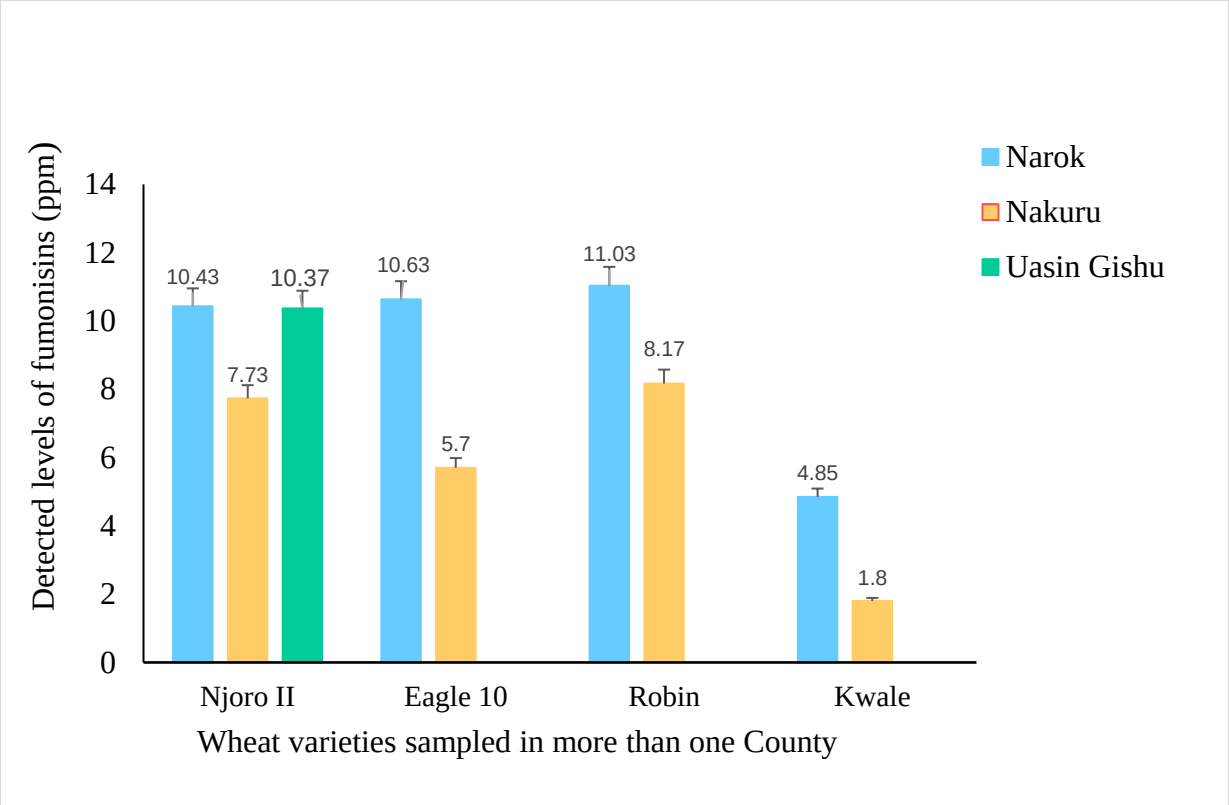


Figure 5-5. Levels of fumonisins in grains of wheat cultivars sampled in high frequence in Narok, Uasin Gishu and Nakuru Counties, Kenya.

5.4 DISCUSSION

Based on the above PCR results, only FUM1 gene was detected. Sixty percent (60%) of the screened *Fusarium* spp. isolates contained the gene, with *F. verticillioides* having the highest percentage occurence frequency. Of all the analyzed *F. verticillioides*, 89% of the isolates contained the gene while 11% did not. The highest occurance frequency of FUM1 was found in *F. verticillioide*s isolated from grains collected in Narok County. Compared to Nakuru and Uasin Gishu Counties, Narok County is drier and, *F. verticilliodes* grows well in such arid environment. The existence of the gene (Tri13DON) that encodes the production of DON was examined in several *Fusarium* species (*F.* c*ulmorum*, *F.* poae, *Fusarium* sp., *F. tricinctum*, and *F. equiseti*). However, none of the examined *Fusarium* spp. contained the gene. Evidently, in a related research work by Wolny-Koładka *et al*., (2015) Tri13DON was detected in *F. poae*. The disparity in the research findings may be the result of intraspecies differences brought on by variations in ecological zones and hence the geographic separation of the *Fusarium* species. Geographical separation causes genetic isolation, which can have a significant impact on the means of reproduction used, resulting in genetic variations and strain responses to ecological conditions. However, with regards to *F. equiseti*, *F. culmorum*, and *F. tricinctum* in which Tri13DON gene was not amplified, the findings are consistent with report by Wolny-Koładka *et al*., (2015). The study reports differences in PCR determined prevalence of genes encoding production of fumonisins and DON and, hence the toxigenic ability in *Fusarium* spp. isolated from the sampled wheat cultivars. Comparable findings about differences in the prevalence of the regulatory pathway genes governing the synthesis of certain mycotoxins generated by *Fusarium* spp. in wheat crops have been reported (El Yazeed *et al.*, 2011; Sadhasivam *et al*., 2017).

Tolerable limits of fumonisins can be exceeded in the grains when crop management approaches at various wheat production steps favour toxin formation and when under unfavorable weather conditions. The high incidences of FUM1 gene in the evaluated *Fusarium* spp. isolates necessitated additional investigation into the presence and concentrations of fumonisins in the respective wheat grains, particularly at the time of harvest. Hence, detectable levels of fumonisins occurred in 76% of the analyzed wheat grain samples. Chehri and colleagues (2010) also found that due to infestation of wheat grains by *F. verticillioides* and other causal fungi, more than 68.2% of the analyzed wheat grains contained significant levels of fumonisins. Notably, according to EU Commission Regulations- EC, NO. 1881/2006 (European Commission, 2006) and [JECFA,56(2001),74(2011) (JECFA, 2017)] the levels of fumonisins detected were generally lower than the acceptable maximum limit (2000–4000µg/kg) in unprocessed cereal grains such as maize (*Zea mays*) and other grains or flour. However, the presence of such toxins in the grains at harvest should be a concern for food safety measures considering the health risks associated with the consumption of such contaminated grains in both human and domesticated animals over a long period of time. This is because in Kenya, some farmers prefer to boil freshly harvested wheat grains for consumption while domesticated animals like cattle and goats feed on wheat remains on farms immediately after harvesting.

Wheat grains from Nakuru County had the lowest levels of fumonisins when compared to those from Uasin Gishu and Narok Counties. Uasin Gishu County had the highest levels and yet *F. verticillioides* isolated from the wheat grains sampled in the County had a lower (25%) occurrence frequency of FUM1 gene. In contrast, Narok County in which the isolated *F. verticilliodes* had a higher (54%) occurrence frequency of FUM1 gene less fumonin levels were detected in the grains. Such differences could possibly be due to variation in the existence of fumonsin producing

chemotypes in the three regions of study. Previous studies have shown that the main producers of fumonisins are *F. verticillioide*s, *F. proliferatum* and, several other members that harbor the entire fumonisin gene cluster involved in the biosynthesis of the toxins (Leslie & Summerrell, 2006; Proctor *et al*., 2008). It has also been reported that chemo-type differences have a significant influence on pathogen fitness (Zhang *et al*., 2012) and, they have also been shown to produce varying amounts of fumonisins (Proctor *et al*., 2013). These reports support the findings of the current study in that among the members of *F. verticillioides* isolated some did not harbor FUM1 gene while those that contained the gene resulted into either detectable or non-detectable levels of fumonisins in their respective host wheat grains. Also important is the fact that some of the *Fusarium* spp isolates that did not fall under any of the four clades in the phylogenetic tree shown in the above results occurred in grains sampled from Narok County.

Other than intraspecific variation in the populations of *F. verticillioides* isolated from the sampled wheat grains, there may have been other factors that influenced and determined the production and accumulation of the detected fumonisin levels. The cultivation of maize and wheat on nearby farms or on the same farms but in different seasons was, for instance, frequently observed in wheat fields during the collection of wheat samples in the Uasin Gishu region. Such agricultural practice facilitates the growth and spread of *Fusarium* spp. in food crops that are vulnerable to it (Keller *et al*., 2011; Njeru *et al*., 2014; Njeru *et al*., 2016). This may account for the larger population of fumonisin producers seen in this area. Additional variations resulting from agro-economic factors included, among others, the quality of the wheat seeds planted, fertilization, ploughing, hallowing, and fungus control measures implemented by individual wheat farmers. The two main wheat types that were sampled from the wheat fields in Uasin Gishu County were Robin and Njoro BWII. The increased incidence of fumonisins from the area may be related to the fact that the same wheat

cultivars had a higher occurrence frequency of *F. verticillioides* compared to all the other wheat cultivars analyzed. In comparison to the grains of other wheat types, some of the wheat cultivars tested, including Sunbird, Kenya Tai, Kingbird, and K. Wren had negligible or undetectable levels of fumonisins. Apparently, all these cultivars were collected exclusively in Nakuru County and hence the low overall fumonisin levels detected in the grains from the region.

Fusarium verticillioides was the main fumonisin producing fungus isolated from most of the sampled wheat cultivars. Therefore, the occurrence and levels of fumonisins detected is majorly due to the high prevalence of the fungal strain in the region. This finding is supported by existing research reports on the worldwide prevalence of fumonisins contamination in cereals and, mainly due to infestation by *F. verticillioides* (Cendoya *et al*., 2018; Rheeder *et al*., 2002; Sampietro *et al*., 2010). Similar to this, instances of measurable fumonisin levels have been reported in western Kenya, including some areas of Uasin Gishu (Mutiga *et al*., 2015). While maize crop is the host plant for pathogenic *Fusarium* spp. that has received the most attention in the research studies listed, the current study concentrated on wheat.

There existed noticeable differences in the occurrences and levels of fumonisins in certain wheat cultivars assessed in this study. For instance, the grains of Kwale wheat cultivar sampled in Narok and Nakuru Counties had minimal (4.85ppm and 1.8ppm) quantities of fumonisins, respectively. The same wheat cultivar was noted to have some degree of resistance to the generation and buildup of *Fusarium* toxins like DON in one of the earlier investigations done in Kenya by Otieno & Njeru, (2014). One of Kenya's modified wheat cultivars, Kenya Wren, has traits that make it resistant to wheat rust (Kamwaga *et al*., 2016). It is important to note that neither *Fusarium* spp. nor fumonisins were detected in the examined grains of these particular wheat cultivar. This finding might be attributed to the fact that these wheat cultivars (Kwale and Kenya Wren) may be having

some degrees of tolerance or resistance to *Fusarium* spp. infestation, emanating from their traits for resistance against wheat rust.

Incidentally, there were also cases of undetectable levels of fumonisins in grains from which FUM1 containing *Fusarium* spp. had been isolated. Some of the possible explanation to this phenomenon may include the prevailing ecological factors that may have affected the expression of the gene (Alexander *et al*., 2009) in the regions studied and other internal genetic factors. Important in the modulation of fumonisins production is drought related stress during the development and maturation of wheat grains and, hence a contributing factor as well to infestation of wheat by *F. verticillioides* and fumonisins production (Ferrigo *et al*., 2014, 2016; Medina *et al*., 2015). For a period of over a year, starting from 2016 through to the year 2017, Kenya experienced a period of drought (Schmidt *et al*., 2017) that affected many crops including wheat. The reduced amounts of rainfall that prevailed may have consequently resulted into low water level related stress in the crop hence predisposing it to susceptibility to infection by *F. verticillioides* and the subsequent production of the fumonisins. It has been reported that *F. verticillioides* has low pathogenicity (Parsons & Munkvold, 2012) and high adaptability to hot conditions (Ferrigo *et al*., 2016).

5.5 CONCLUSION

Mycotoxigenic ability of *Fusarium* spp. populations infecting different cultivars of wheat sampled in three major wheat-producing Counties within the Kenyan Rift Valley was ascertained. Tri13DON gene encoding formation of DON was not detected in all the *Fusarium* spp. isolates suspected to contain the gene. Fumonisins and FUM1 gene was detected in wheat grains and *Fusariu*m spp. respectively in the three regions of study. The gene has a wide spread in populations of *F. verticillioides* infecting the improved wheat cultivars in the regions studied. However, a

higher occurrence frequency of the gene was detected in *Fusarium* spp. infecting wheat in Narok County than in Nakuru and Uasin Gishu Counties. The quantities of detected fumonisins were not directly correlated with the frequency of the FUM1 genes in each region. Seventy-six percent (76%) of the wheat samples evaluated contained fumonisin contamination; however, the amounts were within the permitted range for consumption. The toxins were found in higher concentrations in the wheat grains sampled in Uasin Gishu County. Not all of the wheat cultivars evaluated in the study had detectable levels of the analyzed mycotoxins. Finally, yet importantly, it is advised to take proper precautions while handling freshly harvested wheat grains to lessen or eliminate any potential long-term health hazards that may arise from consuming freshly harvested, unprocessed whole wheat-based foods by both people and domesticated animals.

CHAPTER SIX

A SURVEY ON THE OCCURRENCE AND LEVELS OF DEOXYNIVALENOL AND FUMONISINS IN MARKET WHEAT PRODUCTS SAMPLED IN NAROK TOWN, NAKURU CITY AND NAIROBI, THE CAPITAL CITY OF KENYA

6.1 INTRODUCTION

More than two-thirds of global wheat is used for human food, 20% is used for livestock feed and, another 3% to 5% each for seed, industrial uses, and other use. However, mycotoxin contaminants are a major risk associated with wheat consumption. Mycotoxins are a group of secondary metabolites produced by certain populations of fungi and are among the major contaminants in cereals and cereal flour-based products. Common classes of harmful mycotoxins are aflatoxins, ochratoxins A, fumonisins, trichothecenes, and zearalenone (Alshannaq & Yu, 2017; Lee & Ryu, 2017; Savi *et al*., 2015; Stanciu *et al*., 2015; Wagacha *et al*., 2016). Consumption of mycotoxin contaminated human food and feeds has been reported to cause diseases in the consumers. In animals, fumonisins have been reported to cause neurotoxic, hepatotoxic, nephrotoxic effects and to be carcinogenic in man (International Agency for Research on Cancer, 2002; Voss *et al*., 2006) and specifically linked to esophageal cancer (Kimanya, 2015). In Kenya, high incidences of esophageal cancer patients seeking medical care at Moi referral hospital had been reported, with the incidences being higher in patients from the Nandi community compared to other communities in Kenya, as a result of exposure to fumonisins (Wakhisi *et al*., 2005). Exposure to fumonisins has also been associated with cardiovascular diseases (Kigen *et al*., 2017).

The prevalence of fumonisins producing *Fusarium* spp. has been reported in cultivars of wheat cultivated in Kenya (Kheseli *et al*., 2021). In another study using whole wheat grains and wheat

flour collected from retail markets in the main cities of Punjab and Pakistan, fumonisin B1 (FB1) were analyzed and 90% of the samples were positive for the secondary metabolite with 63% of these positive samples having FB1 concentrations higher than the European Union maximum limit (Fatemeh *et al*., 2016).

DON is a major mycotoxin from the trichothecene group. It inhibits protein synthesis, impairs nutrient intake, affects hematopoiesis, induces neuroendocrine effects, and affects growth, reproduction and immune function after chronic exposure (Payros *et al*., 2016). *Fusarium graminearum,* a pathogen in wheat causes *Fusarium* head blight (FHB) and is the main producer of Trichothecenes (Khan *et al*., 2020).

Wheat (*T. aetivum*) as indicted earlier is a crop in the graminaceae family and a widely cultivated grass for its seed. The seed is a cereal grain that is consumed by 2.5 billion people in 89 countries and it is a significant global staple food. In low and middle income countries wheat is the only grain that provides more proteins and has more calories than rice (Shewry & Hey, 2015). The grains are usually milled into either finely processed white wheat flour or rough whole-wheat flour. Occasionally, the grains may be washed and boiled for human consumption as a whole meal. The grains and their husks may be given as feeds to domesticated animals such as cattle. Different regions in the world have varying legal standards for whole grain flour that is permitted. In this study, the definition of whole grain is according to the recommendation by Health Grain Forum (Ross & Andrew Ross, 2017) and Whole Grain Initiative (Grain *et al*., 2020), while whole wheat flour food stuffs refer to food products elaborated with flours ranging from wheat.

Based on the region of the world, wheat can be eaten as bread that is flat, leavened, unleavened, or processed into pasta, or couscous. However, more often than not, the flour resulting from ground wheat is used chiefly in the production of bread, muffins, noodles, varied kinds of pasta (macaroni

or spaghetti), different types of biscuits, cookies, pastries, cakes, cereal bars, sweet and savory snack foods among many others such as crackers.

Both refined wheat flour and whole-wheat flour have significant amounts of valuable human nutrients in the following approximate measures respectively: Carbohydrates- g/100g - 71. 31 and 71.2, Fiber- 2.7 and 10.6, protein 10.33 and 15.1, lipids mg/100g 0.98 and 2.73, Calcium mg/100g- 15 and 38, Iron mg/100g 1.17 and 3.86, Magnesium mg/100g 22 and 138, Phosphorus 108 and 152 and Potassium 107 and 372 (Gómez *et al*., 2020). Wheat flour products have other nutritional values. For example, based on evidence in epidemiological studies, the outcome of consuming products of whole-grain flours include effects on reducing the risks of loss of lives and incidences of a broad range of illness that are not contagious (Reynolds *et al*., 2019). Among these advantages are reduction in risks associated with diseases such as type 2 diabetes, certain types of cancer and cardiovascular diseases (Aune *et al*., 2016; Zhu & Sang, 2017). Additional positive effects have been reported or seen partly in their influence on human microbiota (Gérard *et al*., 2019).

Bread is one of the well-liked wheat based products eaten all over Kenya and especially among the younger population. In comparison to other wheat flour products (Weegels, 2019) reported that the main food of choice to address difficulties with future food availability would be bread, particularly Whole Grain (WG). However, the quality and healthy nutritional value of wheat flour and the related respective products are occasionally affected and determined by various factors. Annually about 25% of harvested crops such as wheat crop are occasionally contaminated by harmful secondary metabolites such as mycotoxins, resulting into economic losses to agricultural and industrial commodities (Brites *et al*., 2018; Kamle *et al*., 2019; Marin *et al*., 2013). Risks and inconveniences that come with the consumption of grains and wheat flour products especially whole-grain products include agrochemical contaminants, heavy metals, pesticide residues, and

mycotoxin (Bianchini *et al*., 2015) that can be localized in the grain's outer cover. Such contaminants may persist in greater levels due to the fact that there is no component separation during the grinding process of whole-grain wheat flour products as compared with refined white wheat flour (Gómez *et al*., 2020). Consequently, consumption of wheat- based products containing such contaminants have been associated with far-reaching life endangering health consequences (Sabillón & Bianchini, 2016) to the public.

According to a report by (Giertz *et al*., 2015), wheat and rice are Kenya's most important cereal crops. However, the only crop for which the area under cultivation has dropped in the recent decades and the yield has become more variable is wheat. Yet, because of the high cost of corn flour and the elimination of Value Added Tak (VAT) on wheat flour, more consumers are switching to wheat-based products and the use of wheat in the manufacture of livestock feeds. Additionally, new private investments in the industry have increased local milling capacity by around one million metric tons (Townsend & Gitonga, 2019). All these translate to more demand for the commodity hence the widening local wheat supply deficit. Consequently, increased importation of wheat in the country is unavoidable. Mycotoxin-producing pathogenic fungi in general, frequently affect Kenya's wheat production and, the world. The meager agricultural activities such as improper wheat drying methods, handling techniques, packaging material, storage and transport conditions contribute to increased danger in growth of pathogenic fungi and mycotoxin production (Marin *et al*., 2013) and, contamination in the subsequent wheat food products.

Previous studies in Kenya have reported elevated prevalence of mycotoxin producing fungi in wheat crops (Muthomi *et al*., 2008; Njeru *et al*., 2016, 2016). Coupled with the increased wheat importation, frequent scrutiny or inspections of mycotoxins levels in wheat flour brands (WFB)

and wheat flour products (WFP) is crucial in improving and enhancing satisfactory control strategies to reduce the associated health risks and save lives. It is in this respect that the study assessed occurances and the levels of fumonisins and deoxynivalenol in different wheat flour products common on the Kenyan market. The survey was carried out using samples collected from Narok town, Nakuru city and Nairobi (the capital city) of Kenya.

6.2 METHODOLOGY

6.2.1 Materials and methods

6.2.2 Sampling of wheat flour products and preparation of test samples

Sampling of wheat flour and products was done in local grocery stores and supermarkets between May and June 2021, according to procedures described by Bramley, (2016), with slight modifications. The criteria used to purchase the wheat flour included packaging size ranging between 1kg and 2kg, branded wheat flour (both whole grain wheat flour and refined white wheat flour). Samples having the same batch code and within the same shelf life were taken for every sampled commodity. Eight (8) brands of wheat flour milled in Kenya, namely; WFB1, WFB2, WFB3, WFB4, WFB5, WFB6, WFB7 and WFB8 were sampled. In some cases, both whole grain and refined white wheat flour were sampled. Five wheat flour products [spaghetti, noodles, indomie, Weetabix, biscuits (both local and imported) and, bread] were sampled. Nine incremental samples of wheat flour weighing 1kg or more were taken from three randomly selected supermarkets distributed within each area of study. All samples were stored away from sunlight and moisture, in clean dry leak-proof food quality plastic bags before analysis. The analysis involved 72 samples of wheat flour brands (WFB) and 72 wheat flour products (WFP).

6.2.3 Preparation of test samples

For every commodity approximately 150g of the test sample was analyzed, three replicates of each weighing at least 50g. To obtain 150g in the laboratory, nine (9) packets of every type of wheat flour sample, each weighing 1kg were classified into three categories. Each set comprised of replicates of three (3) packets sampled from different stores or supermarkets. The three packets of wheat flour samples in each category were then poured into sterile plastic bag and mixed

thoroughly to produce one homogenous sample that was aseptically weighed into several portions of 150g in labeled sterile zip-locked bags. Similarly, packets of each category of wheat flour products were classified into aggregates of three based on their replicate numbers. The packets of each replicate were blended/ground under sterile conditions to produce a powdered form of the products, followed by weighing to produce packages of 150g in sterile labeled zip-locked bags for analysis. Each set of wheat flour products was milled at a time after which the blender was sterilized using 70% ethanol before blending the next set of wheat product. Prior to mycotoxin analysis, all the samples were kept in the refrigerator at 4°C and away from light.

6.2.4 Extraction of fumonisins

Methanol (90% methanol) was used for extraction of the toxins. Preparation of the extraction solvent for each test sample was achieved by mixing 4mL of deionized water with 36mL of reagent grade methanol. Twenty grams (20g) portion of each wheat flour brand, ground to very small particles equivalent to the size of fine instant coffee particles capable of passing through a 20-mesh screen was weighed and used. Forty (40mL) of the Extraction Solvent was added to each weighed sample and then shaken in a sealed container for one minute. The ratio of sample to extraction solvent was 1:2 (w/v). Each of such preparation was allowed to settle before filtering 5-10mL of the extract using Whatman 1 filter paper. The filtrate was then diluted in distilled water at a ratio of 1:20.

6.2.5 Fumonisin analysis using ELISA method

The assay was conducted at room temperature using fumonisin ELISA Kit and, according to the manufacturer's instructions (Helica Biosystems Inc.). A gentle stream of distilled water was used to wash the contents of a Tween Packet Phosphate buffer into a 1-Liter container to reconstitute the

final buffer solution (PBS). Each of the samples, both the Standard and test samples were placed in one Dilution Well in a microwell holder. Next, an equal number of Antibody Coated Microtiter Wells were placed in a second microwell holder. One hundred microliter (100µL) of the Conjugate Solution A (green) was then dispensed into the appropriate dilution wells, followed by one hundred microliter (100µL) of clear Conjugate Solution B. Next, 100µL of each Standard and test sample solutions were then added to appropriate Dilution Well containing Conjugate mixture using a new pipette tip for each and, mixed three times using priming pipette. Throughout the assay the location of each Standard and Sample were recorded accordingly. One hundred (100µL) of contents from each Dilution Well was then transferred to a corresponding Antibody Coated Microtiter Well using a new pipette tip for each, and incubated at room temperature for 10 minutes. The contents of the mixture from the microwells were then decanted into an appropriate discard basin. The microwells were washed five times using PBS Tween wash buffer. The washed microwells were tapped (face down) on absorbent towels to remove the entire residual buffer. Lastly, the required volume (120µL) of Stop Solution was measured and placed in a separate container. One hundred microliter (100µL) of the stop solution was added in the same sequence and at the same pace as the Substrate was added. A microtiter plate reader using a 450nm filter was used to measure the optical density (OD) of each microwell. Using the recorded optical density (OD) for the content of each microwell expressed as a percentage of the OD of the zero (0.0) standard against the fumonisin content of the standard, a dose-response curve was constructed and the unknowns determined by interpolation from the standard curve.

6.2.6 Extraction of Deoxynivalenol toxins

The wheat flour samples and wheat flour products under study were ground to approximately equivalent particle size of fine instant coffee, capable of passing through a 20-mesh screen. To

100mL of distilled water in a container, 20g of each sample was added at 1:5 (w/v) ratio of sample to water. Mixing was done in an orbital shaker for a minimum of 3 minutes, particulate matter was allowed to settle and, 5-10mL of the extract filtered through a Whatman1 filter paper. An aliquot of the collected filtrate/extract was diluted at the ratio of 1:10 with a wash buffer. The final dilution for use in the calculation was 1:50.

6.2.7 Deoxynivalenol analysis using ELISA method

The assay was conducted using Deoxynivalenol ELISA Kit and according to the manufacturer's instructions (Helica Biosystems Inc.). Every chemical/reagent used was maintained at room temperature before use. A Tween packet of PBS- was reconstituted by flashing out the contents with a gentle stream of de-ionized water into a one -Liter container. Each Standard and test sample to be analyzed were placed in one Dilution Well in a microwell holder. Two hundred microliter **(**200µL) of the Conjugate was put in each mixing well. Using a new pipette tip, 100µL of each standard and prepared test sample were added to each appropriate mixing well containing Conjugate and the contents mixed by priming pipettor for three times. A new pipette tip was used to transfer 100µL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. This was followed by incubation for 15 minutes at room temperature. The contents from microwells were decanted and the wells rinsed five using PBS Tween wash buffer. The microwells were then tapped (face down) on absorbent towels to discard residual buffer. To each microwell, 100µL of the substrate reagent was added and incubated away from direct light at room temperature for five (5) minutes. The required volume of Stop Solution (1mL/strip or 120µL/well) was measured and placed in a separate container. Next, 100µL of Stop solution was pippeted in the same sequence and at the same pace at which the Substrate Reagent was added. The optical density (OD) of each microwell was read with a microtiter plate reader using a 450nm filter

and recorded. Setting the zero standards as 100% binding (Bo), percentage binding (% B) for each standard and each of the sample was calculated as a percentage of the zero bindings (%B/Bo). A dose-response curve was constructed using the OD measurements expressed as a percentage (%B/Bo) of the OD of the Zero (0.0) standard against the DON content of the standard. Subsequently, the unknowns were measured by interpolation from the standard curve.

6.2.8 Data analysis

Comparison of the total amount of fumonisin and deoxynivalenol toxins in both wheat flour brands (WFB) and wheat flour products (WFP) collected in the three areas of study was done using One Way ANOVA and Tukey HSD test where applicable. In each category, the average contamination of all the positive samples, the prevalence of mycotoxins and, percentage of contamination of fumonisins and DON in wheat flour products were calculated. Tukey HSD test at 5% significance was used to compare the average means of the mycotoxins detected in the samples.

6.3 RESULTS

6.3.1 Occurrence of fumonisins and DON in brands of wheat flour in Narok town, Nakuru city and Nairobi, the capital city of Kenya

Both DON and fumonisins were detected in the wheat flour brands sampled for analysis in this study. However, some brands of wheat flour did not contain fumonisins while DON was detected in all of them. Overall, DON was the mycotoxin detected in the highest amounts compared to fumonisin levels. The highest levels (5.6µg/kg) of DON were detected in Whole Grain category of Wheat flour. This was detected specifically in WFB6 brand. In the category of refined wheat flour, the highest amount (3.8µg/kg) of DON was detected in WFB4 brand. The lowest level of DON detected in the refined category of flour was 0.4µg/kg in WFB1 and WFB2 brands of wheat flour. On the contrary, the lowest levels of the same toxin that was detected in the Whole Grain category of wheat flour amounted to 1.3µg/kg and, it was detected in WFB7 brand of wheat flour. The highest (0.7µg/kg) fumonisins levels were detected in WFB1 refined category of wheat flour while the lowest (0.2µg/kg) was detected in WFB3 and WFB4 brands of refined wheat flour. The highest amount (0.33µg/kg) of fumonisins detected in the whole grain category of wheat flour occurred in WFB6 brand. It is worth noting that WFB2 and WFB8 brands of wheat flour did not contain detectible levels of fumonisins (Tables 6.1) and that whereas the highest level of DON was detected in whole grain wheat flour, the highest fumonisin levels were detected in refined brand of wheat flour.

Table 6-1: Fumonisins and deoxynivalenol levels in market wheat flour brands (WFB) sampled in Narok town, Nakuru and Nairobi cities, Kenya.

S/N	Wheat Flour Brand (WFB)	Category	Type of mycotoxins and levels (µg/kg)	
			Fumonisins	DON
1	WFB1	RWF	0.7	0.4
2	WFB2	RWF	0	0.4
3	WFB3	RWF	0.2	2.5
4	WFB4	RWF	0.2	3.8
5	WFB5	RWF	0.26	2.1
6	WFB6	WGF	0.33	5.6
7	WFB7	WGF	0.23	1.3
8	WFB8	RWF	0	1.1

KEY: RWF- Refined Wheat Flour, WGF- Whole Grain Wheat Flour.

6.3.2 Occurrences of fumonisins and DON in market wheat-flour products in Narok town, Nakuru city and Nairobi, the capital city of Kenya

Detectable levels of both DON and fumonisins occurred in the sampled wheat flour products. However, the detected levels of the toxins were very low while some of the sampled wheat flour products did not have detectable levels of both DON and fumonisins. The highest detectable amounts of the toxins were 2.8µg/kg and 0.2µg/kg for DON and fumonisins respectively. Both amounts of the toxins were detected in biscuits imported from South Africa and India respectively. However, some of the sampled wheat flour products such as biscuits (WFP2I) and spaghetti (WFP5) (Table 6.2) did not contain detectible levels of DON. Similarly, some of the sampled wheat products such as bread (WFP1), biscuits (WFP2E) and Indomie (WFP4) also contained undetectable levels of fumonisins. Based on the batch details, the three products were manufactured in Kenya, Egypt and Kenya respectively. The lowest level (0.2µg/kg) of DON was detected in bread, manufactured in Kenya. The lowest detectable level of fumonisins was 0.1µg/kg. This level was detected in several products (Table 6.2).

Table 6-2. Fumonisins and DON levels in market wheat flour products sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya.

S/N	Wheat Flour Product	Origin	Mycotoxins analyzed and levels (µg/kg)	
			Fumomisins	Deoxynivalenol
1	Bread (WFP1)	Kenya	0	0.2
2	Biscuits (WFP2I)	India	0.2	0
3	Biscuits (WFP2S)	S. Africa	0.1	2.8
4	Biscuits (WFP2E)	Egypt	0	2.4
5	Biscuits (WFP2K)	Kenya	0.1	1.6
6	Weetabix (WFP3)	Kenya	0.1	1.8
7	Indomie (WFP4)	Kenya	0.0	0.6
8	Spaghetti (WFP5)	Kenya & Italy	0.1	0

KEY: **WFP- Wheat Flour Product; I- India; S- South Africa; E- Egypt and K- Kenya**

6.3.3 Comparison of fumonisins and DON levels in common market wheat flour brands sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya

Following further statistical analysis of the results, DON levels between most of the analyzed wheat flour samples were not significantly different at 0.5% confidence interval. Fumonisins and DON levels were significantly different ($p < 0.05$) among the numerous brands of wheat flour examined. For instance, in the analysis of fumonisin levels, a comparison between WFB1 and WFB2 wheat flour brands resulted into a mean difference of 0.70 with a p-value of 0.02. Detected fumonisins levels in WFB1 brand of wheat flour was 0.07 while WFB2 and WFB8 brands of wheat flour had undetectable levels of fumonisins. Likewise, analysis of deoxynivalenol levels in WFB1 and WFB6 resulted into a mean difference of -5.30 with a p-value of 0.02. Additionally, comparison of DON levels in WFB2 and WFB6 brands of wheat flour gave a mean difference of -5.20 with a p-value of 0.02 (Figure 6.1).

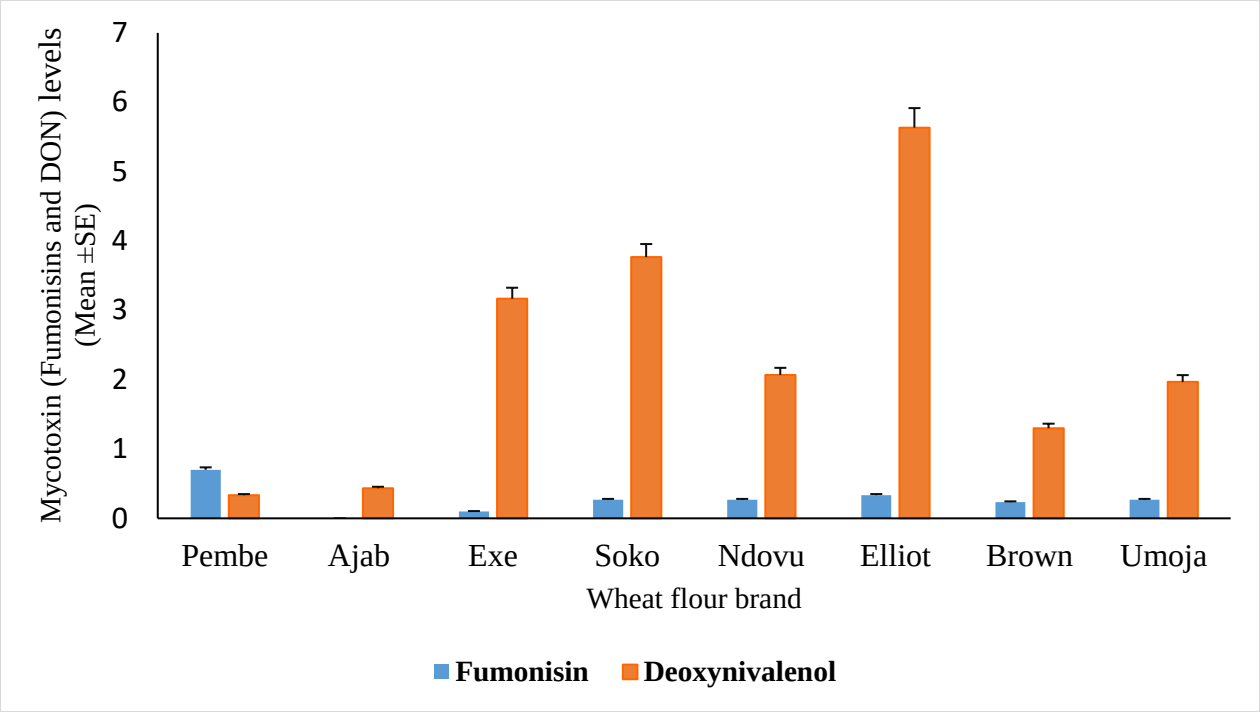


Figure 6-1. Fumonisin and DON levels in market wheat flour brands sampled in Narok town Nakuru and Nairobi cities, Kenya.

6.3.4 Comparison of Fumonisins and DON levels in wheat flour brands and wheat flour products sampled in Narok town, Nakuru city and Nairobi, the capital city of Kenya

Further statistical comparison of fumonisin and DON levels among the various wheat products investigated found no significant differences ($p > 0.05$) [Figure 6.2]. Considering that the highest detected DON level was in wheat flour, more analysis to compare the levels of the mycotoxins between market wheat flour brands and wheat flour products discovered substantial variations in fumonisin levels ($p<0.05$) between wheat brands and wheat products (Figure 6.3). High fumonisin levels were detected in the assessed wheat flour brands whereas low levels were detected in the wheat products. Similarly, significant differences were observed in deoxynivalenol levels between wheat brands and wheat products (Figure 6.3), with wheat brands recording higher levels of the toxins compared to wheat products.

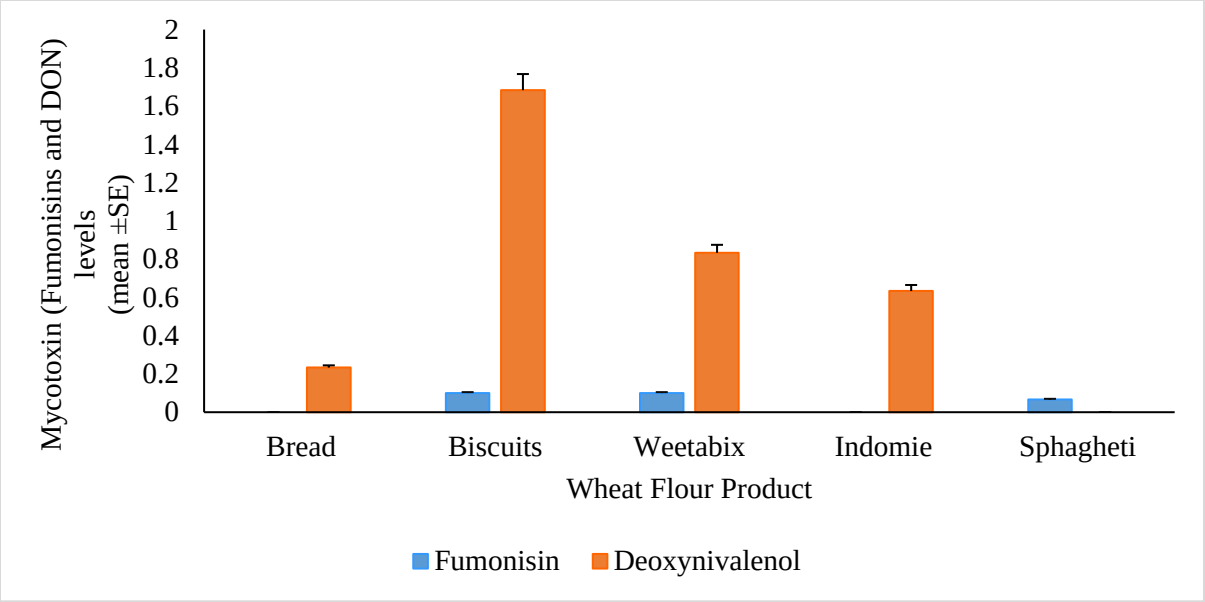


Figure 6-2. A comparison of Fumonisins and DON amounts in selected market wheat-flour products sampled in Narok town, Nakuru and Nairobi cities, Kenya.

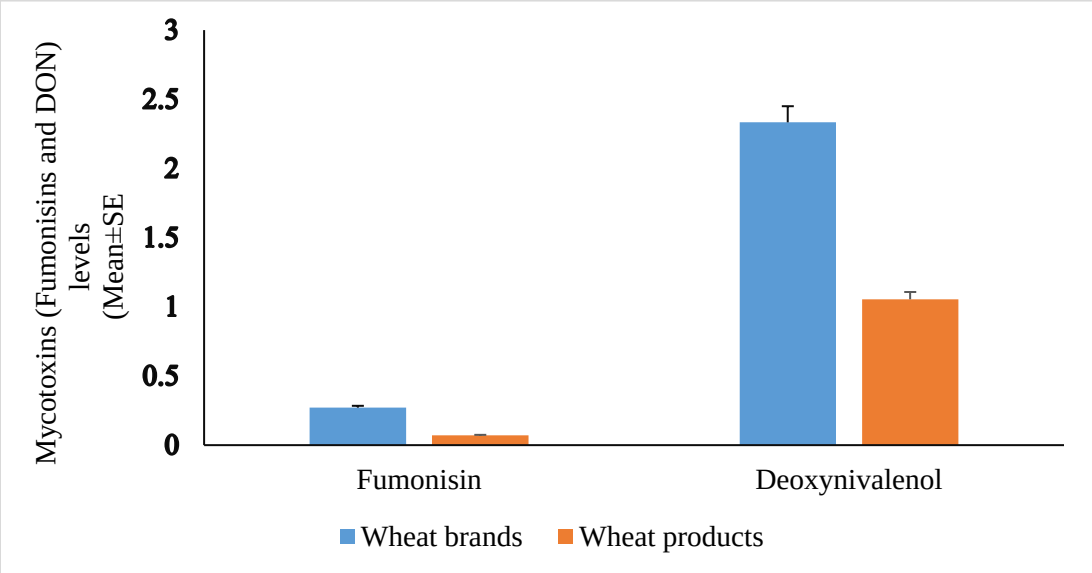


Figure 6-3. A summative comparison of fumonisins and DON amounts in wheat flour brands and wheat flour products sampled in Narok town, Nakuru city and Nairobi, the capital cityKenya.

6.4 DISCUSSION

The study investigated contamination by fumonisins and deoxynivalenol in randomly collected samples of brands of wheat flour and wheat products sold in Narok town, Nakuru city and Nairobi, the capital city of Kenya to ascertain the safety of the wheat food chain. Generally, based on the findings, the amounts of the mycotoxins analyzed were within the recommended levels for domestic use in nearly all the sampled market wheat flour brands and wheat flour products. The results indicate that on average, over 75% of the examined samples contained low amounts of fumonisins and DON. However, none of the 72 analyzed samples of wheat flour and, 72 samples of wheat flour products contained total fumonisins or total deoxynivalenol levels above the acceptable limit levels. According to (2006/583/EC, 2006; Alshannaq & Yu, 2017; European Commission, 2006; FDA, 2017) acceptable levels of DON in wheat products such as bread, pastries, biscuits, cereal snacks and breakfast is 500µg/kg and, 1250µg/kg in unprocessed cereals according to European Commission, 2006. The presence of the targeted toxins (DON and fumonisins) in over 75% of the sampled wheat products is supported by other published reports on incidences and prevalence of mycotoxins in wheat-based products (Andrade *et al*., 2020; Fatemeh *et al*., 2016; Kibugu *et al*., 2019; Martins & Martins, 2001; Roscoe *et al*., 2008; Yazdanpanah *et al*., 2012). However, unlike in the other reports, the low levels of the toxins reported in the wheat-based products investigated in the current study is a novel discovery that emphasizes the safety of the wheat food chain in Kenya in relation to the targeted toxins.

Deoxynivalenol was detected in higher levels in the two categories of the analyzed samples (wheat flour and wheat flour products). It occurred in 75% of wheat flour brands and 86% of wheat flour products sampled. The results is supported by similar findings on the prevalence of DON in wheat flour and wheat flour-based products around the world (Abbas, 2016; Iqbal *et al*., 2020; Kibugu *et*

al., 2019; Machado *et al*., 2017; Marin *et al*., 2013; Nishio *et al*., 2010; Sacco *et al*., 2020). Similar research findings by Wegulo, (2012) reported DON to be the most predominant toxin and probably the most significant mycotoxin in wheat and other small grain crops from an economic stand point. The average DON levels detected in this study ranged between 0.2µg/kg and 5.6µg/kg while minimal fumonisin levels (<1 µg/kg) were detected in 75% of both wheat flour brands and wheat flour products. Incidences of fumonisins in wheat-based products have similarly been reported in other studies (Andrade *et al*., 2020; Fatemeh *et al*., 2016; Roohi, 2012).

Unlike fumonisins, higher DON levels were detected in the assessed wheat flour brands compared to wheat flour products. The highest amount of DON was detected in WFB6 wheat flour brand while the highest amount of fumonisins was detected in WFB1 wheat flour brand. Higher deoxynivalenol levels were also detected in wheat-based products compared to fumonisins. The detected amount of DON in wheat flour products ranged between 0.2 µg/kg and 2.8 µg/kg on average. Among the sampled wheat flour products, South African biscuits contained the highest concentration of DON (2.8 µg/kg). Deoxynivalenol was not detected in samples of biscuits originating from India and, in pasta- spaghetti (origin Kenya and Italy). Similarly, no fumonisins were detected in bread and pasta- indomie (origin- Kenya). Maintaining the accepted mycotoxin limit levels is important to minimize the exposure of humans and other animals (Cheli *et al*., 2021). Consequently, the low toxin levels detected in this study signify the safety of the wheat flour brands and wheat flour products sampled, given that the analyzed mycotoxins were within the legally acceptable national and European Union set limits.

Generally speaking, the scanty amounts of the mycotoxins discovered in the samples examined are in agreement with work by Sabillón & Bianchini (2016) which stated that in various nations, wheat-based products supplied directly to the general population do occasionally include or

contain low but enduring quantities of mycotoxins. However, a number of variables might have played a role in the heterogeneity in the incidence and generally low levels of the mycotoxins examined in the samples. The main producers of DON (Iqbal *et al*., 2020) are the major causative agents of FHB in wheat (Khan *et al*., 2020; Reverberi *et al*., 2021). Hence, the high prevalence of DON in the assessed wheat flour brands and wheat flour-based products. The concentrations reported in this investigation were, nonetheless, within the advised safety levels. Some of the wheat- based products assessed including wheat grains are imported into the country since Kenya is not yet sufficient in wheat production. *Fusarium verticillioides* and *F. proliferatum,* which are common pathogens in maize than wheat, produce the majority of fumonisins (Carbas *et al*., 2021). However, other factors such as the cultivation of such cereals on the same piece of land with wheat in successive years may have contributed to the increased infections of the wheat crop by fumonisin producing fungi. In the previous chapters, infestation of the improved wheat cultivar by *F. verticillioides* was reported to be prevalent. Hence the subsequent incidences of the toxins in wheat grains (Chehri *et al*., 2010; Ferrigo *et al*., 2016) and wheat-based products (Cendoya *et al.*, 2019).

The scanty fumonisins levels detected in this study could also be due to the binding effect of the toxins on food matrix constituents, mainly during thermal processes. Hence the toxins may not be extracted during the usual analytical methods (Berthiller *et al*., 2013; Kovač *et al*., 2018). Wheat flour processing procedures such as physical separation of FHB destroyed kernels, washing of the kernels and pearling (Abbas,' *et al*., 1985; Bullerman & Bianchini, 2007; El-Banna *et al*., 1983; Kushiro, 2008; Savi *et al*., 2016; Vidal *et al*., 2014; Wan *et al.*, 2020) may also have contributed to the detected low toxin levels. Other wheat flour processes such as dry milling and grain fractioning that affect fungal growth and mycotoxin detection (Schaarschmidt & Fauhl-Hassek,

2018), and toxin redistribution in the wheat flour (Nielsen *et al*., 2014; Nishio *et al*., 2010; Tibola *et al*., 2015, 2018) are also critical. Hence, these may be among the other reasons for the differences observed among and between the analyzed samples of wheat flour brands. For example, in comparison to other wheat flour brands, the high amounts of deoxynivalenol detected in the WFB6 wheat brand may be attributed to the fact that it was whole grain wheat flour. During seed, development mycotoxins can be localized in the grain's outer cover and thus may remain in larger amounts in whole grain wheat flour process (Gómez *et al*., 2020). This is because when compared to white wheat flour, there is no fractional separation throughout the milling process (Gómez *et al*., 2020). On the other hand, all the sampled wheat flour brands were packaged in low-density Khaki paper bags, that have been reported to reduce mycotoxins in flour since they do not retain moisture (Opara *et al*., 2016; Sacco *et al*., 2020).

Planting of wheat cultivars that have some level of resistance to kernel damage is another method encouraged for use in the control of mycotoxin-producing fungi (Wegulo, 2012). The Kenya Agricultural and Livestock Research Organization have created wheat genotypes that are suitable for different agro-ecological zones and that has also exhibited a degree of resistance to harmful fungi (Kamwaga *et al*., 2016; Okumu *et al*., 2016). In addition to not having *Fusarium* spp., some the sampled wheat cultivars did not contain detectible levels of the analyzed toxins and, neither were their grains infected with *Fusrium* spp. Hence, this could be another possible reason for the low toxin levels detected in the brands of wheat flour milled in Kenya and the subsequent wheat-flour products in the market.

6.5 CONCLUSION

The study revealed that over 75% of the evaluated wheat flour brands and wheat-flour products in the market had detectable amounts of DON and fumonisins all the sampled areas (Narok town,

Nakuru City and Nairobi, the capital City of Kenya). Higher levels of the analyzed toxins were detected in the wheat flour as compared to wheat flour products. All of the examined samples had greater quantities of deoxynivalenol than fumonisins. However, worth noting is that some wheat flour brands and, both locally manufactured and imported wheat flour products did not contain any of the analyzed categories of mycotoxins. The overall research findings demonstrate that the examined wheat products are safe for household consumption since the levels of fumonisins and DON detected were found to be within permissible levels according EU regulations.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 GENERAL DISCUSION

Wheat is affected by the severe *Fusarium* Head Blight disease of wheat all over the globe (Alisaac & Mahlein, 2023). A multitude of etiological factors influence mycotoxin accumulations (Summerell, 2019). Studies on the population of disease's causative agents are crucial for the establishment of agro-economic policies. For Kenya to produce wheat sustainably, it is also crucial to conduct frequent research on pathogenic fungi in wheat, such as *Fusarium* spp. and the mycotoxins linked to the resultant diseases such as FHB. The government of Kenya has devoted a lot of resources towards the improvement of wheat cultivars to boost crop's produce in the Country (Kamwaga *et al*., 2016). Thus, the study examined the adoption of improved wheat cultivars in three of the main wheat producing Counties (Narok, Uasin Gishu and Nakuru) in the Kenyan Rift Valley. The survey also identified elements influencing famers' decisions on the wheat cultivars to cultivate as well as the most frequent field wheat fungal diseases observed by the wheat growers (Chapter3). *Fusarium* spp. prevalence and phylogenetic diversity in the various the grains of the various wheat cultivars sampled in the three locations were also determined (Chapter 4). This was followed by examining the occurrence of Tri13DON and FUM1 genes encoding the production of deoxynivalenol and fumonisins respectively, from the isolated *Fusarium* spp. In addition, incidences of the respective toxins in the sampled grains were analyzed (Chapter 5). Finally, the occurrence and amounts of fumonisins and DON in randomly selected brands of market wheat flour and wheat flour-based products in Narok town, Nakuru city and Nairobi, the Capital City of Kenya were evaluated (Chapter 6).

The study found a small percentage (0. 23≥10%) of the wheat cultivars developed for cultivation in the three ecological regions (Narok, Uasin Gishu and Narok Counties) were planted during the study period. The predominantly cultivated wheat cultivars in the sampled wheat fields were Njoro BWII, Robin and Eagle 10. However, Njoro BWII was the highly sampled wheat cultivar and consequently the most preferred by farmers in all the three regions of study. The high preference for Njoro BWII wheat cultivar by farmers was due to its promising high weight yield. There were variations in agro-economic factors considered during selection for the cultivars of wheat to cultivate. However, the predominant factors considered by farmers among others were the elevated weight of wheat yield, the affordability and availability of the categories/cultivars of wheat seed cultivars in the market. The choice of wheat cultivar planted was also influenced significantly by the availability of free wheat seeds on offer and preference of farm-saved wheat seeds, either by individual farmers or between farmers.

Every farmer whose wheat farms were sampled stated that wheat rust was the main pathogenic fungus limiting tenable wheat production. Only 1.63% of them mentioned FHB as a significant issue that has an impact on the crop production. Over 90% of the farmers incidentally, referred to all observed occurrences of fungal infestation in the wheat fields, including FHB, as rust. It was clear that the risks of consuming the linked mycotoxins and the impacts of FHB on the crop might not be fully known or understood by the farmers, making them a lower priority. Therefore, the need for creating more awareness about fungal diseases and possible health risks resulting from consumption of mycotoxin contaminated wheat-based foods by both human and domesticated animals.

Further research findings revealed widespread distribution of *Fusarium* spp. (*F. poae*, *F. tricinctum, F. heterosporum*, *F. culmorum, F. equiseti*, *Fusarium sp., F. verticillioides* and *F.*

oxysporum) but with insignificant differences in phylogenetic diversity among the three geographical locations examined. A possible indication that in spite of the three areas being ecologically different, there could be factors enhancing an almost even distribution or spread of the isolated *Fusarium* spp. populations in the wheat cultivars cropped. However, not all the *Fusarium* species identified in this study may be phytopathogenic. Hence, this finding is supported by Summerell, 2019 that many *Fusarium* spp. have been implicated as disease causative agents. Nevertheless, there is need for resolving the status of *Fusarium* species in the light of real disease causative agents.

Additionally, it is important to note that the isolated *Fusarium* spp. occurred often in Njoro BWII wheat grains, most of which did not exhibit any signs of mold contamination. Hence, another possible explanation for the insignificant diversity index in the prevalence of *Fusarium* spp. among the three regions of study. This is because it was the most frequently sampled variety in all the three locations and, it was infected by nearly all the isolated *Fusarium* species. The use of farmer-saved wheat seeds traded among farmers in the three research zones could be another explanation for this incidence. Furthermore, compared to the crop produce of certified wheat seeds, the frequency of the isolated *Fusarium* spp. was higher in the grains of farmer- saved wheat seeds.

The phylogenetic tree showed that *F. verticillioides* and *F. equesiti* were spread throughout multiple sub-clusters. Additionally, they were isolated from more than seventy percent of all the sampled categories of improved wheat cultivars. *Fusarium verticillioides*, a common pathogen in maize in many parts of the world (Mohammadi *et al*., 2016; Rosa Junior *et al*., 2019) was the most often isolated species in all the three locations of study. The high prevalence of this species in wheat may be attributed to the fact that the sampled wheat farms are used for cultivation of maize in other seasons of the year on rotational basis. It is also important to note that in the current study,

F. graminearum, which had been reported in previous studies as one of the causative agents of FHB of wheat in Kenya (Muthomi *et al*., 2012; Wagacha *et al.,* 2016) was not isolated from the examined wheat cultivars. However, according to the authors, only 20% of all the isolated *Fusarium* spp. were *F. graminearum.* Therefore, there is need for additional research into the dynamics of *F. graminearum* population in Kenya and its impact on wheat production.

In order to ensure the safety of the wheat produce at harvest in the light of secondary metabolites' contamination of the grains, it was crucial to determine the implications of the prevalence of the isolated *Fusarium* species. Therefore, analysis of the mycotoxigenic potential of *Fusarium* spp. found in the grains of the studied wheat cultivars revealed a wide range in FUM1 gene frequencies and distribution. However, none of the characterized and identified probable carriers of the gene and, manufactures of DON had the TRI13DON gene, which codes for the production of DON. Based on this finding the effectiveness of managing FHB in Kenya may also depend on establishing what constitutes or make the characteristics of the isolated pathogenic species populations and, how that affects disease detection/diagnosis, management and biosecurity (Summerell, 2019).

Narok County recorded elevated prevalence of FUM1 gene in *Fusarium* spp. populations isolated. However, the fumonisins levels detected in the respective grains were indirectly proportional to the overall occurrence frequency of the gene, similar to the other two regions. For example, the fumonisin levels in the samples from Uasin Gishu County were comparatively high, yet FUM1 gene was least detected in the fungal isolates recovered from wheat grains collected in the region. Essentially, this evidence shows that there are other factors involved in the synthesis and accumulation of fumonisins in addition to the gene. Hence, the need to discover and look into how such variables can be employed in mycotoxin control strategies in the wheat produce.

The variations in quantifiable fumonisin levels among the grains of the different improved wheat cultivars evaluated in this study constituted another important observation. In-spite of the high isolation frequency of *F. verticillioides*, some of the grains had undetectable amounts of fumonisins. This demonstrates that a few of the produced wheat cultivars notably Kenya Wren, Kenya Tai and Sunbird may be tolerant to this harmful *Fusarium* species. It is an observation that merits investigation, particularly when the crop is cultivated in several ecological zones. This is because these three wheat types were sampled only in Nakuru County.

Lastly, the study ascertained the safety of market wheat products based on a case study involving Narok town, Nakuru city and Nairobi, the capital city of Kenya. Despite the many diverse measures put in place to curb mycotoxin contamination in the wheat food chain, the market wheat flour and wheat flour products sampled contained some detectible levels of DON and fumonisins. However, the overall health safety of the analyzed samples of wheat-based products for domestic consumption was reported. The levels of fumonisin and DON detected from the samples were within the acceptable EU limits. Furthermore, some of the wheat products had undetectable amounts of the tested mycotoxins. This possibly could be a reflection of the high quality of the wheat grains (both imported and locally produced) with respect to mycotoxin contamination, efficiency in both transportations of wheat and processing of wheat-based products by the respective companies. Theoretically, it is rational to associate the high prevalence of the pathogenic/mycotoxin-producing fungal species and their identified genes to a high concentration of mycotoxins in the wheat-based foods. In order to consistently reduce the risks associated with mycotoxins in the wheat food chain, other factors connected to the production and flow of the toxins in the food should be given priority.

Finally, since effective processing of products made from wheat can help to reduce the level of the associated mycotoxins, the presence of the mycotoxin producing fungi species in wheat at harvest time is not sufficient for evaluating the risks of the toxic secondary metabolites in wheat-based food products especially for humans. This is because efficient processing of wheat flour products can help to reduce the levels of the associated toxins.

7.2 CONCLUSIONS BASED ON THE RESEARCH OBJECTIVES

1. A small proportion (10%) of the improved wheat varieties were under cultivation. However, abiotic factors suitable for their optimal growth and production did not necessarily form the basis for cultivar selection. Resistance by a cultivar to wheat rust, weight and yield of grains at harvest were key factors considered by farmers. Additionally, Njoro BWII variety is the most preferred variety.
2. *Fusarium* spp. were prevalent in the sampled grains of the various wheat cultivars. However, no significant differences existed in species diversity. *Fusarium verticillioides* and *F. equiseti* were the most prevalent, with some occurring as out groups in the phylogenetic tree, a ppossible indication of interspecies differences.
3. Fum1 gene cluster and fumonisins were detected hence some of the isolates are potential producers of fumonisins. However, TRI13DON gene was not detected in the isolated potential producers of DON.
4. Fumonisins and DON were detected in the market wheat products sampled but the levels were within the recommended/ acceptable EU limits for domestic consumption.

7.3 SUMMARY OF OF THE FINDINGS

1. A small percentage of the improved wheat cultivars released into the market for cultivation in various ecological regions in Kenya, were cultivated by farmers within the study period.
2. Due to its hefty weight grains and high productivity, Njoro BWII was the most popular wheat cultivar among farmers in all the three ecological research regions.
3. Additionally, sampled wheat cultivars were not selected based on their suitability in to specific ecological regions with the suitable abiotic factors required for optimal growth and hence produce.
4. Although the differences in agro-economic factors should determine the farmers' choice of wheat cultivar to cultivate, the yield of the cultivar at harvest and the seed price were the top most determinants.
5. There was overreliance or preference for farm saved seeds because of affordability compared to certified wheat seeds.
6. The main field fungal disease known to wheat farmers and hence a major concern in wheat production was wheat rust. However, most wheat famers seemed to be oblivious of other important types of wheat fungal diseases such as FHB and hence the subsequent implications on wheat food safety.
7. *Fusarium* spp. were prevalent in Narok, Uasin Gishu and Nakuru County. However, not all of them are toxigenic with respect to prevalence of genes encoding production of DON and fumonisins.
8. Wheat grains collected from Uasin Gishu County had higher amounts of fumonisins, while wheat grains sampled from Narok County had a higher prevalence of FUM1 gene.

9. The isolated prospective DON producers, howerver, did not have the gene responsible for production of the mycotoxin.
10. While some wheat types had undetectable fumonisin levels at harvest, others had significant amounts.
11. Wheat grains and wheat remains fed on directly by domesticated animals at harvest time before any form of processing may have long-term detrimental effects on the heath of the consumers due to the occurrence of fumonisins in the contaminated parts of the crop.
12. In comparison to fumonisins, higher quantities of DON were found in both market wheat flour brands and wheat flour products. However, some of the wheat products (both imported and locally manufactured) did not contain detectible levels of the analysed mycotoxins.
13. Levels of the fumonisins and DON in the market wheat products were within the recommended/ acceptable EU and KEBS limits for domestic consumption.

7.4 RECOMMENDATION

1. There should be frequent sensitizations of wheat farmers to adopt improved cultivars of wheat in the light of their characteristics or suitability to specific ecological regions.
2. There is need to encourage farmers to be intentionally involved in the control other fungal diseases such as FHB and consequently be made aware of the negative health effects resulting from intake of mycotoxin contaminated wheat grains/products by both domesticated animals and humans.
3. Continuous tests in the field to measure mycotoxin generation and accumulation in wheat grains as well as the level of resistance of improved wheat cultivars such as Kenya Wren, Kenya Tai, and Sunbird to pathogenic *Fusarium* species.
4. Further research work is recommended to ascertain incidences and prevalence of genes encoding synthesis of trichothecenes in potential fungal populations prevalent in the improved wheat cultivars and to assess their ability to produce the related mycotoxins.
5. Standardized treatment or processing of the wheat grains is advised to minimize any potential harmful long-term consequences of consuming mycotoxin contaminated wheat based food products since over 76% of the sampled wheat grains had at least detectable levels of the fumonisins.
6. More surveys may be done to establish the consistency of the safety status of the assessed wheat-based food products in the Kenyan market due to the changing climatic conditions both in Kenya and in other Countries that supply the country with wheat and wheat based products.

7. Policy making on mechanisms that enhance consisted cultivation of improved wheat cultivars with significant resistance to both wheat rust and FHB to promote wheat food safety.
8. Further research on the dwindling population of *F. graminearium* in wheat crop in the areas under study.
9. Farmers, Plant breeders and agronomists to work together in enhancing the caltivation of developed wheat cultivars to achieve their intended purposes for the benefit of everyone including consumers of wheat and its products.

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APPENDICES

Appendix I Work sheet for collection of wheat Seed samples

County.	Area/center/farm and GPS Coordinates	Previous season	Wheat cultivar planted	Certified/Farm saved seeds	Observed Disease	No. of samples collected
Narok -A						
Uasin Gishu-B						
Nakuru- C						

1. Name of: County……Centre/Area……. Local area/Farm number….. and GPS……...
2. Whether the seeds were certified or none-certified/ locally obtained seeds
3. Cultivar of wheat seeds planted.
4. Category of seeds:
    a. Certified or
    b. None-certified/ Farm Saved seeds
5. Crop planted on the farm in the previous season
6. Which factor do you consider when choosing cultivar of wheat seed to plant:
    a. Weight of yield- Yes/No
    b. Resistance to fungal Diseases-Yes/No
    c. Cost of Seed- Yes/No
    d. Availability of cultivar- Yes/No
    e. None of the above- Yes/ No
    f. All the above- Yes/No

Appendix II Coordinates for wheat sampling sites in Nakuru, Narok and Uasin Gishu Counties

	Uasin Gishu County	
AREA	EASTINGS	NORTHINGS
MOIBEN	35.351483	0.011209
MOIBEN	35.402863	0.007595
MOIBEN	35.382292	0.005668
MOIBEN	35.350812	0.040722
MOIBEN	35.360157	0.791495
MOIBEN	35.384625	0.822235
MOIBEN	35.384513	0.812905
MOIBEN	35.384798	0.814178
MOIBEN	35.384026	0.813215
MOIBEN	35.439590	0.786548
MOIBEN	35.380528	0.528138
MOIBEN	35.370401	0.581091
MOIBEN	35.449990	0.733313
MOIBEN	35.411170	0.645920
MOIBEN	35.356087	0.719093
MOIBEN	35.386078	0.799317
MOIBEN	35.346445	0.799610
MOIBEN	35.343055	0.781109
MOIBEN	35.379006	0.610482
MOIBEN	35.409830	0.649652
MOIBEN	35.406609	0.723193
MOIBEN	35.334944	0.807388
KAPTAGAT	35.579170	0.431667
KAPTAGAT	35.558610	0.455278
KAPTAGAT	35.461670	0.534444
KAPTAGAT	35.424565	0.384006
KAPTAGAT	35.484720	0.518889
KAPTAGAT	35.433610	0.500833
KAPTAGAT	35.678330	0.531111
KAPTAGAT	35.390072	0.395227
KAPTAGAT	35.471082	0.407236
KAPTAGAT	35.497894	0.290778
KAPTAGAT	35.427038	0.342589
KAPTAGAT	35.470560	0.556389
KAPTAGAT	35.605280	0.432778
KAPTAGAT	35.627780	0.417778
KAPTAGAT	35.416012	0.327196
KAPTAGAT	35.426574	0.348602
KAPTAGAT	35.456046	0.339452
KAPTAGAT	35.449554	0.309648
CHEPTERIT-KISES	35.357222	0.326389
CHEPTERIT-KISES	35.304722	0.441389

CHEPTERIT-KISES	35.264480	0.300310
	Narok County	
AREA	EASTINGS	NORTHINGS
NAROK (NR)	35.720283	-1.024337
NAROK (NR)	35.781150	-1.047187
NAROK (NR)	35.748269	-1.074374
NAROK (NR)	35.650901	-1.500592
NAROK (NR)	35.677929	-1.511115
NAROK (NR)	35.688082	-1.522443
NAROK (NR)	35.670981	-1.502343
NAROK (NR)	35.648037	-1.502343
NAROK (NR)	36.099000	-0.811000
NAROK (NR)	36.097000	-0.791000
NAROK (NR)	36.096000	-0.768000
NAROK (NR)	36.092000	-0.747000
NAROK (NR)	36.064000	-0.760000
NAROK (NR)	36.065000	-0.803000
NAROK (NR)	35.886000	-0.989000
NAROK (NR)	35.881000	-1.008000
NAROK (NR)	35.860000	-1.037000
NAROK (NR)	35.978231	-0.659209
NAROK (NR)	36.259269	-1.109366
NAROK (NR)	36.032785	-0.899630
NAROK (NR)	35.666906	-1.001739
NAROK (NR)	35.901961	-1.017063
NAROK (NR)	35.726565	-0.991525
NAROK (NR)	36.044364	-1.095221
NAROK (NR)	35.666706	-1.002739
NAROK (NR)	35.896138	-1.015092
NAROK (NR)	35.896145	-1.015132
NAROK (NR)	35.726576	-0.991587
NAROK (NR)	36.044364	-1.095221
NAROK (NR)	36.097000	-1.097000
NAROK (NR)	36.107000	-1.044000
NAROK (NR)	36.500893	-1.040930
NAROK (NR)	36.006970	-1.105976
NAROK (NR)	36.012462	-1.095745
NAROK (NR)	35.866660	-0.933306
NAROK (NR)	36.076646	-1.094004
NAROK (NR)	36.084744	-1.093921
NAROK (NR)	36.094187	-1.096663
NAROK (NR)	36.099929	-1.096314
NAROK (NR)	35.964741	-0.660323
NAROK (NR)	35.940000	-0.640000
NAROK (NR)	35.977000	0.607000
NAROK (NR)	35.815287	-0.498216
NAROK (NR)	35.992000	-0.850000
NAROK (NR)	35.962285	-0.614876
NAROK (NR)	35.669917	-1.522540

NAROK (NR)	35.678633	-1.523324

	Nakuru County	
AREA	EASTINGS	NORTHINGS
Nakuru	35.946973	-0.330849
Nakuru	35.950721	-0.327923
Nakuru	35.936979	-0.318928
Nakuru	35.966185	-0.223531
Nakuru	35.964757	-0.232097
Nakuru	35.888614	-0.195453
Nakuru	35.881476	-0.203543
Nakuru	35.984745	-0.275403
Nakuru	35.951432	-0.275403
Nakuru	35.851495	-0.252560
Nakuru	35.931445	-0.277782
Nakuru	35.946959	-0.340648
Nakuru	35.944960	-0.343503
Nakuru	35.951956	-0.342932
Nakuru	35.958523	-0.342932
Nakuru	35.949243	-0.348500
Nakuru	35.945817	-0.347501
Nakuru	35.946816	-0.353211
Nakuru	35.954240	-0.355210
Nakuru	35.901619	-0.328810
Nakuru	35.891820	-0.330770
Nakuru	35.904333	-0.347505
Nakuru	35.934938	-0.356550
Nakuru	35.939611	-0.362882
Nakuru	35.981373	-0.344942
Nakuru	35.986197	-0.354591
Nakuru	35.973231	-0.358058
Nakuru	35.961924	-0.347354
Nakuru	35.992981	-0.378713
Nakuru	35.967050	-0.382934
Nakuru	35.982579	-0.388663
Nakuru	36.016500	-0.390020
Nakuru	36.014088	-0.369365
Nakuru	36.016651	-0.357003
Nakuru	35.998560	-0.402985
Nakuru	35.994790	-0.388512
Nakuru	35.981976	-0.377356
Nakuru	35.897549	-0.336348
Nakuru	35.880663	-0.358812
Nakuru	35.892423	-0.361978
Nakuru	35.919861	-0.372531
Nakuru	35.900865	-0.315995

Appendix III Mean prevalence and phylogenetic diversity of *Fusarium* spp. in cultivars of wheat seeds in three Rift Counties, Kenya.

Table 2. LSD test, multiple comparisons of *F. verticillioides, F. oxysporum, F. culmorum, F. tricinctum* and *Fusarium sp.* prevalence

Dependent Variable	(I) Counties	(J) Counties	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Prevalence of *F.verticillioides*	Nakuru	Narok	-7.00000*	1.76383	.007	-11.3159	-2.6841
		Uasin Gishu	1.00000	1.76383	**.591**	-3.3159	5.3159
	Narok	Uasin Gishu	8.00000*	1.76383	.004	3.6841	12.3159
Prevalence of *F.oxysporum*	Nakuru	Narok	-3.00000*	.66667	.004	-4.6313	-1.3687
		Uasin Gishu	3.00000*	.66667	.004	1.3687	4.6313
	Narok	Uasin Gishu	6.00000*	.66667	.000	4.3687	7.6313
Prevalence of *F.culmorum*	Nakuru	Narok	6.00000*	.94281	.001	3.6930	8.3070
		Uasin Gishu	6.00000*	.94281	.001	3.6930	8.3070
	Narok	Uasin Gishu	.00000	.94281	**1.000**	-2.3070	2.3070
Prevalence of *F.tricinctum*	Nakuru	Narok	.00000	.47140	**1.000**	-1.1535	1.1535
		Uasin Gishu	2.00000*	.47140	.005	.8465	3.1535
	Narok	Uasin Gishu	2.00000*	.47140	.005	.8465	3.1535
Prevalence of *Fusarium sp.*	Nakuru	Narok	2.00000*	.47140	.005	.8465	3.1535
		Uasin Gishu	3.00000*	.47140	.001	1.8465	4.1535
	Narok	Uasin Gishu	1.00000	.47140	**.078**	-.1535	2.1535

*. The mean difference is significant at the 0.05 level.

Appendix IV Mean prevalence and phylogenetic diversity *Fusarium* spp. in cultivars of wheat seeds in three Rift regions, Kenya.

Table 3: DescriCTive statistics									
						95% Confidence Interval for Mean			
		N	Mean Frequency	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Prevalence of *F.verticillioides*	Nakuru	3	6.0000	2.00000	1.15470	1.0317	10.9683	4.00	8.00
	Narok	3	13.0000	1.00000	.57735	10.5159	15.4841	12.00	14.00
	Uasin Gishu	3	5.0000	3.00000	1.73205	-2.4524	12.4524	2.00	8.00
Prevalence of *F.equiseti*	Nakuru	3	6.0000	3.00000	1.73205	-1.4524	13.4524	3.00	9.00
	Narok	3	4.0000	1.00000	.57735	1.5159	6.4841	3.00	5.00
	Uasin Gishu	3	9.0000	2.00000	1.15470	4.0317	13.9683	7.00	11.00
Prevalence of *F.poae*	Nakuru	3	4.0000	1.00000	.57735	1.5159	6.4841	3.00	5.00
	Narok	3	2.0000	.00000	.00000	2.0000	2.0000	2.00	2.00
	Uasin Gishu	3	3.0000	2.00000	1.15470	-1.9683	7.9683	1.00	5.00
Prevalence of *F.oxysporum*	Nakuru	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
	Narok	3	6.0000	1.00000	.57735	3.5159	8.4841	5.00	7.00
	Uasin Gishu	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Prevalence of *F.heterosporum*	Nakuru	3	3.0000	2.00000	1.15470	-1.9683	7.9683	1.00	5.00
	Narok	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Uasin Gishu	3	2.0000	.00000	.00000	2.0000	2.0000	2.00	2.00
	Total	9	1.6667	1.65831	.55277	.3920	2.9414	.00	5.00
Prevalence of *F.culmorum*	Nakuru	3	6.0000	2.00000	1.15470	1.0317	10.9683	4.00	8.00
	Narok	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Uasin Gishu	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Prevalence of *F.tricinctum*	Nakuru	3	2.0000	.00000	.00000	2.0000	2.0000	2.00	2.00
	Narok	3	2.0000	1.00000	.57735	-.4841	4.4841	1.00	3.00
	Uasin Gishu	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Prevelence of *Fusarium sp.*	Nakuru	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
	Narok	3	1.0000	.00000	.00000	1.0000	1.0000	1.00	1.00
	Uasin Gishu	3	.0000	.00000	.00000	.0000	.0000	.00	.00

Appendix V Shannon weiner diversity indices table on *Fusarium* spp. in cultivars of wheat seeds in three Rift Valley Counties, Kenya.

Species	Frequency/Prevalence			Nakuru		Narok		Uasin Gishu	
	Nakuru	Narok	Uasin Gishu	Relative prevalence (p_i)	$(P_i)^2$	Relative prevalence (p_i)	$(P_i)^2$	Relative prevalence (p_i)	$(P_i)^2$
F. verticillioides	6	13	5	6/33=0.1812	0.0328	13/28=0.4643	0.2156	5/19=0.2632	0.0693
F. equiseti	6	4	9	6/33=0.1812	0.0328	4/28=0.1429	0.0204	9/19=0.4737	0.2242
F. culmorum	6	0	0	6/33=0.1812	0.0328	0/28=0.0000	0.0000	0/19=0.0000	0.0000
F. poae	4	2	3	4/33=0.1212	0.0147	2/28=0.0714	0.0051	3/19=0.1579	0.0249
F. oxysporum	3	6	0	3/33=0.0909	0.0083	6/28=0.2143	0.0459	0/19=0.0000	0.0000
Fusarium sp.	3	1	0	3/33=0.0909	0.0083	1/28=0.0357	0.0013	0/19=0.0000	0.0000
F. heterosporum	3	0	2	3/33=0.0909	0.0083	0/28=0.0000	0.0000	2/19=0.1053	0.0111
F. tricinctum	2	2	0	2/33=0.0606	0.0037	2/28=0.0714	0.0051	0/19=0.0000	0.0000
	33 isolates	28 isolates	19 isolates		$\sum(P_i)^2 = 0.1417$		$\sum(P_i)^2 = 0.2934$		$\sum(P_i)^2 = 0.3295$
				Nakuru D=1-$\sum(P_i)^2$ =1-0.1417=**0.8583**		Narok D=1-$\sum(Pi)^2$ =1-0.2934=**0.7066**		Uasin Gishu D=1-$\sum(P_i)^2$ =1-0.3295=**0.6705**	

Appendix VI Shannon weiner diversity indices table on *Fusarium* spp. in cultivars of wheat seeds in three Rift Valley Counties, Kenya.

Species	Frequency/Abudance			Nakuru		Narok		Uasin Gishu)	
	Nakuru	Narok	Uasin Gishu	Relative prevalenceprevalence(p_i)	$p_i \ln p_i$	Relative prevalenceprevalence(p_i)	piln pi	Relative prevalenceprevalence(p_i	piln pi
F. verticillioides	6	13	5	6/33=0.1812	-0.3095	13/28=0.4643	-0.3562	5/19=0.2632	-0.3513
F. equiseti	6	4	9	6/33=0.1812	-0.3095	4/28=0.1429	-0.2780	9/19=0.4737	-0.3539
F. culmorum	6	0	0	6/33=0.1812	-0.3095	0/28=0.0000	0.0000	0/19=0.0000	0.0000
F. poae	4	2	3	4/33=0.1212	-0.2558	2/28=0.0714	-0.1885	3/19=0.1579	-0.2915
F. oxysporum	3	6	0	3/33=0.0909	-0.2180	6/28=0.2143	-0.3301	0/19=0.0000	0.0000
Fusarium sp.	3	1	0	3/33=0.0909	-0.2180	1/28=0.0357	-0.1190	0/19=0.0000	0.0000
F. heterosporum	3	0	2	3/33=0.0909	-0.2180	0/28=0.0000	0.0000	2/19=0.1053	-0.2370
F. tricinctum	2	2	0	2/33=0.0606	-0.1699	2/28=0.0714	-0.1885	0/19=0.0000	0.0000
	33 isolates	28 isolates	19 isolates		$\sum p_i \ln p_i$ =-2.008		$\sum p_i \ln p_i$ =-1.4603		$\sum p_i \ln p_i$ =-1.2337
				Nakuru H=-$\sum p_i \ln p_i$ =-(-2.008)=**2.008**		Narok H=-$\sum p_i \ln p_i$ =-(-1.4603)=**1.4603**		Uasin Gishu H=-$\sum p_i \ln p_i$ =-(1.2337)=**1.2337**	

Appendix VII Publictions

1) Kheseli, O. P., Imbahale, S., Okoth, S., Otipa, M., & Wafula, W. V. (2021). Prevalence and Phylogenetic Diversity of Pathogenic *Fusarium* Species in Genotypes of Wheat Seeds in Three Rift Valley Regions, Kenya. *Advances in Agriculture*, *2021*, 1–13. https://doi.org/10.1155/2021/8839147
2) Otieno, P. K., Imbahale, S. S., Wekesa, V. W., Otipa, M., & Okoth, S. (2022). Molecular Determination of Toxigenic Potential of *Fusarium* spp. Isolated from Seeds of Wheat (Triticum aestivum) Genotypes and Evaluation of Levels of Fumonisins in the Grains at Harvest in Three Major Wheat Producing Counties in Kenya. International Journal of Agronomy, 2022, 1428312. https://doi.org/10.1155/2022/1428312

Appendix VIII Conferences attended

S/N	Name of Conference and Date	Title of presentation and form of attendance
1.	6th International Conference for Women in Science Without Borders. Date: 3rd – 5th, November 2021. Venue- Embu- Kenya	Toxigenic Potential of *Fusarium* spp. isolated from seeds of wheat genotypes in Kenya. Virtual presentation
2.	Food safety Conference for Africa. Date: 10th -11th November 2021.	Survey of Fumonisins and Deoxynivalenol in market wheat flour and wheat products in Kenya. Virtual presentation.

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Phanice Khesell Otieno, Susan S. Imbahale, Vitalis Wafula Wekesa, Miriam Otipa, Sheila Okoth. "Molecular Determination of Toxigenic Potential of Fusarium spp. Isolated from Seeds of Wheat (Triticum aestivum) Genotypes and Evaluation of Levels of Fumonisins in the Grains at Harvest in Three Major Wheat Producing Counties in Kenya", International Journal of Agronomy, 2022

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