Research Article

Prevalence and Phylogenetic Diversity of Pathogenic *Fusarium* Species in Genotypes of Wheat Seeds in Three Rift Valley Regions, Kenya

Otieno P. Kheseli [iD],[1] **Imbahale S. Susan** [iD],[1] **Okoth Sheila** [iD],[2] **Miriam Otipa**,[3] **and Wekesa V. Wafula** [iD][4]

[1]*Department of Applied and Technical Biology, Technical University of Kenya, P.O Box 52428–00200, Nairobi, Kenya*
[2]*School of Biological Sciences, University of Nairobi, P.O Box 30197–00100, Nairobi, Kenya*
[3]*Kenya Agricultural Research and Livestock Organization, Kabete, P.O Box 14733-00800, Nairobi, Kenya*
[4]*Flamingo Horticulture (K) Limited (Dudutech), P.O BOX 1927–20117, Naivasha, Kenya*

Correspondence should be addressed to Otieno P. Kheseli; phanicekheseli@yahoo.com

Wheat is a source of nutrients for around 40% world's population and the second most important cereal crop in Kenya. However, *Fusarium* head blight (FHB) hinders sustainable sufficient production of the crop, causing both economic and health losses. With the emerging unfavorable climatic changes, effective disease management strategies and adequate seed system are necessary to meet the deficiency. Current information on prevalence of the causative pathogens in varieties of wheat genotypes is a critical prerequisite to such strategies. This study aimed at determining the prevalence of pathogenic *Fusarium* species in seeds of developed varieties of wheat genotypes in three major wheat-producing regions in Kenya. A total of 260 samples of 18 wheat genotypes from 123 farms were collected. Peptone pentachloronitrobenze agar was used for fungal isolation, while identification of *Fusarium* spp. was based on the gene encoding translation elongation factor 1-α (tef1-alpha) sequence analysis. *Fusarium* spp. isolated include *Fusarium poae*, *F. tricinctum*, *F. heterosporum*, *F. culmorum*, *F. equiseti*, *Fusarium* sp., *F. verticillioides*, and *F. oxysporum*. There was no significant difference in prevalence of *Fusarium* spp. pathogens among the three regions studied. *Fusarium* spp. diversity index for Nakuru was 2.008, Narok was 1.4603, and Uasin Gishu was 1.2337. Wheat produce from farm-saved seeds yielded 66.25% of the isolates, while the produce from certified commercial wheat seeds yielded 33.75% of the isolates. The significant finding of the study is that *Fusarium* spp. associated with mycotoxins that contaminate the wheat food chain seem to be flourishing in all the sampled wheat seed genotypes from the regions studied. Information on the prevalence and diversity of the pathogens on persistence of the disease in the crop is critical in advancing integrative FHB control measures.

1. Introduction

Fusarium spp. is one of the most significant filamentous pathogenic fungal genera that is ubiquitous around the globe. They are field or soil fungi, causing wilts, seedling blights, rots, and cankers in susceptible plants [1]. *Fusarium* spp. contamination is a major agricultural problem that reduces the quality and yield of agricultural products while producing mycotoxins that have virulence factors responsible for many diseases in humans and farm animals [2].

Wheat (*Triticum aestivum*) is a staple source of nutrients for approximately 40% of the world's population and is the most widely grown crop in the world, cultivated on more than 218 million ha. In addition, its world trade is greater than all other crops combined [3, 4]. However, infestation of fungal pathogens in the crop is a serious worldwide problem due to various factors. Unfavorable climatic conditions and agronomy-related issues have been reported to be critical in this respect [5–8]. Pathogenic *Fusarium* spp. is the main cause of *Fusarium* head blight (FHB) that causes devastating

wheat grain destruction with resultant accumulation of mycotoxins.

Globally, various control measures are under investigation by researchers with the aim of effectively protecting the crop from FHB. This has involved the use of different pre-harvest and post-harvest methods, starting from agrotechnical methods that limit the source of primary infection. Post-harvest control measures include physical methods such as proper harvesting that does not damage the grains, crop rotation tillage, fertilization, use of appropriate quality seed material and the timely sowing period [9, 10], education and training of agricultural producers in application of good agricultural practices. Although fungicide efficacy against FHB has been inconsistent due to the short time framework for application as well as limited number of available fungicides [10], their role in control of FHB cannot be ignored among pre-harvest control measures. Chemical control of FHB in wheat influences the disease intensity in spikes and deoxynivalenol contamination in grains [11]. Timely use of the correct dosage of the effective type of fungicides [12–14] has been reported to be an applicable FHB control measure especially during plant flowering. Also included in this category is the use of resistance inducers including biopreparations based on antagonistic microbial agents, endophytes and other biologically active ingredients, and nonchemical fungicides and plowing [15–18]. Selection and use of cultivars with a high level of resistance to *Fusarium* spp. infestation [10, 17] and to lodging [19] cannot be overemphasized. Use of a combination of preharvest measures when conditions are favorable for *Fusarium* spp. infestation is more effective [9, 20] since they limit the survival of the pathogens in debris, reducing their presence in field and infestation severity. However, the ever-changing farm and climatic conditions require application of consistent effective FHB control measures such as the use of wheat genotypes that are highly resistant to *Fusarium* spp. pathogens. The current study investigated the prevalence and diversity of pathogenic *Fusarium* spp. in varieties of wheat genotypes from three major wheat-producing regions in the Kenyan Rift Valley in line with factors determining selection of the cultivated genotypes.

1.1. Background of the Study. Wheat is Kenya's second most important cereal crop after maize and an economically important crop among the large and small-scale farmers [21]. It is grown in areas above 1500 metres above sea level, in southern and upper Rift Valley regions (Nakuru, Narok, and Uasin Gishu) and in the eastern part of Kenya (Nanyuki and Meru). Wheat production is carried out by small, medium and large-scale farmers and the industry, supported by about 20 millers, contributes 1.4% and 30% to overall and cereal GDP [22], respectively. The high costs of farm inputs and land fragmentation among other factors have led to a shift in production from large-scale and medium-scale commercial farms using capital-intensive technology to production mostly by small-scale farmers [23].

Wheat requires a fine planting bed for uniform germination. In this view, farmers are encouraged to have the land thoroughly ploughed and harrowed, free from growing weeds and weed seeds, at least 4 weeks before planting to ensure that the land does not have fresh compost (plant material that is not fully rotten) during planting. Fertilizer application is recommended during planting depending on the soil type as per the soil analysis report. During planting, diammonium phosphate (DAP) at 200–250 kg Ha^{-1} is recommended as determined by factors such as the stage of the crop, since availability of nitrogen is important during tilling, stem extension, and ear emergence.

Diseases, pre-harvest, and post-harvest losses are the major recurring challenges hindering optimal production of wheat. Occurrence of wheat devastating fungal diseases such as wheat rust [24] and FHB [25–27] has been reported as the major setback to wheat production in Kenya. Generally, other than good farm management practices, the main wheat crop protection regime used by farmers in Kenya against all fungal foliar wheat diseases such as wheat rust is the use of fungicides. Foliar diseases can increase the susceptibility of wheat spikes to FHB. Consequently, fungicides become crucial in reducing the effects of the diseases on wheat. However, the low efficacy and limited spectrum of such fungicides create a gap in control of resistant fungal diseases such as FHB. Although disease resistance or tolerance remains a driving goal of Kenya Agricultural and Livestock Organization's (KALRO') wheat research program, it remains a challenge for the recommended wheat varieties [21]. Hence, there is consistent development of wheat genotypes with high stable yields, tolerance to biotic and abiotic stresses, pre-harvest sprouting diseases, and insect pests, so that farmers from different growing regions in the country can select the most suited variety [21]. Therefore, current information on prevalence and diversity of *Fusarium* spp. in the existing and cultivated wheat genotypes is important prerequisite information in integrated management of FHB and the improvement or development of other genotypes with better disease resistant qualities. The current study evaluated the occurrence and diversity of pathogenic *Fusarium* spp. in the developed wheat genotypes in three major wheat-producing regions within the Kenyan Rift Valley and the factors considered by farmers in selecting the cultivated wheat varieties.

2. Materials and Methods

2.1. Study Area. The study was carried out in three regions situated within the Kenyan Rift Valley, the main commercial wheat-growing zone. Figure 1 shows the location of each site generated using the geographical positioning system (GPS). The average rainfall range was between 20 mm–40 mm in Narok, 60 mm–80 mm in Uasin Gishu, and 40 mm–60 mm in Nakuru; whereas, the average temperature range in Narok was 18°C–22°C, in Usain Gishu <18°C, and 18°C–22°C in Nakuru during the study period. These areas have annual rainfall varying between 800 mm and 2000 mm, with the amounts occasionally rising up to 2,500 mm in higher altitudes. Research on smallholder wheat technologies is performed by the National Plant Breeding Station based in Nakuru. The commonly cultivated crops on large-scale
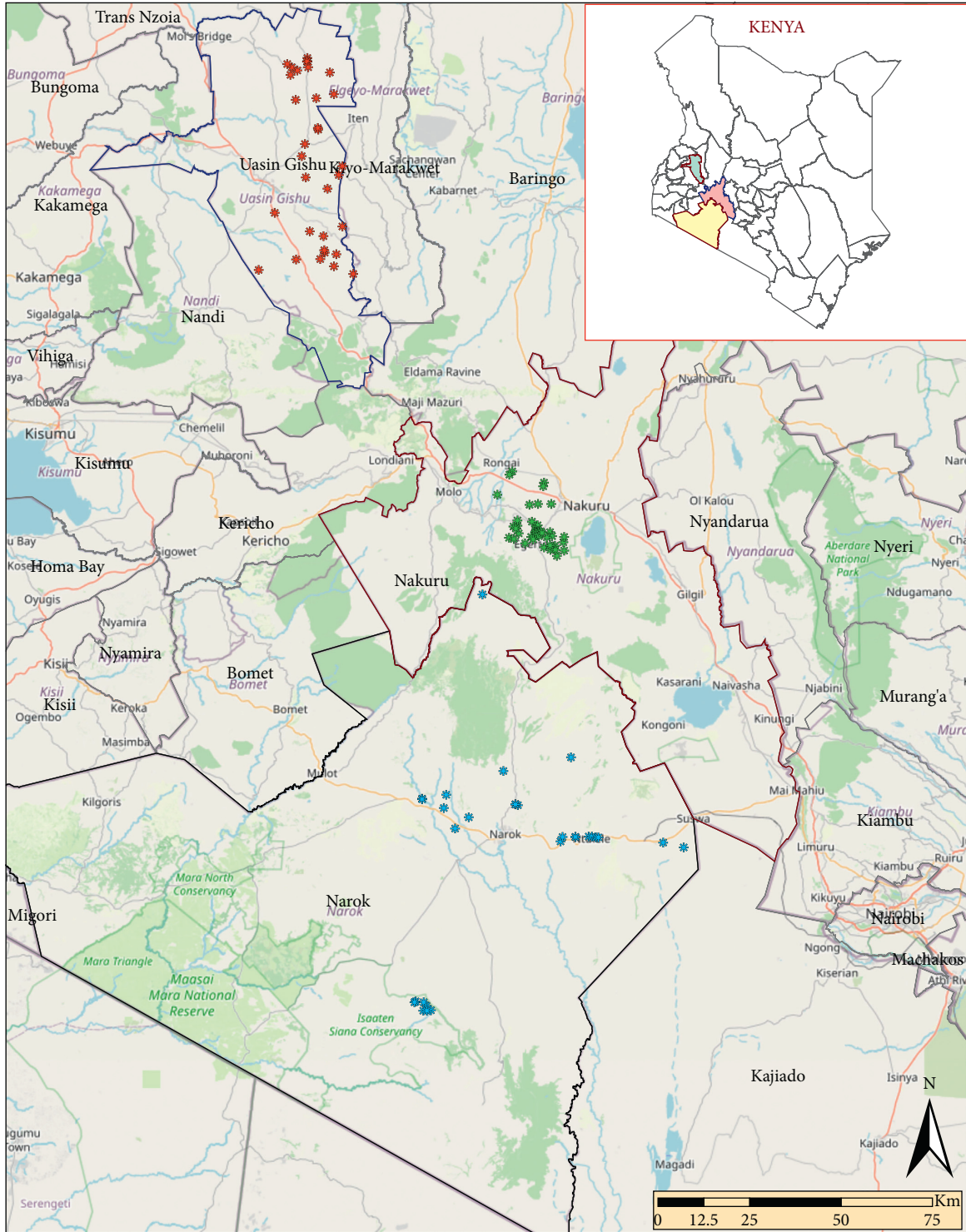
FIGURE 1: The location of each sampling site generated using the geographical positioning system (GPS).

farms in the study regions are wheat, barley, and maize. Other subsistence crops such as legumes (beans), cabbages, kales, and Irish potatoes are also cultivated on small-scale basis. Farms barely stay fallow for 1-2 years before cultivation.

2.2. Field Sampling of Wheat Seeds. The sampling took place between September 2016 and October 2017 as follows: Narok, July 28th to early September, 2016; Uasin Gishu region, November 8, 2016 to early December, 2016, Nakuru region: Nakuru-Naivasha area, February, 2017; and Nakuru-Njoro area, October, 2017. In Nakuru region, most of the sampling was carried out in Njoro, the main wheat-producing and research centre. Cross-sectional purposeful sampling was followed, whereby farms were randomly selected depending on the day of harvesting. Laboratory and file samples were taken from bulks of freshly harvested wheat grains using spear sampling technique [28, 29]. The number of samples collected per farm varied as determined by farm size and ranged from 3 to 21 samples per site. Each wheat variety was sampled in replicates of three per sampling site and every sample weighed approximately 500 gm. Samples were packed in khaki papers and labelled and transported in cooler boxes to the laboratory, where they were stored at 4°C for isolation of *Fusarium* species. The sampling process included gathering information from wheat farmers on the genotypes of wheat seeds cultivated and criteria used in selection of the wheat genotypes, the use of farm-saved and certified commercial wheat seeds and last, common fungal diseases observed on the crop in the fields.

2.3. Preparation of Wheat Samples for Isolation and Characterization of Fusarium spp. To obtain representative samples from multiple samples procedures described by the authors of [28, 29] were used but with slight modifications. Multiple samples from specific farms and sites were aseptically poured into a sterile container (polythene paper bag) and manually mixed to make one homogenous composite sample. The composite sample was then manually reduced into portions of 500 gm and labelled and randomly selected for use as test and file samples. Samples that weighed 500 gm from collection sites were used directly as test samples after mixing. The methods developed by the authors of [30, 31] were adopted for use in the process of isolation and morphological characterization of *Fusarium* species. Approximately one hundred and fifty seeds of each test sample were randomly picked and soaked in 2% of NaOCl for 2 minutes. The seeds were then rinsed three times, each time in fresh sterile distilled water and dried on sterile muslin cloth in a laminar flow. Ten sterile seeds from each of the three replicates of every sample were randomly picked and plated in triplicates on selective media, peptone pentachloronitrobenze agar, and incubated at 25°C for 4–7 days. These constituted 90 plated seeds for each sample. Germinating fungal colonies were subcultured on tap water agar at 25°C for a period of 7–14 days for sporulation. Spores of each isolate were examined using a microscope to ascertain

distinctive characteristic features unique to *Fusarium* species. To obtain pure colonies, spores of each *Fusarium* spp. isolate were subjected to 10^{-4} fold serial dilution and cultured on water agar at 25°C for 18 hours. Hyphal tips of each fungal colony formed were subcultured in triplicates on three different media: potato dextrose agar (PDA), synthetic agar (SNA), and carnation leaf agar (CLA) under two different temperatures, 25°C and 30°C, with alternating 12 hours of darkness and 12 hours of fluorescent light. This was carried out for further morphological characterization following procedures by the authors of [32, 33]. Pure *Fusarium* spp. isolates were then stored as spore suspensions in 15% glycerol at −80°C in Kenya Agricultural and Livestock Research Organization (KALRO) biotechnology laboratory for molecular characterization.

2.4. Molecular Characterization of Fusarium spp

2.4.1. DNA Extraction. Spores of each isolate were cultured on PDA at 25°C and alternating 12 hours of fluorescent light and darkness. DNA of each isolate was extracted between day 7 and day 14 depending on the quantity of mycelia using a Zymo Research Fungal/Bacterial DNA Mini Prep Kit (Epigenetics, Hatfield, South Africa) and according to the manufacturer's instructions. For optimal performance, beta-mercaptoethanol (user supplied) was added to the genomic lysis buffer to a final dilution of 0.5% (v/v), i.e., 500 μl per 100 ml. In the first step, approximately 10 mg–20 mg (wet weight) of fungal cells that had been resuspended in up to 200 μl of isotonic buffer (e.g., PBS) were added to a ZR BashingBead™ lysis tube (0.1 mm and 0.5 mm), and 750 μl BashingBead™ buffer was added to the tube before capping tightly. The preparation was then secured in a bead beater fitted with a 2 ml tube holder assembly and processed at a maximum speed of ≥5 minutes. Processing time varied based on sample input. The ZR BashingBead™ lysis tube (0.1 mm and 0.5 mm) was then centrifuged in a micro-centrifuge at 10,000 x g for 1 minute. Up to 400 μl of the supernatant was transferred to the Zymo-Spin™ III-F filter in a collection tube and centrifuged at 8,000 x g for 1 minute, and the Zymo-Spin™ III-F filter was discarded. Next, 1,200 μl of genomic lysis buffer was then added to the filtrate in the collection tube and mixed well. 800 μl of the mixture was added to a Zymo-Spin™ IC column 2 in a collection tube and centrifuged at 10,000 x g for 1 minute. The flow through from the collection tube was discarded, and the previous step (step 6) was repeated. 200 μl DNA pre-wash buffer was then added to the Zymo-Spin™ IC column in a new collection tube and centrifuged at 10,000 x g for 1 minute. 500 μl·g^{-1} DNA Wash Buffer was added to the Zymo-Spin™ IC column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IC column was then transferred to a clean 1.5 ml microcentrifuge tube, and 20 μl (10 μl minimum) of DNA elution buffer was added directly to the column matrix and incubated for 1 minute. This was followed by centrifugation at 10,000 x g for 30 seconds to elute the DNA, using a Zymo Research Fungal/Bacterial DNA Mini Prep Kit (Epigenetics, Hatfield, South Africa) and according to the manufacturer's instructions.

2.4.2. Polymerase Chain Reaction (PCR) Amplification of Fusarium spp. DNA.

A set of primers targeting the gene encoding translation elongation factor 1-α (tef1-α)- Ef1 (forward primer: 5'-ATGGGTAAGGA (A/G) GACAA-GAC-3') and EF2 (reverse primer: 5'-GGA (G/A) GTAC-CAGT (G/C) ATCATGTT-3') was used to amplify 700 bp fragment [34]. The PCR was performed in 25 μl reactions which included 1 μl of a 1 : 10 or 1 : 100 DNA dilution, 1 U RedTaq DNA polymerase (Sigma–Aldrich Company, Milan, Italy), 2 μl RedTaq buffer supplemented with 1.7 μl of 22 mM MgCl$_2$ for a final concentration of 3.0 mM, 10 mM de-oxyribonucleotide) and 1.0 μM of each primer: reverse and forward (ef1 and ef2). Reactions were run in a Mastercycler ep-gradient (BioRad, California, USA) with a thermal profile of 4 min at 94°C followed by 35 cycles of 60 s at 94°C, 60 s at 57°C and 1 min at 72°C followed by 72°C for 5 min. The amplified DNA was electrophoresed in 1.5% (w/v) Tris-acetate ethylene diamine tetraacetic acid (EDTA) agarose gels. Amplicons were visualized at 700 bp–710 bp using a 1 kb bp DNA ladder (Bioline) as a size standard. A concentration of 1% agarose gel was used. To make the gel, the agarose was boiled at 100°C for 5 minutes in a conical flask and left to cool to 55°C. Then, 0.3 μl of ethidium bromide was added while swirling the flask to enable the gel mix with ethidium bromide. The mixture was poured into a gel tank with the combs on and left to solidify. Molecular marker (2 μl) was added to one well, and DNA (4 μl) plus the loading dye was added to other wells and the arrangement was noted. Distilled water was used as a control. The gel was run for 45 minutes at 80 V and viewed under Geldoc (BioRad, Molecular Imager Gel Doc XR-CLASS, Imaging System, California and USA). A qPCR to estimate the severity of infection for each given Fusarium spp. per sample was not performed since our interest for DNA analysis was to ascertain the species identity of the isolates.

2.4.3. Nucleotide Sequencing and Analysis of tef1-α Nucleotide Sequences.

Amplification products of TEF-1 gene were purified using a QIAquick Gel Extraction Kit according to manufacturer's instructions (Qiagen manual). Sequencing of both strands was performed in the ABI 3700 DNA Sequencer in the Sequencing Service Unit of Macrogen (Netherlands). The ef1-α (tef1-α) raw nucleotide sequences were assembled, contigs generated, and consensus sequences retrieved using Geneious version 11.1.5 software. Basic Alignment Search Tool for Nucleotide Sequences (BLASTN), https://blast.ncbi.nlm.nih.gov/Blast.cgito, was used in retrieving sequences with high similarity index with those from National Centre for Biotechnology Informatics (NCBI) database. The retrieved sequences were aligned together with the consensus sequences of the isolates using ClustalW, a matrix-based algorithm build in Geneious version 11.1.5 software. A phylogenetic tree was generated to infer relatedness of the aligned 49 sequences. Maximum likelihood (ML) analysis was performed and a phylogenetic tree (Figure 2) was built through the Tamura–Nei model. One thousand bootstrap replicates were performed for assurance of stability and robustness of each of the branch

[35, 36]. The tree with the highest log likelihood (−31270.16) was shown. The percentage of trees in which the associated taxa clustered together was shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with the superior log likelihood value. There were 4167 positions in the final dataset. Evolutionary analysis was conducted in MEGA X [35].

2.5. Data Analysis on Prevalence and Diversity of Fusarium spp.

Data was analyzed in SPSS using one-way ANOVA to compare prevalence of each of the Fusarium spp. isolated. The analysis was done with respect to overall occurrence and types of Fusarium spp. in each of the sampled wheat genotypes among the three regions, at 0.05 significance level. Fusarium spp. with significantly different means among the regions were subjected to further analysis using the LSD test for multiple comparisons to identify any differences in overall occurrences between any two regions. To determine species richness and their relative abundance in each region, diversity indices were computed using Simpson's and Shannon's diversity equations. Evaluation of the prevalence of Fusarium spp. between wheat seeds arising from farm-saved seeds and certified commercial seeds was also performed using descriptive statistics.

Simpson's diversity index (D) is given by

$$D = 1 - \sum (p_i)^2. \tag{1}$$

While Shannon's Weiner diversity index (H) is given by

$$H = -\sum p_i \ln p_i, \tag{2}$$

where "D" stands for Simpson's diversity index, "H" stands for Shannon's diversity index, "$\sum$" is the sum of and "p_i" is the relative abundance of each species, and "ln" is the natural logarithm.

3. Results

3.1. Wheat Genotype Preference by Farmers in the Rift Valley.

Two hundred and sixty (260) samples of different genotypes of wheat seeds (Table 1) were collected from 123 wheat farms. Eighteen wheat seed genotypes were classified out of the 260 samples of wheat seeds collected. Sixty-six percent (66.25%) was the produce of certified commercial wheat seeds and 33.75% was the produce of farm-saved wheat seeds (Table 2). Agro-economic factors that determined farmers' choice of wheat seed genotype to cultivate varied from potential weight of wheat yield, resistance of wheat grains to pests in storage, affordability of wheat seed genotype, resistance of wheat genotype to wheat rust, availability of the seeds of genotypes released in the market and free wheat seed genotypes offered for research purposes or for production of certified wheat seeds. Only 1.6% of the farmers whose wheat seeds were sampled cited FHB as a serious problem that affects the crop. However, the disease effects
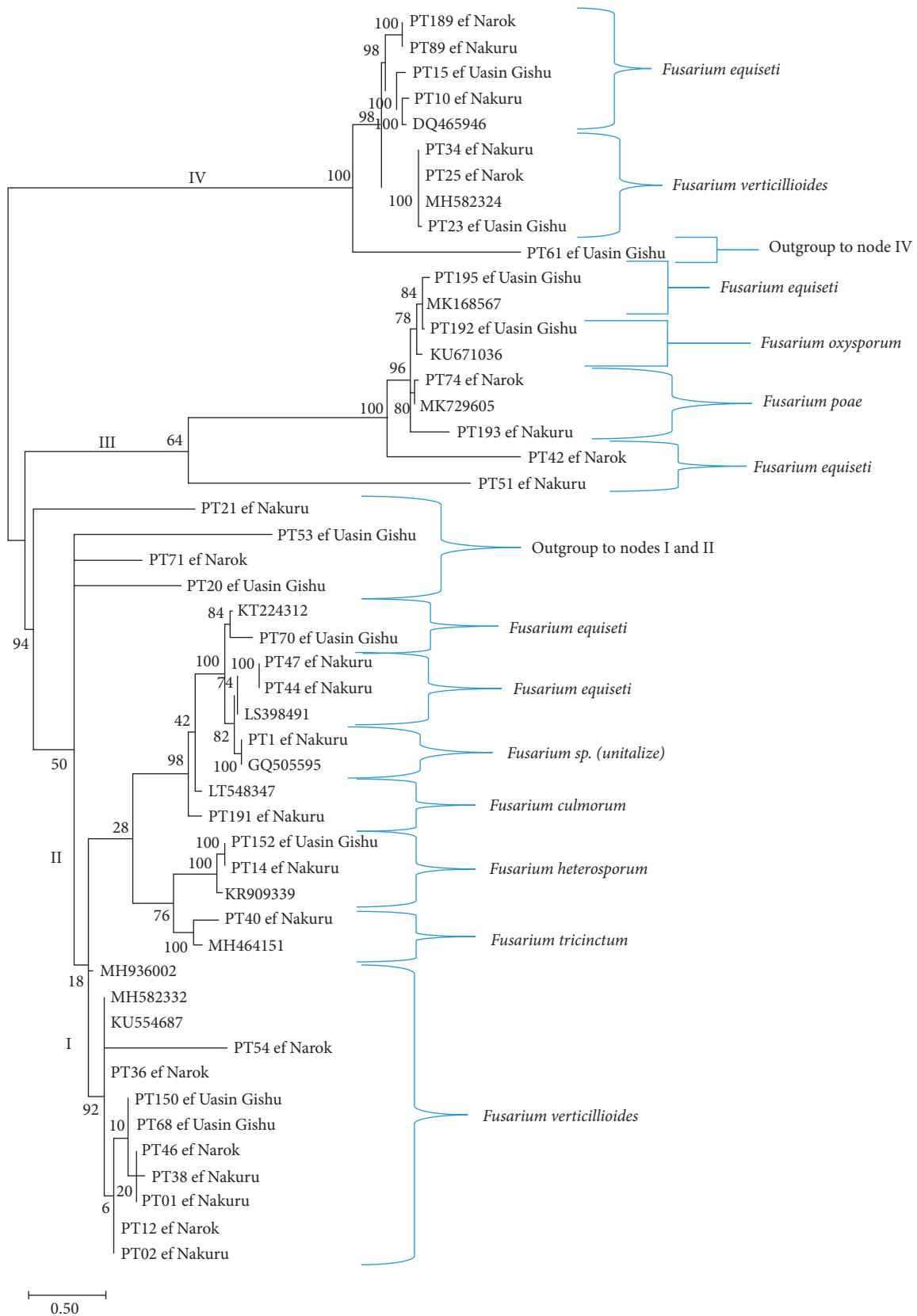
FIGURE 2: Phylogenetic tree of *Fusarium* spp. isolated from freshly harvested genotypes of wheat seeds in three Rift Valley regions, Kenya. The analysis was based on tef1-α gene sequences and by the maximum likelihood method with 1,000 bootstraps.

TABLE 1: Wheat genotypes sampled in three Rift Valley regions, Kenya.

Wheat genotype	Region of sampling and no. of samples			Total no. of samples
	Narok	Uasin Gishu	Nakuru	
1. Robin	15	9	9	36
2. Eagle 10	9	3	6	18
3. Ibis	3	0	3	6
4. Njoro II	30	82	15	127
5. Korongo	6	0	3	9
6. Farasi	3	0	0	3
7. Mwamba	6	0	3	9
8. Hawk	3	0	3	6
9. Ruiru	6	0	0	6
10. Simba	0	3	0	3
11. Ngamia	0	0	3	3
12. Duma	0	0	3	3
13. Kingbird	0	0	3	3
14. K. Tai	0	0	3	3
15. Yombi	0	0	3	3
16. K. Wren	0	0	3	3
17. Kwale	0	0	3	3
18. K. Sunbird	0	0	3	3
19. Undisclosed	8	4	4	16
Total	89	101	70	260

TABLE 2: Produce of certified and farm-saved genotypes of wheat seeds and overall occurrence of *Fusarium* spp.

S/N	Wheat genotype	Occurrence of certified and farm-saved wheat seed produce						Percentage (%) occurrence of *Fusarium* spp.	
		CT	FS	CT	FS	CT	FS	CT	FS
1	Njoro II	+	+	+	+	+	+	2.5	25
2	Unidentified	−	+	−	+	−	+	−	6.25
3	Eagle 10	+	−	−	+	+	−	3.75	7.5
4	Robin	+	−	−	+	+	−	1.25	5
5	Mwamba	−	−	+	−	−	−	2.5	−
6	K. Korongo	+	−	−	−	−	−	−	6.25
7	Ruiru	−	−	−	+	−	−	−	5
8	Hawk	−	−	−	+	−	−	1.25	3.75
9	K. Ibis	−	+	−	+	−	−	−	6.25
10	Duma	+	−	−	−	−	−	5	−
11	Kwale	+	−	−	+	−	−	3.75	−
12	Fahari	−	−	+	−	−	−	2.5	2.5
13	Kingbird	+	−	−	−	−	−	1.25	−
14	K. Tai	+	−	−	−	−	−	1.25	−
15	Yombi	+	−	−	−	−	−	2.5	−
16	K. Wren	+	−	−	−	−	−	0	−
17	Simba	+	−	−	−	+	−	2.5	−
18	Sunbird	+	−	−	−	−	−	1.25	−
19	Ngamia	+	−	−	−	−	−	2.5	−
	Overall % occurrence of *Fusarium* spp.							33.75	66.25

K, Kenya; T, total; "+," produce of wheat genotype sampled; "−," produce of wheat genotype not sampled; CT, certified commercial wheat seeds; FS, farm-saved wheat "seeds."

were incidentally observable on some farms and some wheat grains. All (100%) farmers whose wheat seeds were used in this study cited stem rust as the major fungal disease affecting sustainable wheat production.

3.2. *Fusarium* spp. Complex in Genotypes of Wheat Seeds at Harvest Time. Based on the identification key the authors of [33], 80 fungal isolates belonging to the genus *Fusarium* were identified. Distinctive morphological features of mycelia, pigment formation and conidial cells' formation were used to determine both similarities and differences among the isolates of *Fusarium* spp. Results following PCR amplification of DNA for each positive isolate exhibited single clear amplified band, with fragment total size of between 700 bp and 710 bp (Figure 3). Analysis and comparison of the resultant sequences with sequences from National Centre for Biotechnology Informatics (NCBI) database using Basic Alignment Search Tool for Nucleotide Sequences (BLASTN), https://blast.ncbi.nlm.nih.gov/Blast.cgito, established species identities of the fungal isolates as *Fusarium poae*, *F. tricinctum*, *F. heterosporum*, *F. culmorum*, *F. equiseti*, *Fusarium* sp., *F. verticillioides* and *F. oxysporum*. Mean prevalence of *Fusarium* spp. among the three counties was not significantly (all $p > 0.05$) different (Figure 4). However, exceptions in prevalence were observed at the species level with respect to *F. verticillioides*, *F. oxysporum*, *F. tricinctum*, *F. culmorum*, and *Fusarium* sp. among the 3 regions after LSD pairwise comparisons. These exceptions were observed in the occurrence of the following species: *F. verticillioides* between Nakuru and Uasin Gishu ($p = 0.591$), *F. culmorum* between Nakuru and Uasin Gishu ($p = 1.00$), *F. tricinctum* between Nakuru and Narok ($p = 1.00$), and *Fusarium* sp. between Narok and Uasin Gishu ($p = 0.078$). Diversity indices for *Fusarium* spp. in the three regions were as follows: Nakuru County, 2.008; Narok, 1.4603; and Uasin Gishu, 1.2337. Based on descriptive statistics, wheat produce of certified seeds had a minimum of 0 amounts of *Fusarium* spp., while the maximum was 16 with a mean of 3.42. Wheat produce of farm-saved seeds had a minimum of 0 amounts of *Fusarium* spp. isolates while the maximum amount was 22 with a mean of 6.31. The overall
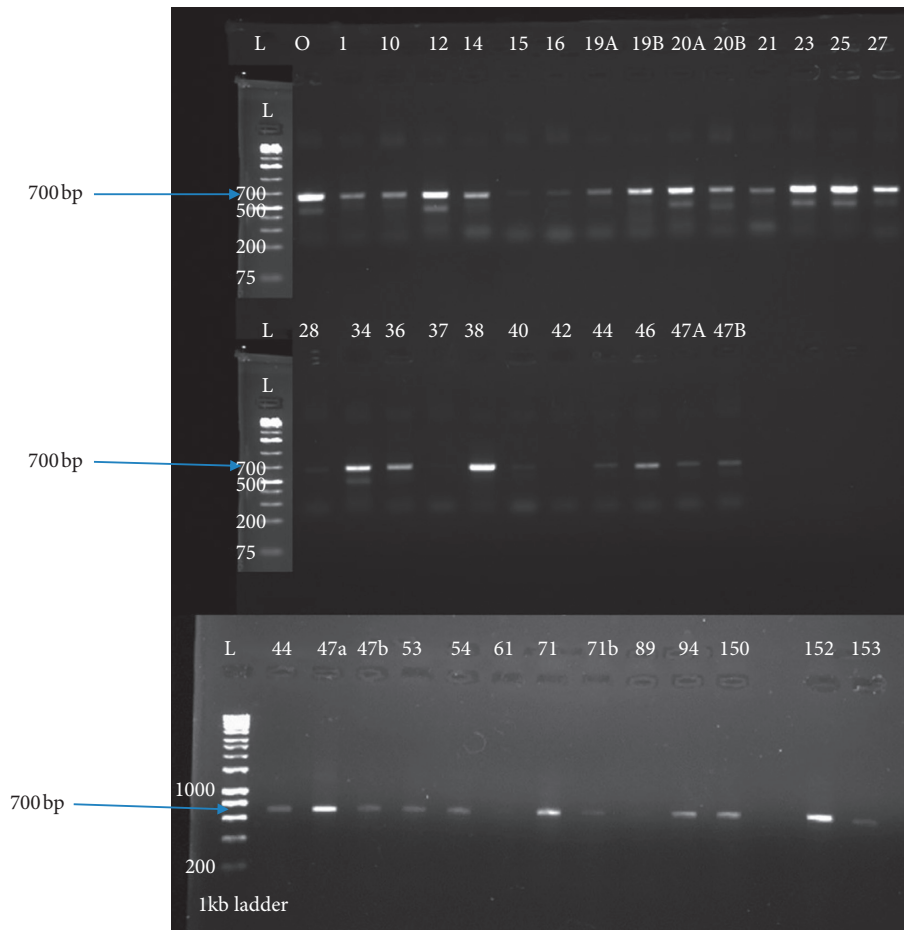
FIGURE 3: Agarose 1% gel electrophoresis of PCR-amplified elongation factor 1-alpha gene of *Fusarium* spp. isolated from different genotypes of wheat seeds in three Rift Valley regions, Kenya. Amplification band of 700-710 bp shows positive results for identity of *Fusarium* spp.

amounts of *Fusarium* spp. per wheat variety in the category of farm-saved seeds were widely spread (SD = 6.626) and hence implicative of a higher prevalence of *Fusarium* spp. compared to those resulting from varieties of certified commercial wheat seeds (Table 2).

3.2.1. Phylogenetic Analysis of the Fusarium spp. Isolates. A phylogenetic tree generated to infer relatedness of the aligned sequences was constructed based on bootstrap values of over 75%, while the identity of the isolates was based on identity matrix values lying between 95% and 100% in similarity with reference sequences in the NCBI. This analysis further confirmed the identity of the species and phylogenetic relations, revealing interspecies variation and intraspecies variation among members of *F. verticillioides* and *F. equiseti*, while some other fungal isolates emerged as outgroups. The phylogenetic tree divided the strains into four major clusters, namely, I, II, III, and IV (Figure 2). *Fusarium* strains identified under cluster I included *F. verticillioides* strain CNRG455, GeneBank accession (cc) no. MH936002; *Fusarium* sp. strain MCR2228, GeneBank accession no. MH582332; and *F. verticillioides* strain F28,

GeneBank accession no. KU554687. Identity matrix values for the strains ranged between 97.85% and 100% and were all supported by the bootstrap value of 100%. Group II comprised of five *Fusarium* spp. (*F. verticillioides* strain CM-CNRG455, GeneBank accession no. MH936002; *F. tricinctum* strain PPRI20693, GeneBank accession no. MH464151; *F. heterosporum,* GeneBank accession no. DAOMC235644; *F. culmorum* strain E24, GeneBank accession No. LT548347; *Fusarium* sp. isolate NRRL 20722, GeneBank accession no. GQ505595; and *F. equiseti* strain URM: 6788, GeneBank accession no. LS398491). The highest identity matrix value in this cluster was 99.85%, supported by bootstrap values of 100% for all the species. Four (4) *Fusarium* spp. isolates (PT20, PT21, PT53, and PT7) in this cluster had low identity matrix and hence were treated as outgroups to node I and node II. Group III was supported by a bootstrap value of 100% and constituted of subclusters that divided the members into 3 *Fusarium* species: *F. poae* strain Montana II, MK729605; *F. oxysporum*, KU671036; and *F. equiseti* isolate LQ144, MK168567 with similarity matrices of 97.67%, 89.15%, and 100%, respectively. In this cluster, *Fusarium* spp. isolate PT42 and PT51 emerged as out groups under the identity of *F. equiseti* isolate LQ144, MK168567.
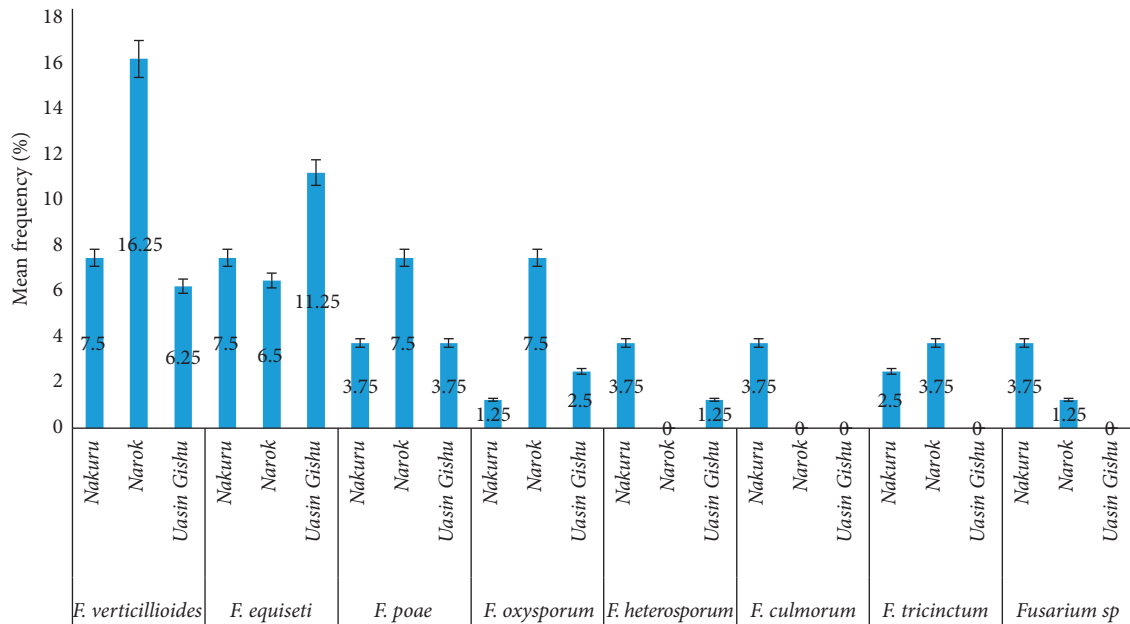
FIGURE 4: Means on prevalence of pathogenic *Fusarium* spp. in genotypes of wheat seeds at harvest time in three Rift Valley regions, Kenya.

Group IV *Fusarium* spp. strains had a bootstrap value of 100% and comprised of one-subcluster and one outgroup, PT61, whose reference accession identity was *F. equiseti*. This isolate was so prevalent in Uasin Gishu region. The subcluster was further divided into two groups, both of which had bootstrap values of 100%. One of the groups comprised of *F. equiseti* isolate SAT73, with identity reference Gene-Bank accession no. DQ465946, while the other group consisted of *F. verticillioides* strain MRC929, GeneBank accession no. MH582324.

3.2.2. Diversity of Pathogenic Fusarium spp. in the Wheat Seed Varieties. The phylogenetic analysis shown in Figure 2 shows that *F. verticillioides*, *F. equiseti, Fusarium poae, F. oxysporum, F. tricinctum, Fusarium* sp., and *F. culmorum* comprised the pathogenic *Fusarium* spp. complex diversity prevalent in the sampled genotypes of wheat seeds (Table 3). *Fusarium verticillioides* and *F. equiseti* isolates were distributed across more than one cluster in the phylogenetic tree and were isolated from over 70% of all the wheat genotypes studied. Percentage prevalence of *F. verticillioides*, *F. equiseti* and *F. poae* in all the wheat seed genotypes was as follows: 94%, 77%, and 50%, respectively, compared to the prevalence of the other *Fusarium* spp. isolated. All the eight *Fusarium* spp. were more prevalent in Njoro II and Eagle 10 wheat seed genotypes, while Kenya Wren is the only wheat genotype that was not infected with *Fusarium* species (Table 3). Other than Robin wheat genotype, both Njoro II and Eagle 10 wheat genotypes were sampled from all the three regions of study in the following proportions: 44%, 15%, and 9.5% for Njoro II, Eagle 10, and Robin, respectively. All of them were infected with over 50% of the isolated *Fusarium* species. *Fusarium graminearum* was not isolated from any of the wheat samples.

4. Discussion

The findings of this study suggest that freshly harvested wheat produce of both farm-saved and commercially processed wheat seeds was significantly infected with pathogenic *Fusarium* spp. that may inherently affect plant growth from germination. When such *Fusarium* spp. infected grains are used as seed for the next crop without decontamination, poor stands, poor vigor, and/or seedling blights can occur, substantially reducing optimal production of the crop. *Fusarium* spp. infected grains may as well be contaminated with mycotoxins that affect both human beings and domesticated animals that consume wheat products and wheat remains in the form of fodder, respectively. Farm-saved wheat seeds had a higher prevalence of *Fusarium* spp. compared to certified commercial wheat seeds despite the fact that they formed only 22.7% of the wheat grains sampled. *Fusarium* spp. infected grains in wheat debris also form an initial point for FHB contamination on farms and may contribute to propagation of FHB in the next crop especially in case of poor preharvest and postharvest wheat cultivation practices. Therefore, farmers should consistently use good quality, certified and decontaminated seeds to avoid initial fungal contamination by infected seeds [37, 38] and the subsequent disease effects [39]. Decontamination of seeds has been reported to increase grain yield and reduce amount of deoxynivalenol (DON) in wheat [40].

Ninety-four percent (94%) of the wheat seed genotypes studied were infected with *Fusarium* spp., the major causative agents of FHB. This high prevalence indicates the susceptibility of some of the developed wheat genotypes to the disease. However, the prevalence and distribution of the fungal pathogens within the eighteen wheat genotypes sampled varied. Njoro II, Kenya Eagle 10, and Robin wheat genotypes were dominant in all the three regions, and they

TABLE 3: Pathogenic *Fusarium* spp. diversity in genotypes of wheat seeds in three Rift Valley regions, Kenya.

Fusarium species	Wheat genotypes and the occurrence of *Fusarium* spp.																			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1. *F. verticillioides*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	—	18
2. *F. equiseti*	✓	✓	✓	✓	—	✓	✓	✓	✓	✓	✓	✓	✓	✓	—	✓	✓	—	—	15
3. *F. poae*	✓	✓	—	✓	✓	—	✓	✓	—	—	✓	—	—	—	✓	—	✓	—	—	9
4. *F. oxysporum*	✓	✓	✓	✓	✓	✓	—	✓	—	—	—	—	✓	—	—	—	—	—	—	8
5. *F. tricinctum*	✓	✓	—	—	—	—	✓	✓	—	—	✓	—	✓	—	✓	—	—	—	—	7
6. *F. heterosporum*	✓	✓	—	—	—	—	✓	—	—	—	—	✓	—	—	—	—	—	—	—	4
7. *Fusarium* sp.	✓	✓	—	—	—	✓	—	—	✓	—	—	—	—	—	—	—	—	—	—	4
8. *F. culmorum*	✓	✓	—	—	✓	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
No. of *F.* species	8	8	4	4	4	4	5	4	3	2	4	3	2	4	2	3	2	1	0	

A, Njoro II; B, Eagle 10; C, unidentified wheat genotype; 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; T, total number of affected wheat varieties; "✓," isolated; "—," not isolated.

were all infected with over 50% of the isolated pathogenic fungi. Worth noting is the finding that all wheat genotypes (Kenya Tai, Kenya Sunbird, Kenya Wren, and Kenya Kingbird) developed for resistance against fungal diseases such as stem rust and yellow rust had less than 50% or had zero overall occurrence of *Fusarium* spp. However, Eagle 10 wheat genotype was an exception in this respect. Njoro II, farmers' most preferred wheat genotype, recorded the highest (25%) rate of *Fusarium* sp. infestation in the category of farm-saved seeds compared to 3.75% that occurred in certified commercial genotypes (Eagle 10 and Kwale) of wheat seeds. The occurrence of *F. culmorum*, *F. poae*, *F. equiseti*, *F. tricinctum*, *Fusarium* sp., and *F. verticillioides* in almost all the wheat genotypes studied confirms the problem of pathogenic *Fusarium* spp. in the wheat crop in the regions. *Fusarium oxysporum* and *F. heterosporum* are among the less frequently or sporadically isolated *Fusarium* spp. in cereals such as maize [41] and hence, their occurrence in the studied wheat genotypes should be a concern for further research. Of significance was the absence of *F. graminearum*, the main causative agent of FHB in the isolated populations of *Fusarium* spp. isolates. This finding contradicts with existing research findings on occurrence of *Fusarium* spp. on wheat kernels in both Narok and Nakuru counties, in which *F. graminearum* has been previously reported [26, 27]. The absence of *F. graminearum* might be due to the prolonged drought that existed in years 2016 and 2017 [42] when the crop was in season and did not favor the growth of the species. It may also be due to the decline in the population of this strain in the areas studied. The high prevalence of *F. verticillioides*, one of the causative agents of pink ear rot, in all the three regions of the study is in agreement with the existing reports on the wide distribution of the species [32, 33] and the report that it is the most common species isolated worldwide from diseased maize [43]. Its high prevalence in wheat is alarming, since it is a globally important pathogen of agriculture and livestock [44] and harmful fumonisins. The occurrence of *Fusarium* spp. in Uasin Gishu is significant due to scanty or lack of similar published data in the region compared to Narok and Nakuru counties.

The prevalence and diversity of the *Fusarium* spp. in the three regions was similar despite the fact that the regions are located in fairly different agro-ecological zones. This could be partly explained by the similarity in unfavorable changing climatic conditions, agronomy-related factors and farm practices in wheat production. The factors included cultivation of succeeding crops such as maize, barley and wheat, sharing of wheat farm tillage and harvesting equipment without strict observation of disease preventive measures, poor management of weeds, less fertilization, continuous land use, unskilled manpower and use of unprocessed low quality farmers' saved wheat seeds. Similar role of agro-economic factors in occurrences and propagation of FHB pathogens in wheat has been reported [45–48]. Infected wheat, barley, and maize debris usually act as the initial FHB inoculum [46, 49] on wheat farms. Consequently, consistent cultivation of these succeeding crops without proper farm management predisposes the wheat crop to the pathogens. Nakuru region recorded all the isolated *Fusarium* spp. which might be explained by two main reasons. First, there are many potentially susceptible wheat varieties cultivated in the area for research purposes. Second, the relatively warm and wet humid climatic conditions that characterize the region are favorable to FHB infestation.

Njoro II was the farmers' most preferred wheat variety because of its potential to produce high yields and to resist wheat rust. Only 1.63% of the farmers whose wheat seeds were sampled had knowledge about other fungal diseases such as FHB, while the majority had no idea. Over 90% of farmers recognized all fungal wheat diseases as "Wheat Rust" even where symptoms were for a different fungal infection. In Uasin Gishu, the farmers' minimal adoption of new wheat seed varieties was evident. Only four wheat genotypes (Njoro II, Robin, Simba, and Eagle 10) were cultivated. Existing reports suggest that limited knowledge on wheat seed management and adoption of new seed varieties [50], technical inefficiencies such as lack of farm equipment, lack of capital for farm inputs and application of less than recommended levels of fertilizers and use of low quality farm-saved seeds [22] hinder maximum potential of wheat farms. Therefore, awareness creation and education of farmers on the control of fungal wheat diseases and the related effects of mycotoxins in wheat production remains critical.

5. Conclusion and Recommendations

In conclusion, *Fusarium* spp. associated with mycotoxins that contaminate the wheat food chain seem to be rampant

in the genotypes of wheat studied with no significant difference in both prevalence and diversity in the three regions of study. However, the variation in percentages of *Fusarium* spp. isolated from each wheat genotype provides evidence that some of the developed wheat genotypes might be more susceptible to pathogenic *Fusarium* species complex. The findings from this study also emphasize the impact of unprocessed and low quality farm-saved wheat grains in production of the wheat crop and its products. Wheat farmers are much more informed about wheat rust as compared to FHB. There is therefore a need for consolidative effort by stakeholders in wheat production to create awareness and educate farmers on control and management of all fungal diseases for sustainable wheat crop production. There is a need for increased extension services to farmers with the aim of inclusion in controlling FHB infestation in wheat crop and awareness on its effects from both economic and health perspective. Since only 10% of wheat genotypes developed for various purposes in Kenya is in use, there is a need for constant feedback from farmers on the criteria for selection of wheat genotypes. This information is helpful to wheat seed-producing companies in improving the resistance potential of existing varieties and/or development of new wheat genotypes with stronger resistance.

Data Availability

The data used to support the research findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

OPK, OS, and WVW conceptualized and designed the study. OPK carried out field data collection. ISS and MO assisted OPK with sample collection and data analysis. OPK and ISS drafted the manuscript. OS, ISS, and WVV critically reviewed the manuscript. All authors read and approved the manuscript for publication.

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