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Research Article

Diversity of *Fusarium* Species Infecting Maize Grown In Nakuru County, Kenya

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Abstract

Maize (Zea mays L.) is one of the famous food crop in Kenya. It is always contaminated by fungi, *Fusarium* spp. produces mycotoxins, Fumonisins (FBs) which occurs worldwide in maize infected by *Fusarium verticillioides*. The most common mycotoxin is Fumonisin FB_1. Intake of this toxin above 2.0 mg/kg body weight/day has a role to play in development of Oesophangeal cancer. No data has been found to be readily available on the genetic diversity of *Fusarium* species in various maize genotypes in Kenya. This study was to determine the genetic diversity of *Fusarium fungi* infecting maize in Nakuru County-Kenya. A Purposive sampling where maize grains were collected from 277 farmers. *Fusarium* species were isolated confirmed using molecular techniques, DNA sequencing of a gene, Translation elongation factor 1-alpha (TEF-1). The data was edited and aligned using vector NTI software and blasted in NCBI data base. *Fusarium* was identified using colour, followed by spore morphology on Potato Dextrose Agar (PDA). DNA sequence analysis revealed presence of *F. temperatum*, *F. boothii*, and *F. verticillioides*. The finding revealed infection of *Fusarium* ear rot on symptomless maize kernels. This is to enlighten the public, farmers, and agricultural officers on the infections of maize by specific *Fusarium* sp., hence the need to grow less infected maize genotypes H614, H6218 and H6210.

Keywords: Diversity; Fungal infection; *Fusarium sp*; Maize genotypes

Introduction

Kenya's primary food crop is maize (Zea mays L.), with a production of 39.0 million (90 kilogram) bags in 2014 [1]. Nakuru County used an area of 86,504 hectares of land to produce 1,765,714 90kg bags in total that year [1]. A number of factors have limited Kenya's ability to produce maize, including soil acidification from the continuous use of Di-ammonium Phosphate (DAP) fertilizer, the inability to obtain improved and certified seeds, and presence of diseases such as maize ear rot and Maize Lethal Necrosis Disease (MLND) among others. Table 1 shows common maize varieties.

Genotype	Yield/Acre (90 Kg Bags)	Altitude (M)	Days to Maturity
H6213	52	1700-2100	160 - 190
H6210	50	1700-2100	160 - 190
H628	46	1500-2100	150 - 180
H614	38	1500-2100	160 - 190
H624	32	1000-1800	140 - 180
H629	28	1000-1800	100 - 140
H6218	52	1700-2100	160 - 190
Pioneer	38	1500-2100	160 - 190

Table 1: Maize genotypes recommended by the Kenya seed company for Nakuru County.

Note: Source: Kenya Seed Bulletin (2016).

Maize productivity is severely limited by the ear rot fungus Fusarium, Aspergillus, Penicillium, and Sternocarpella in particular [2,3]. Studies by Duan et al. [4], documented the severity of maize ear rot, which is largely caused by *F. verticillioides* and *F. graminearum* species. China reported three species complexes of *F. graminearum*, *F. meridionale*, and *F. boothii*, along with the TEF-1α gene sequence [4].

Fusarium verticillioides can infect maize by a variety of mechanisms, including roots, insect pest-caused lesions in the seed, and silk produced by flying conidia [5,6]. When an infection spreads throughout the roots of maize seedlings, it first shows no symptoms. However, as the crop matures, the infection spreads throughout practically the whole plant, resulting in leaf blight, stalk rot, and ear rot. Corn kernels become infected when contaminated silk is used [7,8]. The endosperm and embryo are the two main tissues that Fusarium verticillioides colonizes in maize. In the endosperm, it results in increased levels of FB1 [9]. It has been found that in tropical and subtropical climates, Fusarium verticillioides produces large levels of fumonisin [10-12]. Fumonisins have been found in rice in addition to products derived from maize [13,14]. Maize may also contain other mycotoxins such as citrinin, cyclopiazonic acid, penicillic acid, ochratoxin A, zearalenone, trichothecenes (T-2), and Deoxynivalenol (DON or vomitoxin).

Kenya's National Government and the corresponding County governments in corn-growing regions have started taking action to boost maize production. Among these steps include providing farmers with alternate fertilizers and certified seedlings. In the North Coast region, the Kenyan government is also carrying out a second pilot phase of the Galana/Kulalu irrigation project. A additional 500,000 hectares of maize were predicted to be produced in this second phase in MY 2016-2017. Furthermore, in order to minimize postharvest losses through reducing aflatoxin contamination, the Kenyan national government has constructed drying and storage facilities in the corn-growing

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regions. Under the Kenya Cereal Enhancement Programme (KCEP), farmers in Nakuru County received gunny bags for storage after receiving maize seeds for planting.

Identification of *Fusarium* species has been done using morphological characterization [15]. Using morphological approaches, Hafizi and his fellow researchers were able to determine the morphological diversity of the *Fusarium* species. Morphological identification has been based on single-spore isolate coloured on the media and macrospore characteristics [16-18]. Using a single spore ensures that only one strain is examined, since natural sources contain many morphologically distinct *Fusarium* strains.

Morphological characteristics, identification of *F. verticillioides* has been done based on cultural practices according to Leslie et al. [18]. This was done by looking at the colony formed on the media and microscopic examination of the macrospores and microspores. *Fusarium verticillioides* produce abundant single-celled micro-conidia in distinctive long chains [18,19]. In a normal conidium development, Wall Building Zone (WBZ) is present to initiate and assists in the development of the conidium [20]. It was reported that besides use of morphological characteristics, *F. thapsinum* from *F. verticillioides* and *F. graminearum* are effectively differentiated by use of molecular markers.

Fusarium temperatum, was identified and characterized in Spain, Poland, Argentina, France and China. It was found to be pathogenic in maize seedlings and stalks [21]. *Fusarium temperatum* was distinguished from *F. subglutinans* within *Fusarium Fujikuroi* Species Complex (FFSC) by use of translation elongation factor-1 gene (*tef*-1α). Studies of mating compatibility and metabolite profiling have also been used in identification of *F. temperatum.* Isolates of *F. temperatum* was described after isolation from maize in Argentina [22,23]. Other countries where *F. temperatum* had been isolated include; Spain, France and Poland [21,24]. It had also been isolated in China and proved to be pathogenic and able to produce fumonisins in infected maize ears [25].

Using DNA sequence-based identification provides a much stronger inference. The strength of the inference depends on the sequences available in the databases. An exact sequence match with a known isolate in the database is considered very close to unambiguous species identification. However, this is not always the case. A query sequence that is different by one or few bases may be an allelic variant not present in FUSARIUM-ID from a known species. It may also be that the sequence is from a new species that is not represented in FUSARIUM-ID or it corresponds to a species that is currently poorly defined. Molecular identification of *F. verticillioides* strains have been carried out at the gene level using polymerase chain reaction [26].

Materials and Methods

Maize Samples Collection

During sample collection, maize grains which were 12 weeks Harvest Time Points After Physiological Maturity (HTPAPM) according to Alakonya et al. [27], were collected from each farmer. Bulk samples were made consisting of similar maize genotypes collected from each of the farmers in Molo Sub-County. Similarly the same maize genotypes collected from each farmer in Njoro Sub-County were bulked. This gave a total of 13 bulk samples, since five maize genotypes which were previously given to farmers in Njoro, formed five bulks samples; while eight maize genotypes previously distributed to the farmers in Molo formed eight bulk samples. In each bulk sample, an eighth of a kilogram of maize grain of each genotype was collected from each of the farmers figure 1.

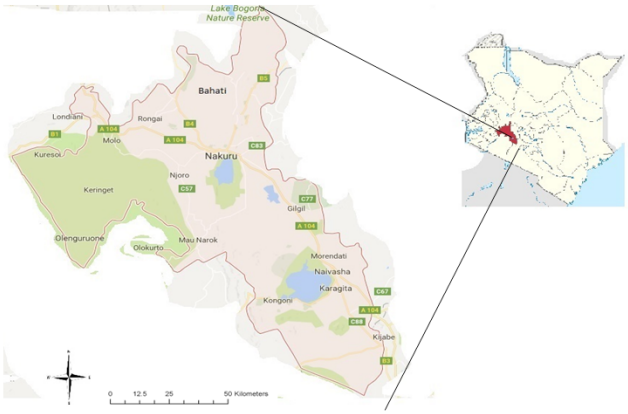

Figure 1: Nakuru County map (2016).

Note: Source: https://cgspace.cgiar.org/bitstream/handle/10568/80458/Nakuru.pdf?sequence

Isolation and Identification of *Fusarium* Species from Maize Kernels

Symptomless maize kernels collected from the farmers stores were taken to Kenyatta University, Microbiology laboratory for mycological isolation of *Fusarium spp.* The media used was, Pentachloronitrobenzene (PCNB) agar media, which is a selective media for promotion of growth of only *Fusarium* fungi by inhibiting growth of other fungi species. Data on the incidence was first normalized and the percent incidence of *Fusarium* sp. on the maize genotypes was computed as;

$$\text{Percentage incidence (\%)} = \frac{No\ of\ grains\ infected\ with\ Fusarium\ sp.}{Total\ number\ of\ grains\ plated} X100$$

A total of fifteen grams of each maize genotype (about 25-30 maize grains) showing no symptoms of fungal infection were collected from each bulk sample. Five grains of each genotype were picked from each bulk sample, surface sterilized by immersion in 70% ethanol for five seconds followed by 1.0% NaOCl for 90 seconds. The kernels were rinsed in three changes of sterile distilled water for 20 seconds, blot dried between two sterile filter papers and inoculated on petri dishes containing Pentachloronitrobenzene agar (PCNB) medium. This was replicated five times. The inoculated petri dishes were incubated at 25°C for 5 days to allow growth of the fungi and the samples of each of the maize genotype in each petri dish was analyzed for percentage incidence of *Fusarium spp.* infection. Each colony formed from each of the kernels was sub-cultured by transferring to Potato Dextrose Agar (PDA) slants for identification.

Morphological Identification of *Fusarium* Isolates Using Colour

The first identification was based on colour morphology of the mycelium according to procedure by Nelson et al. [16], after 10 days growth on PDA slant. In the second step, carnation leaf agar cultures were used for identification based on morphology of the micro and macroconidia according to Leslie et al. [18]. Microscopic observation of the spores was carried out at the Department of Veterinary

Anatomy, University of Nairobi, using Leica compound microscope (Leica DM4, P DM 2700, Leica microsystems, Buffalo groove, U.S.A) which was mounted in-build camera. This microscope uses a computer Programme LAS EZ version 1.6.0 which enabled documentation of macrospores and microspores observed. Percentage incidence of each *Fusarium* species in the different maize genotypes was computed.

DNA Extraction

Extraction of the DNA from each *Fusarium* isolate as a representative sample was carried out using procedure described for plant DNA extraction. This was achieved by culturing the fungus directly in an Eppendorf tube and suppressing the phenolization step. In this method, a 1.5ml Eppendorf tube was filled with a 500µl of liquid Potato-dextrose broth medium. The culture was started by inoculating a single hyphal thread of each of the *Fusarium* fungal isolate and incubated for 72 hours at 25°C. This method is faster and less prone to contamination procedure. The mycelial mat from each of the isolate was pelleted by centrifugation for 5 minutes at 13,000rpm in a microfuge, washed with 500µl of TE buffer and pelleted again. The TE was then decanted and 300µl of extraction buffer added. The mycelium was crushed with a conical grinder (Treff AG, Degersheim, Switzerland), fitting exactly the tube and centrifuged by hand potter at 200rpm for 10 minutes. After that, 150µl of 3.0M sodium acetate, (pH 5.2) was added and tubes placed at -20°C for ten minutes. Tubes were then centrifuged in a microfuge at 13,000rpm and the supernatant transferred to another tube. An equal volume of isopropanol was added. After 5 minutes at room temperature, the precipitated DNA was pelleted by centrifugation in a microfuge at 13,000rpm. This was therefore washed using 70% ethanol and then air dried on paper towel for 20 minutes. The pellet was re-suspended in 50µl of TE for assessment of the DNA quality was determined by running in a 1.0% agarose gel at 80 volts for 30 minutes together with a known size, 1kb universal gene ruler (Biolab).

1 X TE Buffer, pH 8.0 (1mM Na_2 EDTA and 10mM Tris base) was made as 50X stock solution and diluted as required for use. To prepare 1 litre of 50X stock solution: In a 2 litre beaker, 700ml of double-distilled water was added, 60.6g of Tris base (i.e 500mM) and 18.6 g of Na_2 EDTA (i.e 50mM). This was stirred vigorously to dissolve on a stir plate. After the Tris and the EDTA were both in solution, obtained pH was adjusted to pH 8.0 using concentrated Hydrochloric Acid (HCl) and the final volume made to 1 litre with double-distilled water.

Lysis bufferwas used at (pH 8.0), 50mM Tris, 50mM EDTA, 3% SDS, 1% β-mercaptoethanol, and 0.1mg/ml Proteinase K were mixed. To obtain 500ml of buffer; to a 1L beaker was added 350ml of double-distilled water, 25ml of 1M Tris-HCl (pH 8.0) stock. Tris was allowed to dissolve and mix. EDTA ($Na_4EDTA{\cdot}2H_2O$; 50ml of 0.5 M EDTA stock) was added and continued mixing (pH≥8 for the EDTA to stay in solution). After which 150g SDS (sodium dodecyl sulfate (Sigma cat. no. L-6026) was added and continued to mix until dissolved. Solution was transferred to a graduated cylinder and a final volume adjusted to 500ml by adding distilled water. β-mercaptoethanol at 1.0 % (v/v) and Proteinase K (Sigma cat. no. P-6556) at 0.1mg/ml was therefore added to the solution just before use.

PCR Amplification and Sequencing

Fusarium isolates were identified by sequencing the translation elongation factor-1 alpha gene (α-TEF gene) region. DNA was extracted by CTAB method. The PCR was carried out using forward primer, TEF 1-α gene, EF1, 5'GTGGGGCATTTACCCCGCC3' and reverse primer EF2, 5'ACGAACCCTTACCCACCTC3'. A PCR reaction mixture (25µl) for TEF 1-α gene consisted of 12.8µl milli Q H_2O, 2.5µl 10x buffer, 2.5µl $MgCl_2$, and 0.5µl dNTP mix (10mM each). A mount of 0.8µl forward primer EF1 (10mM), 0.8µl reverse primer EF2 (10mM), 0.1µl Taq (Biotech) and 5µl DNA sample was used. The PCR conditions were; initial denaturation at 95°C for 75 seconds, 35 cycles of denaturation at 95°C for 15 seconds, annealing temperature at 53°C for 30 seconds and a primer extension at 72°C and final extension at 72°C.

Sequencing Alignment and Phylogenetic Analysis

Using Molecular Evolutionary Genetic Analysis software, (MEGA 7; http://www.megasoftware.net), the ITS sequences were edited and aligned. To assess the relationships between genetic traits of the identified *Fusarium* species, ITS sequences were used to generate phylogenetic tree by grouping the isolates into clusters. The aligned sequences were therefore deposited in the GenBank database.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model [28]. The tree with the highest log likelihood (-1474.6907) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 7.

Data Analysis

Molecular DNA sequence data obtained was edited and aligned using vector NTI software and further BLAST on the NCBI sequence database used to find best matches for each obtained sequence for every isolate. Phylogenic and molecular evolutionary analysis was carried out using Mega version 7 [29,30]. Neighbour joining approach for geographical representation of pairwise nucleotide sequence divergence among the fungi strains was used.

Results

Fusarium Fungi Identified Using Colour on PDA Slants

Visual observation of *Fusarium sp.* from each maize grains isolated on each petri dish which contained PCNB agar and thereafter sub-cultured onto Potato dextrose Agar slants, revealed differences in the colour of *Fusarium* mycelium on the media (Figure 2(A and B). Figure 3(A and B) is showing *F. verticillioides* on petri dish revealing a rapidly growing white, becoming tinged with purple aerial mycelium.

Result of Enumeration of *Fusarium spp*. Identified Using Mycelia Colour

Eight different *Fusarium* spp. identified using colour of the mycelium on PDA media were; *F. dimerum, F. equseti, F. solani,*

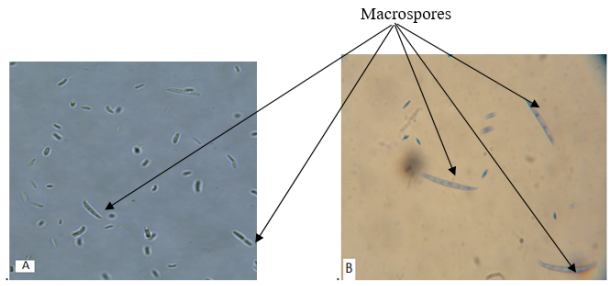

Figure 2: Macrospores (x400). (A): *F. temperatum*; (B): *F. verticillioides*.

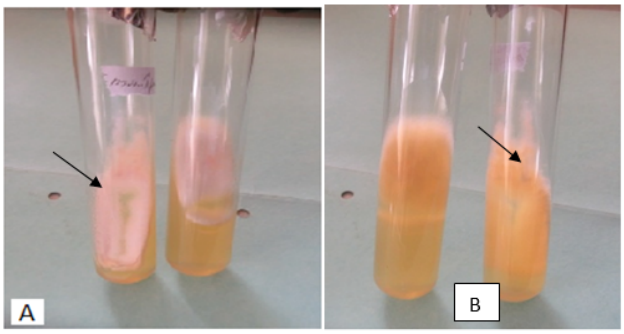

Figure 3: *F. verticillioides* on PDA slant. A: Front view; B: Back view.

Note: Arrows showing white aerial mycelium.

F. verticillioides, F. oxysporum, F. culmorum, F.graminearum and unknown *Fusarium* species. Number of *Fusarium* species that was recorded in the different maize genotypes varied. The result showed that H6213 maize genotype was infected by six out of the eight *Fusarium* species (75.0%), Pioneer, H626 and H614 were infected by 37.5 % of the *Fusarium* species. H6210 and H624 maize genotypes were only infected by *F. verticillioides* as shown in table 2.

Fusarium Species Spore Morphology

Spore identification of 10 day old *Fusarium* cultures grown on CLA medium showed 8 different *Fusarium sp*. Microscopic observations of the macrospores were as shown in Figure 4. The *Fusarium* sp. of interest i.e. *Fusarium verticillioides* macrospores were slender, uniform in size and thick-walled. The macrospores were straight to moderately curved with the ventral straight surface and smooth dorsal side. They had 5-6 septate which are distinct as shown in figure 3B. Figure 3A shows a *F. temperatum* isolate macroconidia having a typical four-septa with a foot-shaped basal cell, figure 3.

Using morphological identification, Fusarium species found infecting maize varieties were as shown in table 2.

Maize Genotype	Number of *Fusarium* Species (n = 8)	Percent
H6218	4 *(F. solani, F. verticillioides, F. oxysporum, Unknown F. species)*	50.0
H6213	6 *(F. solani, F. verticillioides, F. oxysporum, F. culmorum, F. graminearum, Unknown F. species)*	75.0
H6210	1 *(F. verticillioides,)*	12.5
H614	3 *(F. verticillioides, F. oxysporum, F. graminearum,)*	37.5
H626	3 *(F. verticillioides, F. graminearum, Unknown F. species)*	37.5
H624	1 *(Unknown F. species)*	12.5
H629	4 *(F. equiseti, F. verticillioides, F. graminearum, Unknown F. species)*	50.0
Pioneer	3 *(F. dimerum, F. culmorum, Unknown F. species)*	37.5

Table 2: *Fusarium* species infecting maize genotypes.

Molecular Identification of *Fusarium* Species Infecting Maize

On gel electrophoresis, it was observed that *Fusarium* species' DNA extracted from the fungal mycelium had a high molecular weight, pure DNA. The DNA samples were quantified on 1.0 percent Agarose gel electrophoresis using 100ng/µl of 1kb standard Lambda DNA. The DNA quantification revealed that the concentration ranged from 10 to 30ng/µl of isolated DNA, figure 4.

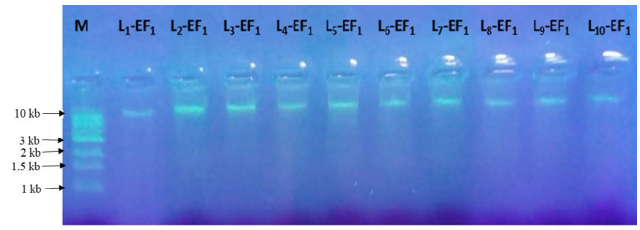

Figure 4: DNA bands in 1.0 % Agarose gel.

Note: Lanes L_1-EF_1 to L_{10} -EF_1, *Fusarium spp*. Isolates; M, Gene ruler.

Figure 4, shows images of quality DNA bands on 1.0% agarose gel. It shows the sizes of high molecular weight, genomic DNA of 10 samples in relation to Molecular marker 1kb. Lane 1, Unknown *Fusarium* species; Lane 2, *F. solani*; Lane 3, *F. dimerum*; Lane 4, *F. Culmorum*; Lane 5, unknown B; Lane 6, *F. oxysporum*; Lane 7, *F. graminearum*; Lane 8, *F. equiseti*; Lane 9, unknown *Fusarium* species; Lane 10, *F. verticillioides*.

Fusarium Species Identified Using Molecular DNA Sequences

Based on the *Fusarium* DNA sequences obtained, the result confirmed that; sample L10-EF-1 was *Fusarium verticillioides* isolate with an NCBI data base, which showed a percentage similarity of 97.2% to *F. verticillioides* isolate KF562141. Sample L7_EF-1 was *Fusarium boothii* which showed a similarity of 96.1% to isolate KX269067 in the NCBI data base. Samples L1_EF-1, L2_EF-1, L4_EF-1 and L6_EF-1 were strains of *F. temperatum* and which showed percentage similarity of between 96.5-99.1% as shown in table 3.

Phylogenetic Tree Showing Diversity of *Fusarium sp*

Figure 2 shows a phylogenetic tree based on TEF-1α gene showing three *Fusarium* isolate clusters (cluster I, II and III). Number shown at the nodes in the dendrogram indicates the percentage bootstrap support values for 1000 interactions. Cluster I comprised of the isolates L1_EF1, L2_EF1, L4_EF1 and L6_EF1. Cluster II comprised of L10_EF1 while cluster III had L7_EF1. Samples marked in red colour are the research samples while the rest were sequences from the Genebank NCBI accessions. The figure is drawn to scale, with bootstrap values showing number of substitutions per site. GenBank accession numbers for each mitogenomic sequences are shown in parentheses. The tree confirmed mainly three groups; group I; *F. temperatum*, group II; *F. verticillioides* and group III which had *F. boothii*.

Sample	Bit Score	Grade (%)	Hit Start	Hit End	Close NCBI Accession	Description
L1_EF1	1175.59	97.5	656	8	KC964112	*F. temperatum,*
L2_EF1	1201.44	98.2	666	14	KC964117	*F. temperatum,*
L4_EF1	1166.36	99.1	649	7	KC964119	*F. temperatum,*
L6_EF1	1160.36	96.5	649	14	KC964119	*F. temperatum,*
L7_EF1	1125.73	96.1	38	667	KX269067	*F. boothii*
L10_EF1	1188.52	97.2	13	655	KF562141	*F. verticillioides*

Table 3: DNA sequences using Mega 7 Programme.

Figure 5 shows the genetic relationship between *F. verticillioides* (sample L10_EF-1), *F. boothii* (sample L7_EF-1) isolates and *F. temperatum* (sample L1_EF-1, L2_EF-1, L4_EF-1 and L6_EF-1). Among the group of *F. temperatum,* sample L1_EF-1 was much closer to L2_EF-1 while sample L4_EF-1 and L6_EF-1 formed a subgroup.

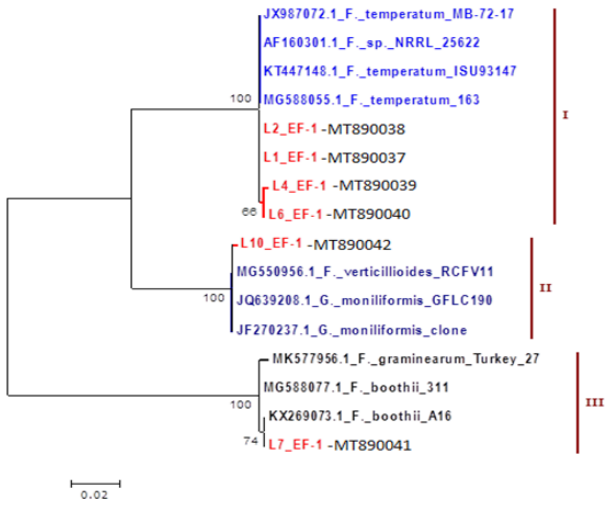

Figure 5: *Fusarium* species' phylogenetic tree based on *TEF-1α* gene sequences.

Branch bootstrap values indicating the number of times out of 1000 (in this case), the same branch was obtained when repeating the phylogenetic reconstruction on a re-sampled set of the data. Evolutionary analyses were conducted in MEGA 6 and show the tree with the highest log likelihood.

Fusarium spp. Speciation

Six out of the ten cleaned DNA samples amplified during PCR reaction. These were sample L1_EF-1, L2_EF-1, L4_EF-1, L6_EF-1, L7_EF-1 and sample L10_EF-1. The remaining four (Sample L3_EF-1, L5_EF-1, L8_EF-1 and L9_EF-1) did not amplify during PCR amplification. Results of the six *Fusarium* spp. samples' DNA sequences based on the National Center for Biotechnology Information (NCBI) database searching using individual sequences confirmed that sample L10_EF-1 isolate belong to *F. verticelloides* and had 99.69 % identity with 93 % query cover. Four of the DNA sequences (sample L1_EF-1, L2_EF-1, L4_EF-1, and L6_EF-1) were *Fusarium temperatum,* which is within the *Gibberella fujikuroi* species complex and closely related to *Fusarium subglutinans*. Sample L1_EF-1; *F. temperatum* had 98.93% identity with 94% query cover, Sample L2_EF-1; *F. temperatum* had 99.96% with 94 % query cover and, sample L4_EF-1 and L6_EF-1; *F. temperatum* had identity of 98.72 % with 80 % query cover. Sample L7_EF-1 was *Fusarium boothii* (formerly *F. graminearum*) and had identity of 98.51 % with 86 % query cover (Figure 2).

Discussion

Morphological Identification of *Fusarium* spp

In the present study, the first identification of *Fusarium* species was carried out using morphological characteristics. Identification was first based on colour differences of *Fusarium* fungi on PDA slants as shown in figure 2. Similar identification had been reported by Nelson et al. [16]. This was followed by microscopic identification that was carried out on the spores formed by the different *Fusarium* species *in-vitro* on CLA media indicated in figure 3 using the procedure by Leslie et al. [18]. These two morphological methods of identification relied on cultural characteristics and were dependent on visual assessment by the eye. This is by looking at the colony formed on the media and microscopic examination of the macrospores and the microspores. Research by Leslie et al. [18], and Nelson et al. [16], used different methods of identification of *Fusarium* species. Using the two methods of identification, *F. verticillioides* produced abundant single-celled micro-conidia in distinctive long chains [18]. Glenn et al. [20], used powerful microscopes to view the small hyaline micro-conidia, a rising from morphologically simple phialides, as distinct characteristics. Studies have shown that in a normal conidium development, a specific zone at the phialide apex, referred to as Wall Building Zone (WBZ) was present to initiate and assists in the development of the conidium [20]. Morphological identification of *F. temperatum* was based on the macro-conidia usually having four-septate with a foot-shaped basal cell. This makes a distinct difference from its most closely related species *F. subglutinans* and others found within *Gibberella fujikuroi* complex species. In the present study, seven morphologically identified *Fusarium* species and one unknown *Fusarium* sp. were isolated from the eight maize genotypes. These *Fusarium* species were therefore subjected to further identification using a molecular technique of DNA sequencing.

Molecular Analysis Using DNA Sequences

Molecular identification of *Fusarium spp*. in this study, which was carried out using DNA sequence comparisons of the translation elongation factor 1-α gene regions, clearly showed that the *Fusarium* isolates infecting maize in Nakuru County were; *F. verticillioides, F. temperatum* and *F. boothi.* Strains of *F. temperatum*are closely related to *F. subglutinans* and *F. boothi* which belongs to *F. Graminearum* Species Complex (FGSC) as had been reported previously by Aoki et al. [31]. Findings from the present study therefore reveal that *F. temperatum* is reported for the first time in maize samples in Kenya. This result indicated that, besides the use of morphological techniques in identification [18], the most accurate method of identification is by

using molecular DNA sequence analysis. In the present study, the five *Fusarium* species that were identified using morphological characteristics were confirmed to be only three (*F. temperatum*, *F. verticillioides* and *F. boothii*) by using DNA sequences analysis as shown in phylogenetic tree figure 5.

Conclusion

Visually, ear rot symptomless maize kernels used for human consumption in Nakuru County, Kenya, were infected by more than one *Fusarium* spp.

The most dominant *Fusarium* species in ear rot symptomless maize genotypes in Nakuru County was *F. verticillioides* which had 75.0% dominance of the maize genotypes. Using morphological characteristics, the lowest dominating *Fusarium sp*. was *F. dimerum* and *F. equiseti* which recorded 12.5% dominance in the maize genotypes.

Molecular analysis in identification of *Fusarium* spp. is the most accurate method in genetic diversity determination and it revealed presence of a novel *F. temperatum* and *F. boothii*.

In an *in vitro* culture, the use of the colour of the mycelium and pigmentation of the medium, is the preliminary method for the identification of *Fusarium* species. After preliminary identification, further authentication of the various *Fusarium* species by using DNA sequencing techniques gives the accurate results to confirm *Fusarium* species identity.

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Conflict of Interest

This work was done with the knowledge of the author and no organization made any contradiction on the findings of the research. There was no conflicting opinion on publication of this research.

Data Availability Statement

Data used for this study were primary data obtained by the researcher. The data can be made available as already presented in the document and any other detailed information is available with the Author.

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