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Characterisation and Divergence of *Fusarium* Species in Certain Varieties of *Zea mays* L.

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Abstract: ***An experiment was conducted to characterize and evaluate the diverse Fusarium species associated with some varieties of Zea mays L. Root samples of thirteen maize varieties were collected in three replicates from different seed multiplication fields in International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, Ibadan. Isolation of Fusarium species from the samples were done by inoculating five cut sections of 3mm of the maize roots on sterilized Potato Dextrose Agar (PDA) medium for fungal growth and all plates were incubated for 5 days at 25 °C. The fungal isolates were sub-cultured and single spore isolation was done to obtain pure culture. Morphological characterization of the isolated Fusarium species was done following standard procedures. Molecular identification was conducted by PCR assay using ITS1 and ITS4 primers. The Fusarium species were analyzed by DNA sequence. Data obtained were subjected to analysis (ANOVA) using Generalized Linear Model Procedure (GLM) of statistical analysis software (SAS). Means were separated using Duncan's Multiple Range Test (DMRT) at p ≤ 0.05. Thirty-nine Fusarium isolates (all of which were four species), were obtained from the thirteen maize varieties. These include thirty isolates (three strains) of F. verticillioides from all the thirteen maize varieties, four isolates (two strains) of F. fredkrugeri from four varieties, three isolates (two strains) of F. oxysporum from three varieties and two isolates (two strains) of F. solani from two maize varieties. Fusarium verticillioides was the predominant species with an incidence of 76.9% followed by F. fredkrugeri (10.3%), F. oxysporum (7.7%) and F. solani (5.1%). Cultural characteristics of some isolates of the same strain from different maize varieties were not the same. Radial growths of the F. verticillioides, F. oxysporum, F. fredkrugeri and F. solani differed significantly (p≤0.05) at the different days of incubation. Macroconidia as well as the microconidia of isolates of different species differed significantly in length, and also in breadth (p≤0.05). Macroconidia as well as the microconidia of some isolates of the same species from different maize varieties also differed significantly in length, as well as in breadth (p≤0.05). On agar, sometimes growth performance of one particular Fusarium strain obtained from different maize varieties can differ. This could be the first reported case of Fusarium fredkrugeri isolated from maize roots.***

Keywords: Morphological characterization, Molecular characterization *Fusarium* species, *Zea mays* L. and maize varieties

1. Introduction

Maize (*Zea mays* L.) has been severally reported to be the world's most important cereal after wheat and rice (Verheye 2010, Zhang *et al.*, 2010, Sanchez-Garcia *et al.*, 2010, Lai and Guo 2011, Luo *et al.*, 2011, Kumar and Jhariya 2013, Saxena *et al.*, 2013, Ranum *et al.*, 2014, Sliwinska and Bewley 2014, Schnable 2015, Hofmann *et al*., 2016, Ognakossan *et al.*, 2018). It is documented to serve as a source of food, and income for many populations in different countries of the world including Nigeria (Tandzi and Mutengwa, 2020). Globally, its production was approximated to produce 1.2 billion tons on roughly 194 million hectares of land with about 70 million tons generated on more than 33 million hectares in Sub-Saharan Africa (FAOSTAT, 2020, Yarnell, 2008). However, its production is being affected by several abiotic and biotic factors including diseases caused by different pests and pathogens (Orsi *et al*., 2000). Pathogens such as *Penicillium* species, *Aspergillus* species, and *Fusarium* species are among the genera found on maize and can affect them both in the field and during storage (Orsi *et al*., 2000).

Fusarium species have been generally reported as widespread pathogens of maize that can cause root, stem and ear rot (Munkvold and Desjardins, 1997). Their impact on maize has resulted in yield losses of between 50 - 80% on the farm and during storage (Kossou and Aho, 1993). They are known to be among the several pathogenic fungi causing severe diseases in maize both in the field and post-harvest. They (*Fusarium* pathogens) are common and can pose a threat to plant development throughout the growing season. Seed rot, root and stem rot, ear and kernel rot, and rudimentary ear rot are all significant diseases caused by *Fusarium* infections in maize (Kabeere, *et al.*, 1997). Some members of the species are known to produce secondary metabolites (mycotoxins) that have carcinogenic, teratogenic, immunosuppressive, and estrogenic effects on humans and animals (Orsi *et al*., 2000). Trichothecenes, zearalenone, fumonisins, and moniliformin are the most prevalent mycotoxins produced by these fungi in afflicted maize (Nelson, *et al.*, 1993). Certain *Fusarium* species are known to cause significant decrease in yield of maize and major hazard to human and animal health from consumption of mycotoxin contaminated maize. Crops, most of the times, get polluted with *Fusarium* mycotoxins as the infection progresses, which are often hazardous to plants, animals, and humans (Placinta *et al.*, 1999, Bennett and Klich, 2003; Richard, 2007; Streit, *et al*, 2012).

Phytopathogenic and toxigenic features of *Fusarium* species are known to affect yield, nutritional value, and hygienic quality of agricultural products from arable crops all over the world. *Fusarium* spp. infection of maize (Zea mays L.) and small-grain cereals is of particular concern because of the importance of such grains as food and feed production (Placinta *et al*, 1999; Bennett and Klich, 2003; Richard, 2007; Streit, *et al.*, 2012). The study was done to characterize and evaluate the diverse *Fusarium* species associated with some varieties of *Zea mays* L.

2. Methodology

2.1 Collection of maize roots samples

Thirteen varieties of healthy maize roots were collected from two fields in IITA Ibadan (Table 1). The samples were collected in three replicates in sterile sample bags and taken to the laboratory for isolation.

Table 1: Details of the maize root samples collected

SN	Sample ID	LGA	Location	Latitude	Longitude	Altitude
1	DR 197	Akinyele	Ibadan	N07°29.281	E003°54.115	689
2	EEI 29	Akinyele	Ibadan	N07°29.337	E003°54.193	706
3	EEI 34	Akinyele	Ibadan	N07°29.341	E003°54.191	698
4	EEI 81	Akinyele	Ibadan	N07°29.280	E003°54.109	678
5	EEI 128	Akinyele	Ibadan	N07°29.318	E003°54.201	699
6	EI 135	Akinyele	Ibadan	N07°29.299	E003°54.163	695
7	EI 1361	Akinyele	Ibadan	N07°29.246	E003°54.071	707
8	EI 1386	Akinyele	Ibadan	N07°29.239	E003°54.068	704
9	EEI 176	Akinyele	Ibadan	N07°29.280	E003°54.104	635
10	QI 297	Akinyele	Ibadan	N07°29.326	E003°54.171	698
11	QI 386	Akinyele	Ibadan	N07°29.280	E003°54.104	708
12	STR 104	Akinyele	Ibadan	N07°29.337	E003°54.190	700
13	STR 105	Akinyele	Ibadan	N07°29.321	E003°54.160	699

2.2 Isolation of *Fusarium* species from the collected samples

The samples were sterilized in 1% sodium hypochlorite (NaOCl) for 1 min. Samples were transferred to sterile distilled water (SDW) for 3 minutes and rinsed twice followed by drying on sterile filter paper. A sterile scalpel was used to cut 3 mm pieces and five pieces of the cut sections were inoculated equidistant on sterilized Acidified Potato Dextrose Agar (APDA) plates. The plates were sealed with parafilm and incubated at 25 ± 2 °C for 5 days. The growing fungi were subcultured to obtain pure cultures. All experiments were done in triplicates.

2.3 Single spore isolation of the *Fusarium* species

To obtain a pure culture, single spore isolation of the *Fusarium* species was done by transferring small fragments of each culture into 40ml vials containing 10ml sterile distilled water, vortexed at 3600rpm for 2min and 50µl of the solution was picked into 9mm petri dish containing sterile water agar and then spread on the plate with a sterile spreader. The plates were incubated at 25 °C for 24 hours. With the aid of a light microscope, spores observed on the plates were picked singly with a sterile scalpel and transferred to fresh 9mm petri dish containing sterile PDA. The plates were sealed with parafilm and incubated at 25 °C for 5 days when pure culture of each fungus was observed growing on the plates.

2.4 Data Collection

The collected data were the radial growth rate of the *Fusarium* isolates, macroscopic characteristics, (such as colour, pigmentation, conidia shape and size, phialides and chlamydospores etc.). A pure culture of each *Fusarium* species was inoculated at the center of APDA plates and incubated at 25°C. Diameter (D) of the growing fungus was measured daily till plate was fully ramified. Radial growth rate area was calculated for each isolate using the formula below;

Radial growth rate = $^{D}/_{2}$ x 100

2.5 Microscopic identification

Pure cultures were transferred on carnation leaf agar (CLA), and the mycelium was inoculated close to sterile carnation leaf pieces. After 10 days of growth, the morphological characteristics (such as macroconidia, microconidia and chlamydospores shapes) were observed using the method of Burgess *et al.* (1994) as well as Leslie and Summerell, (2006) under a light microscope (Olympus model BX-51) and photographed using Olympus camera model DP50 U-CMAD3 with an image analyser-SIS programme.

2.6 Molecular characterisation

2.6.1 DNA Extraction

To extract the Genomic DNA of the isolates. Mycelia from the isolates was scraped using a sterile spatula and transferred to a sterile mortar where 500 µL CTAB extraction buffer was added. A sterile pestle was used to mix the mycelia and buffer. The mixture was then transferred into 1.5ml centrifuge tube and placed in water bath at 65°C for one hour. 750 µL Chloroform (24:1) was added and mixed using a vortex mixer followed by centrifugation at 12,000 rpm at 25°C for 15 minutes. The supernatant was extracted, and 300 µL cold Isopropanol was added to the supernatant in order to precipitate the DNA and the content inverted thrice to mix before keeping the tubes overnight at -20°C. DNA was pelleted by centrifugation at 12,000 rpm at 4°C for 15 minutes. Pellets was washed with 700 µL 70% ethanol by vortexing and incubated at -20°C for 10 minutes. After which it was centrifuged at 13,000 rpm at 25°C for 5 minutes. The ethanol was removed and the pellets were air dried; 100 µL 0.1 × Trics – EDTA was added to the dried pellets to dissolve them and then placed on ice for 30 minutes. The

pellets were then vortexed briefly so as to determine the DNA concentration using Nanodrop spectrophotometer.

2.6.2 PCR amplification

Primers ITS1 (Forward primer) and ITS4 (Reverse primer) were used to amplify a fragment of rDNA including ITS1 and ITS4 and the 5.8S rDNA gene. PCR amplifications were performed in a total volume of 50 µl by mixing 100 ng of the template DNA with 0.2 mM concentrations of each primer, 200 µl concentrations of each deoxynucleoside triphosphate, and 2.5 µl of Taq DNA polymerase in GeneAmp 103 PCR Buffer II (100 mM Tris-HCl, pH 8.3; 500 Mm KCl) (Perkin-Elmer). These reactions were subjected to an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 minutes. Annealing was later done at 55°C for 30 seconds, followed by extension at 72°C for 1 minute, and a final extension for 7 minutes at 72°C in a thermal cycler (Seegene). The amplified DNA products were analysed by gel electrophoresis on agarose gel (1%) in 1 strength TAE (1X TAE) buffer and ran for 50 minutes with 110 volts. The DNA fragments were observed using a UV light photo documenter.

2.6.3 PCR Purification for sequencing

PCR product (23 µl) was transferred into Eppendorf tubes and 2.5 volume of 95% ethanol was added into the tubes and inverted several times. It was incubated at -20 ^{o}C refrigeration for 1 hour and spinned at 13000 rpm for 10 minutes. The supernatant was discarded and 500 µl of 70% ethanol was added, followed by a 2 minutes' centrifugation at 13000 rpm. The supernatant was discarded, and the pellet-containing tubes were air dried for 15 minutes; 25 µl of nuclease free water was added to the tube containing the pellets in order to dissolve it. A Nanodrop spectrophotometer was used to verify the purity and amount of the DNA.

2.6.4 Sequencing

Only the amplified products of the ITS gene region were excised from the agarose and purified through silica columns (EZ-10 Spin Column DNA Gel Extraction Kit BS354, Bio Basic Inc.). Once the purified PCR fragments were obtained, these samples were sent for sequencing at IITA Bioscience Center where the Big DyeTerminator 3.1 kit (Applied Biosystems, Foster City, CA) was used. The search for similarity between DNA sequences were made through the BLAST program, with which the nucleotide sequences under study were compared with the databases of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/), identifying the homology values.

2.7 Statistical analysis

The data collected were subjected to analysis (ANOVA) using Generalized Linear Model Procedure (GLM) of Statistical Analysis software (SAS). Means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

3. Results

3.1 Isolated *Fusarium* species and their Occurrence

A total of thirty-nine *Fusarium* species were isolated from the roots of thirteen maize varieties (EI 128, EI 1361, EI 135, EI 1386, EEI 29, EEI 81, EEI 34, EEI 176, STR 104, STR 105, DR 197, QI 386, QI 297). The thirty-nine *Fusarium* isolates were all of four *Fusarium* species. These include thirty isolates (three strains) of *F. verticillioides*, four isolates (two strains) of *F. fredkrugeri*, three isolates (two strains) of *F. oxysporum* and two isolates (two strains) of *F. solani*. The *Fusarium* isolates included; thirty *F. verticillioides* (DR 197, EEI 29_1, EEI 81_2, EI 1386_1, STR 104, DR 197_1, EEI 29_2, EI 128, EI 1386_2, STR 104_1, DR 197_2, EEI 34_1, EI 128_1, QI 297, STR 104_2, EEI 135_2, EEI 34_2, EI 128_2, QI 297_1, STR 105, EEI 176_2, EEI 81, EI 1386_1, QI 297_2, STR 105_1, EEI 29, EEI 81_1, EI 1361_2, Q1 386_2 and STR 105_2) from all the thirteen varieties, four *F. fredkrugeri* (EI 1361, EEI 135, EEI 176 and QI 386) from four varieties (EI 1361, EI 135, EEI 176 and QI 386), three *F. oxysporum* (EEI 34, EEI 176_1, and QI 386_1) from three varieties (EEI 34, EEI 176 and QI 386) and two *F. solani* (EI 1386 and EEI 135_1) from two varieties (EI 1386 and EI 135). *Fusarium verticillioides* had the highest occurrence in the roots of the maize varieties, followed by *F. fredkrugeri*, *Fusarium oxysporum and F. solani* in that order (Figure 1).

3.2. Morphological characterization

Cultural characterization

The *Fusarium verticillioides* cultures were characterized by a white or creamy cottony mycelium with a white to pink to purple pigmentation on Potato Dextrose Agar (Plate 1 - 3). The *Fusarium solani* cultures (EI 1386 and EI 135) had a white and cottony mycelium with yellowish white colour at the bottom of the Potato Dextrose Agar (Plate 4 - 5). The culture of *Fusarium oxysporum* on PDA had white or creamy aerial mycelium and a yellow to brown, to purple and pink pigment at the bottom (Plate 6 - 8). The *Fusarium fredkrugeri* cultures (EEI 176_3, EI 135_1, EI 1361_2 and QI 386_2) had an abundance of white aerial mycelium with the surface cultures varying in colour from greyish lilac to pale violet and a vinaceous buff under-surface on PDA (Plate 9 - 11). Table 2 gives the radial growth (mm) of the *Fusarium* species at day 11 after incubation. Generally, the growth of *Fusarium solani* was significantly lower than those of the other three (*F. oxysporum*, *F. verticillioides*, and *F. fredkrugeri*) which were not significantly different from themselves. Table 3 shows the ANOVA table for radial growth of the *Fusarium* isolates at day 11 after incubation. The F values for model ($P>0.0123$), and *Fusarium* species ($P>0.0024$) were highly significant. Figure 2 compares growth of the 30 *F. verticillioides* isolates after 11 days of incubation. Most of the isolates recorded maximum growth at the 9th day after incubation. However a few others including isolates EI 1386_2, EEI 29, and QI 386_2 recorded maximum growth at the 11th day after incubation. Isolates EI 128, EEI 29_1, EEI 81, EI 128_2, and STR 105 recorded slightly better growth compared to others. Figure 3 compares growth of the 3 *F. oxysporum* isolates after 11 days of incubation. All the three

isolates recorded maximum growth at the 11th day after incubation. Isolates EEI 34 and QI 386_1 had better growth than EEI176_1. Figure 4 compares growth of the four *F. fredkrugeri* isolates after 11 days of incubation. All the four isolates recorded maximum growth at the 11th day after incubation. Isolate EEI 176 had the best growth while isolate EI 1361 had the least growth. Figure 5 compares growth of the two *F. solani* isolates from the two varieties after 11 days of incubation. Both of the isolates recorded maximum growth at the 11th day after incubation. Isolate EEI 135_1 had better growth than EI1386.

Table 4 gives the means comparison of the growth parameters of macroconidia of the isolated *Fusarium* species. The lengths of macroconidia of *F. verticillioides* from EI 128 R3, and EI 1386 R1 as well as *F. solani* from EI 135 R3 were significantly longer than those of the other *Fusarium* isolates, most of which did not differ significantly in length. However, the lengths of macroconidia of *F. fredkrugeri* from EEI 176 R3, EI 1361 R2 and QI 386 R2 were significantly shorter than those of the other *Fusarium* isolates. The breadth of macroconidia of *F. verticillioides* from EEI 81 R3 was significantly longer than those of the other *Fusarium* isolates, most of which did not differ significantly in breadth. However, the breadths of macroconidia of *F. verticillioides* from EI 1386 R1, QI 297 R1, EI 1361 R2 and QI 386 R2 were significantly shorter than those of the other *Fusarium* isolates. Table 5 gives the means comparison of the growth parameters of microconidia of the isolated *Fusarium* species. The lengths of microconidia of *F. solani* from EI 135 R3 and EI 1386 R2 were significantly longer than those of the other *Fusarium* isolates. The lengths of microconidia of *F. verticillioides* from QI 297 R1 and DR 197 R3 were also significantly longer than those of *F. verticillioides*, (from DR 197 R1), *F. oxysporum* (from QI 386 R1) and *F. fredkrugeri* (from QI 386 R2, EI 135 R1, EEI 176 R3 and EI 1361 R2). However, the lengths of microconidia of *F. fredkrugeri* from EEI 176 R3 and EI 1361 R2 were significantly shorter than the those of the other *Fusarium* species most of which did not differ significantly in length. The breadths of microconidia of *F. fredkrugeri* from EI 1361 R2, EEI 176 R3 and EI 135 R1 and *F. verticillioides* from EI 1386 R1 were significantly shorter than those of the other *Fusarium* isolates, most of which did not differ significantly in breadth. Table 6 gives the ANOVA table for the microconidia and macroconidia of the isolated *Fusarium* species. The F values for models (P>0.0001) for the length and breadth of both macroconidia and microconidia were all highly significant. The F values for *Fusarium* species (P>0.0001) for the length and breadth of both macroconidia and microconidia were as well all highly significant. Other key features observed from *F. fredkrugeri* isolate grown on Carnation Leaf Agar medium (CLA) were the rare macroconidia with zero to one septate for also the microconidia, with the presence of chlamydospores formed singly in chains (Table 7a). Key features of *F. oxysporum* on CLA were the presence of chlamydospores formed singly, macroconidia ranging from 1-3 septation, and microconidia with 0-1 septation (Table 7a). *Fusarium solani* had chlamydospores formed in chains, macroconidia ranging from 1-4 septation and microconidia with 0-1 septation as their key features on CLA (Table 7a). *Fusarium verticillioides* grown on CLA medium had macroconidia ranging from 1-4 septate and microconidia with 0-1 septate and the absence of chlamydospores as their key features (Table 7b to 7d and Plates 12 and 13).

3.3. Molecular characterisation of the *Fusarium* isolates

The Amplification of the Polymerase Chain Reaction (PCR) used gave 550 bp amplicon from 22 representative isolates chosen out of the 39 isolates from the roots of the different maize varieties (Plate 14). Table 8 gives the homology and gene bank accession number of the *Fusarium* species used for the phylogenetic analysis and origin of the species. Molecular characterisation of the *Fusarium* species (*Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium solani* and *Fusarium fredkrugeri*) was done by using the ITS universal primers, ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC GCT TAT TGA TAT GC). The analyses of ITS sequence by BLAST showed that *Fusarium oxysporum* had a homology range of 97.27% - 100%, *Fusarium verticillioides* had a homology range of 96.93% – 99.80%, *Fusarium fredkrugeri* had a homology range of 97.64% - 99.61% and *Fusarium solani* had a homology range of 98.45% - 99.43%.

3.4 Phylogenetic analysis

Figure 6 gives the phylogenetic tree constructed using the generated sequences. The isolates formed two major clades with sequences clustering with *F. verticillioides* or *F. oxysporum* or *F. fredkrugeri*. The phylogenetic tree derived by analyzing the isolate sequences assigned the species of *Fusarium* to well separated groups with high bootstrap values of 99%. Isolates MYI 3 (EI 1386 R2) and 5 (EI 135 R3) were clustered with *F. solani*, isolates MYI 6 (QI 386 R1) and MYI 18 (EEI 176 R2) clustered with *F. oxysporum*, isolates MYI 9 (EI 1361 R2), MYI 1 (EEI 176 R3), MYI 14 (EI 135 R1) and MYI 22 (QI 386 R2) clustered with *F. fredkrugeri* and isolates MYI 7 (STR 105 R1), MYI 15 (STR 104 R3), MYI 8 (DR 197 R1), MYI 11 (EI 1386 R1), MYI 2 (EEI 34 R3), MYI 4 (EEI 81 R3), MYI 16 (DR 197 R2), MYI 20 (QI 297 R1), MYI 10 (EI 128 R3), MYI 13 (STR 105 R1), MYI 21 (QI 297 R3), and MYI 19 (EEI 29 R1) clustered with *F. verticillioides* (Figure 6).

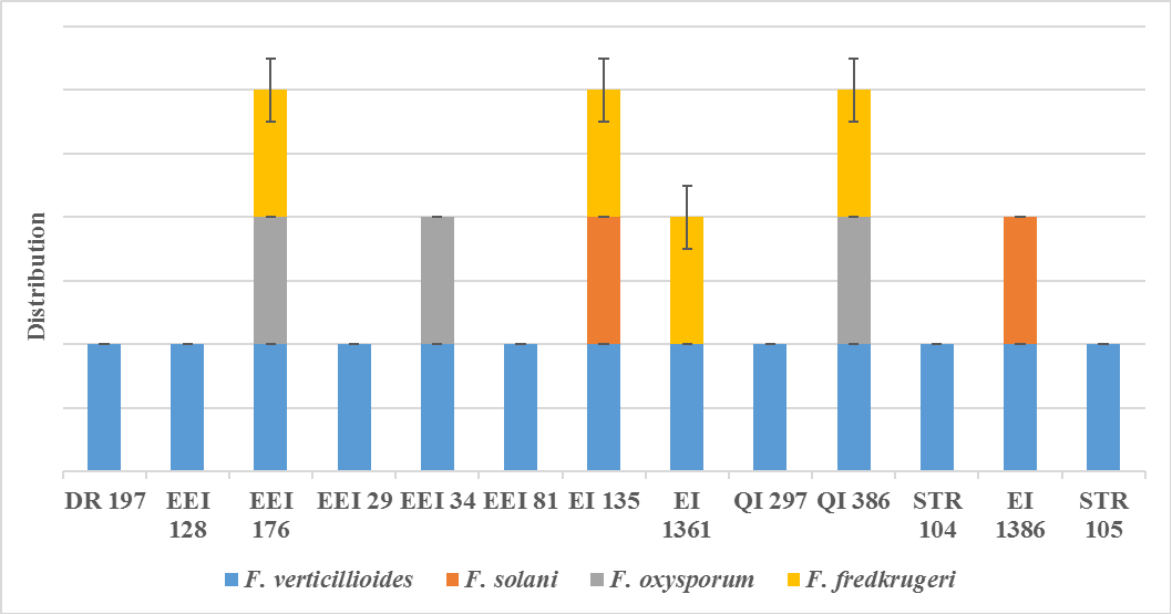

Figure 1: Occurrence of the *Fusarium* species in the roots of the thirteen maize varieties.

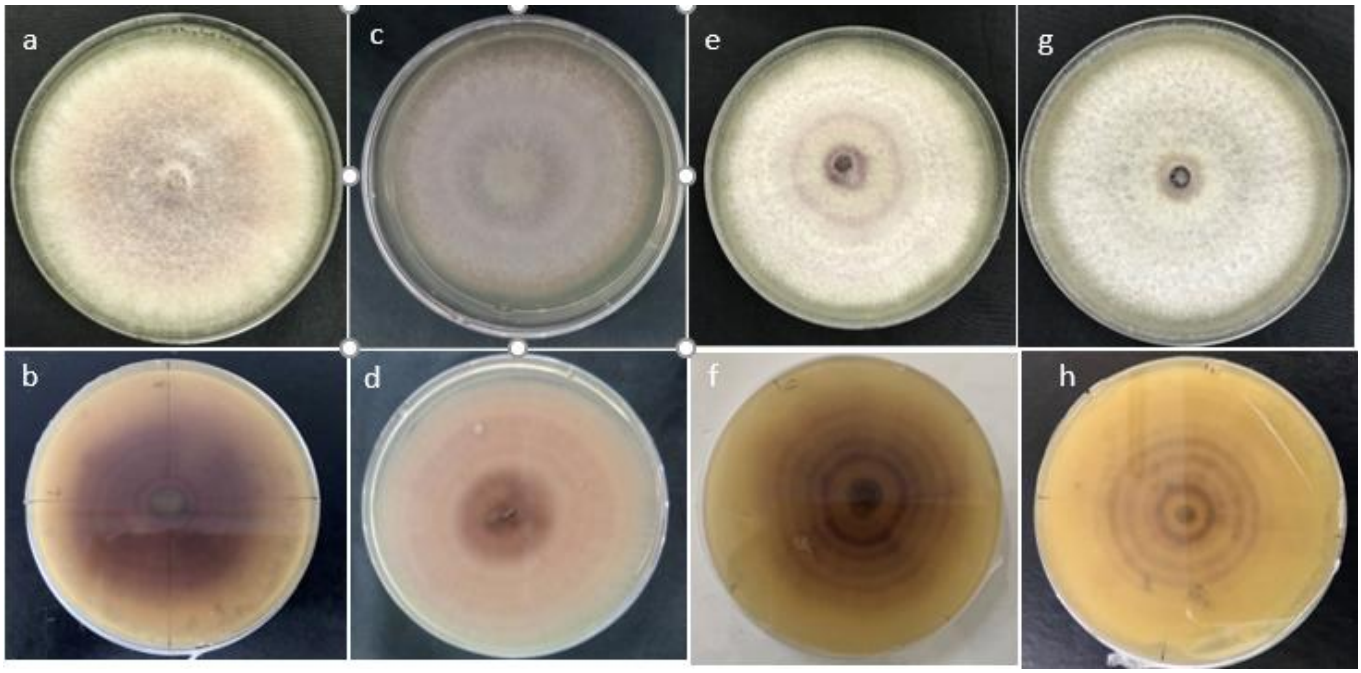

Plate 1: *Fusarium vertcillioides* a&b, c&d, e,f,g&h isolated from roots of maize varieties DR197, EI1361 and EEI81, respectively

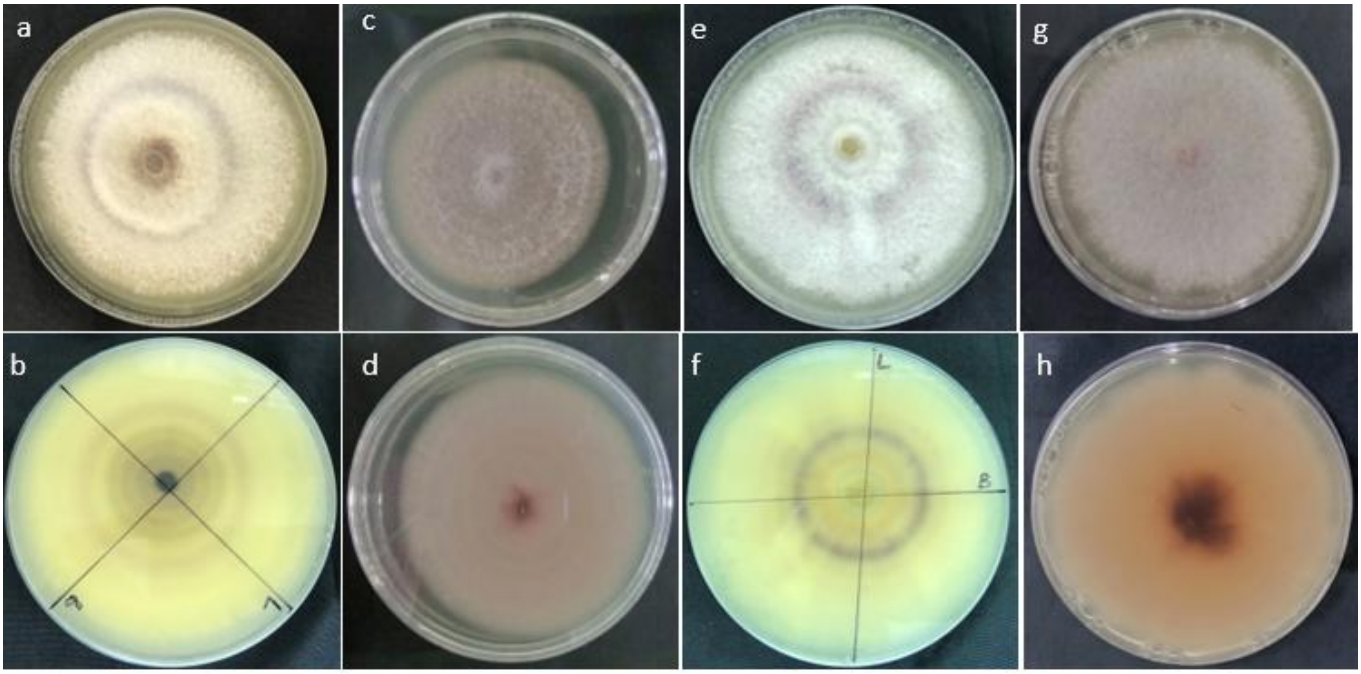

Plate 2: *Fusarium vertcillioides* a,b,e&f, c&d, g&h isolated from roots of maize varieties EI129, STR105 and DR197, respectively

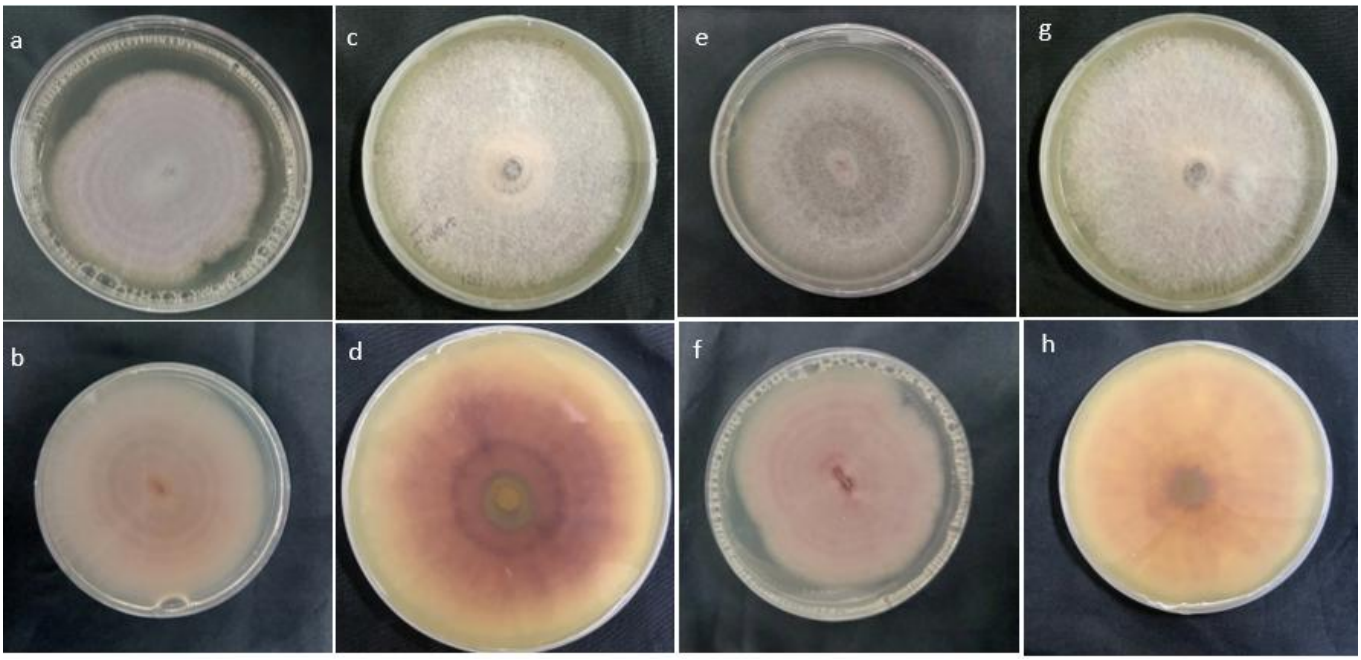

Plate 3: *Fusarium vertcillioides* a&b, c&d, e&f, g&h isolated from roots of maize varieties EEI176, EEI29, EEI34 and QI386, respectively

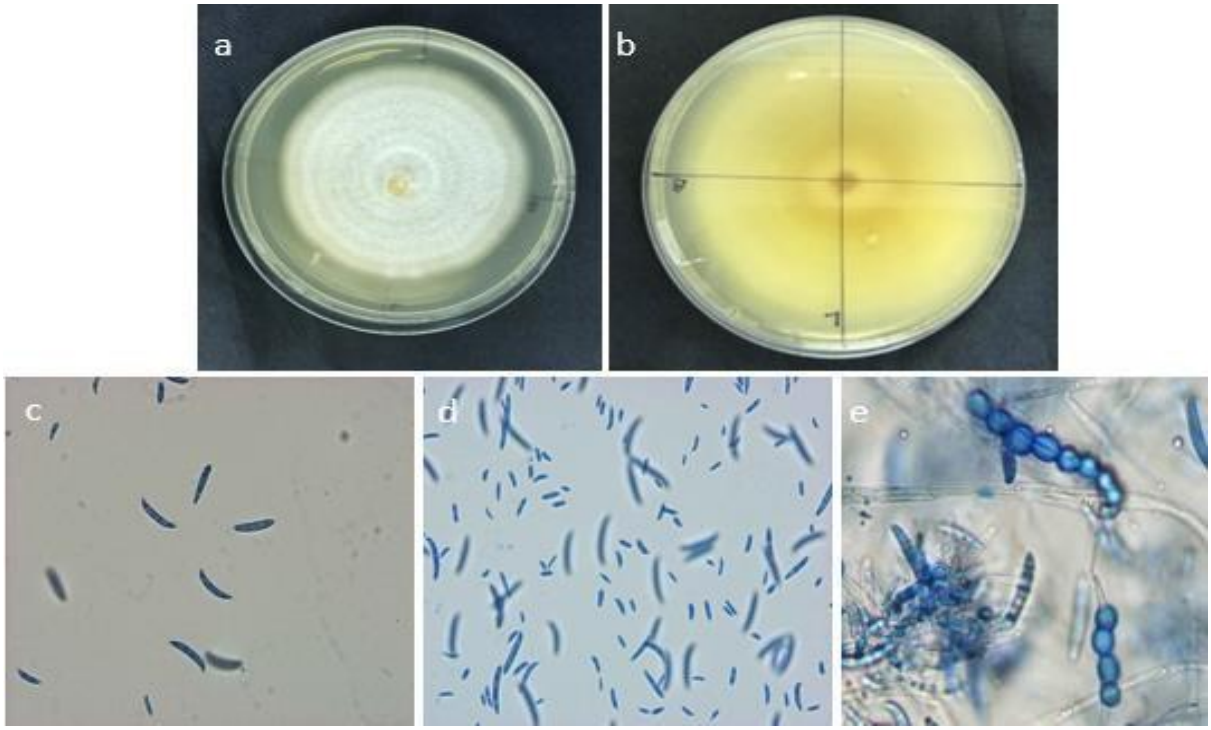

Plate 4: *Fusarium solani* colony morphology (a) Front view, (b) Back view, (c) macroconidia and (e) chlamydospores obtained from roots of maize variety EI1386

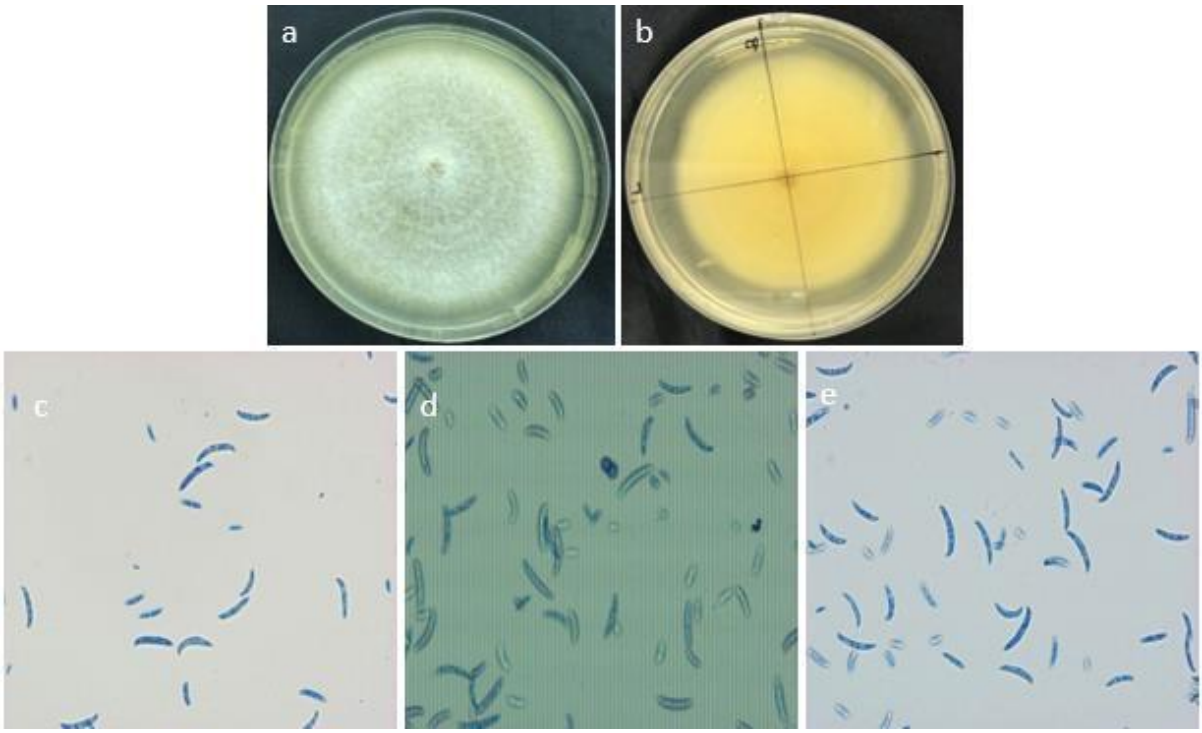

Plate 5: *Fusarium solani* colony morphology (a) Front view, (b) Back view, and (c-e) macroconidia obtained from roots of maize variety EI135

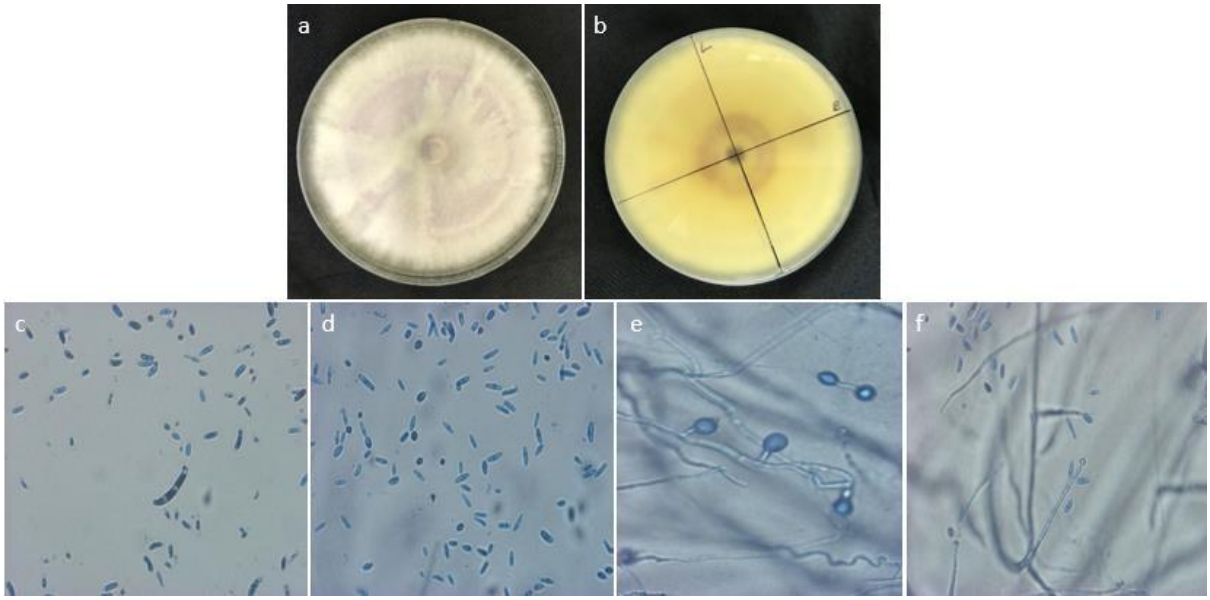

Plate 6: *Fusarium oxysporum* colony morphology (a) Front view, (b) Back view, (c) macroconidia, (d) microconidia (e) chlamydospores and (f) monophialides obtained from roots of maize variety EEI34

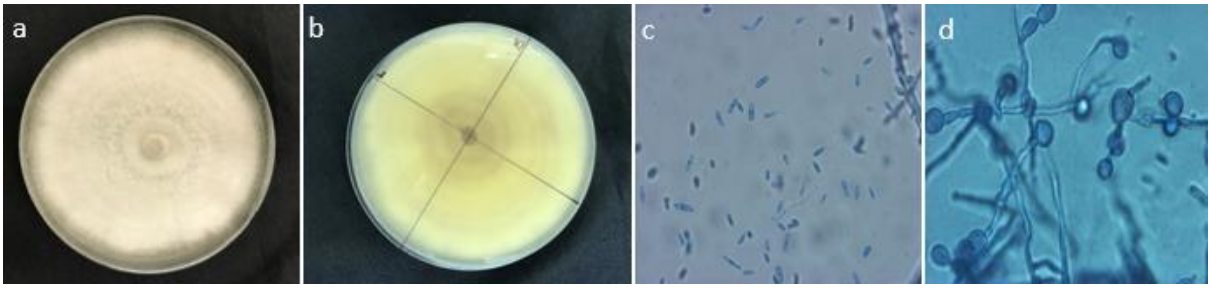

Plate 7: *Fusarium oxysporum* colony morphology (a) Front view, (b) Back view, (c) microconidia, and (d) chlamydospores obtained from roots of maize variety EEI176

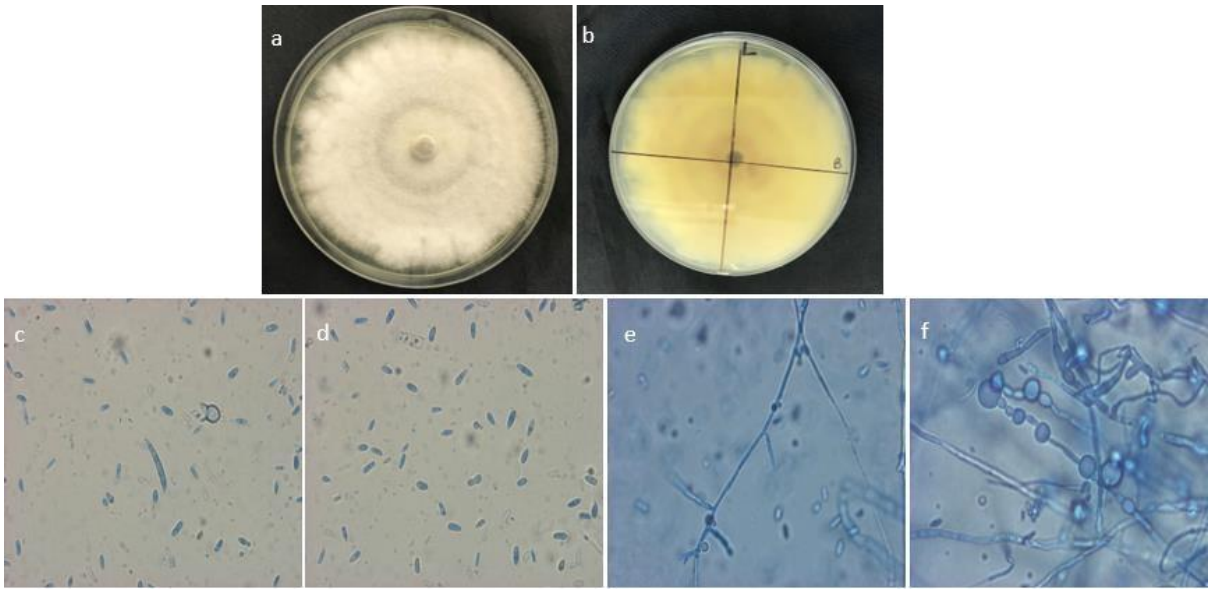

Plate 8: *Fusarium oxysporum* colony morphology (a) Front view, (b) Back view, (c) macroconidia, (d) microconidia (e) monophialides and (f) chlamydospores obtained from roots of maize variety QI386

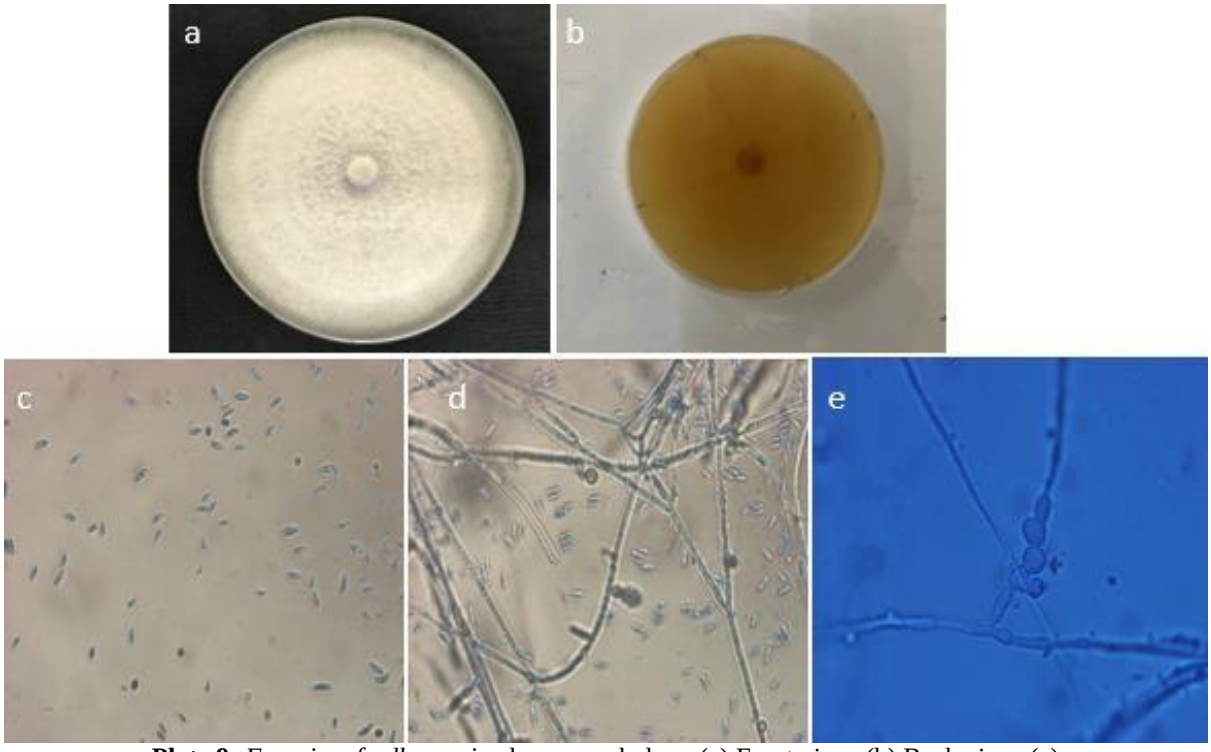

Plate 9: *Fusarium fredkrugeri* colony morphology (a) Front view, (b) Back view, (c) macroconidia and microconidia, (d) monophialides and (e) chlamydospores obtained from roots of maize variety EI1361

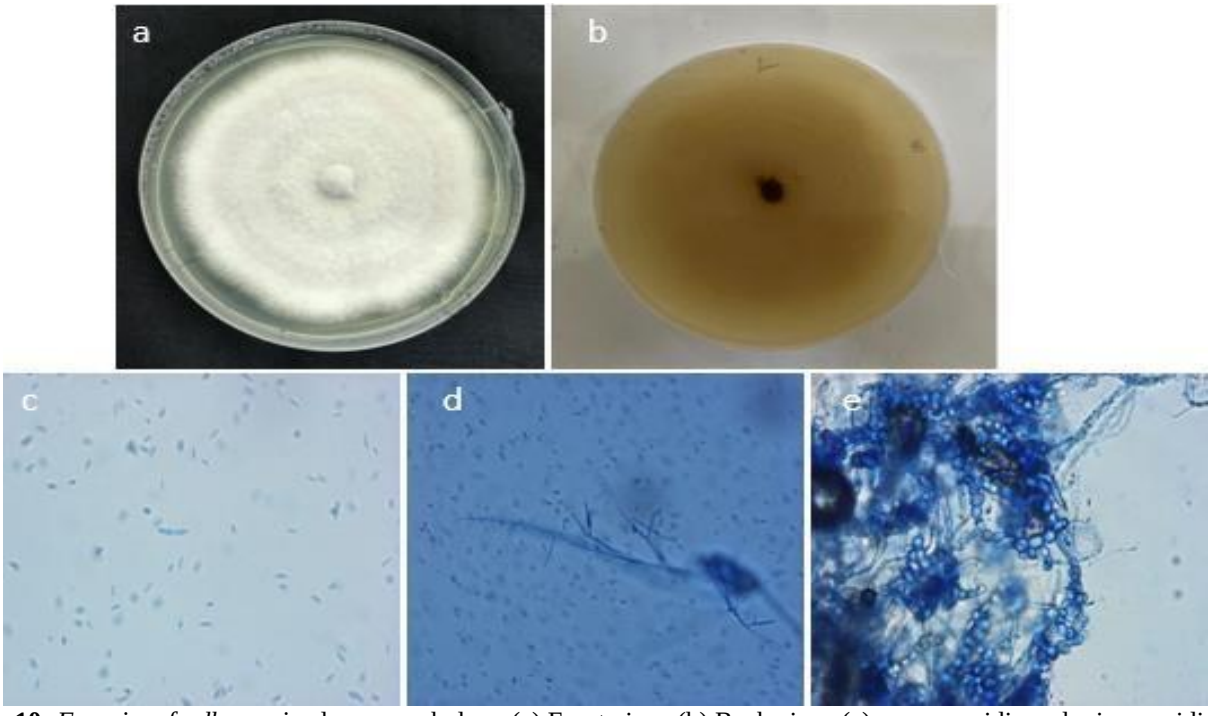

Plate 10: *Fusarium fredkrugeri* colony morphology (a) Front view, (b) Back view, (c) macroconidia and microconidia, (d) monophialides and (e) chlamydospores obtained from roots of maize variety EI135

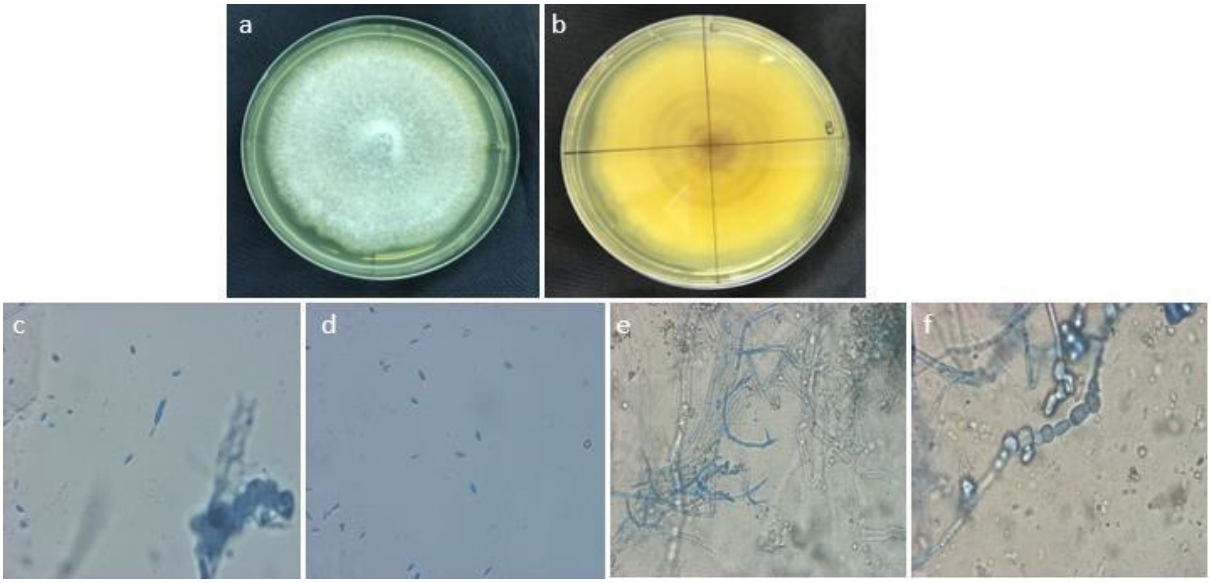

Plate 11: *Fusarium fredkrugeri* colony morphology (a) Front view, (b) Back view, (c) macroconidia, (d) microconidia (e) monophialides and (f) chlamydospores obtained from roots of maize varieties EEI176 and QI386

Table 2: Radial growth of the *Fusarium* species at day 11 after incubation

Fusarium species	Means of radial growth (mm)
Fusarium oxysporum	57.44[a]
Fusarium verticillioides	56.50[a]
Fusarium fredkrugeri	47.63[ab]
Fusarium solani	40.00[b]
$LSD_{0.05}$	10.36
R^2	0.12

Means with different letters in a column are significantly different ($p \leq 0.05$).

Table 3: ANOVA table for the Radial Growth Rate of *Fusarium* species at day 11

Source of variation	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2254.58	450.92	3.07	0.0123**
Replicates	2	1.25	0.62	0.00	1.00
Fusarium species	3	2253.33	751.11	5.12	0.0024**
Error	111	16287.78	146.74		
Corrected Total	116	18542.36			

**** = Highly significant, * = Significant**

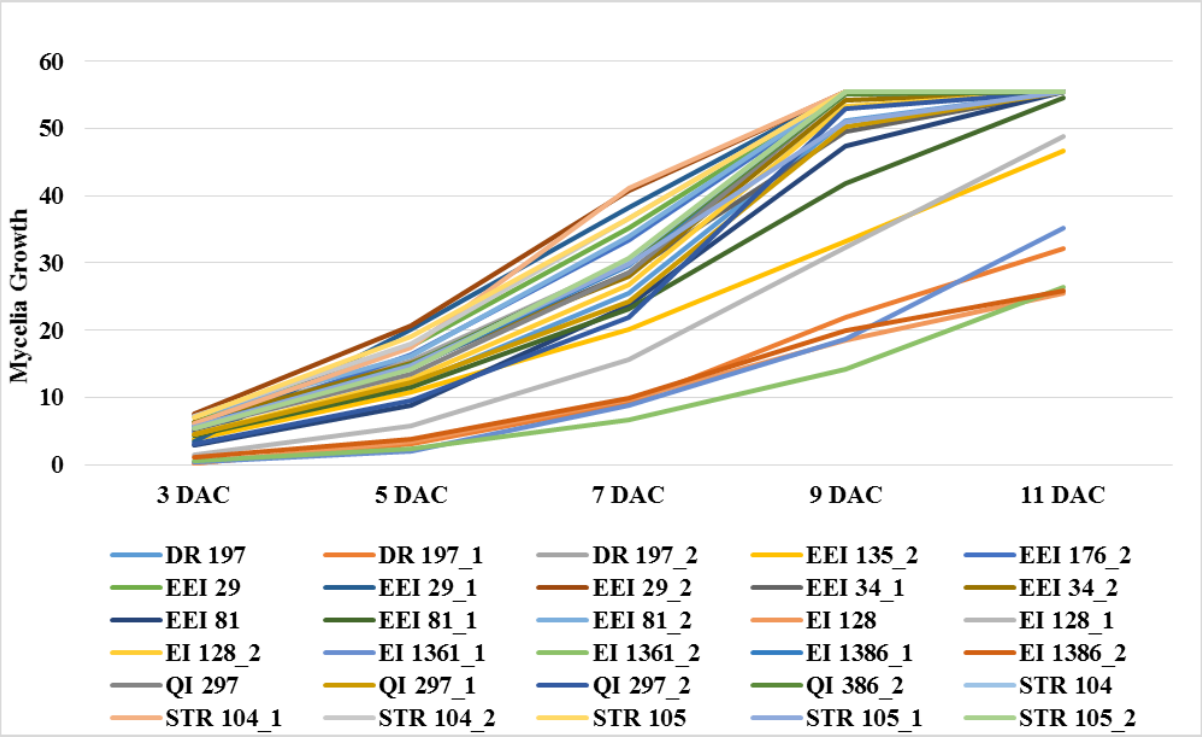

Figure 2: Growth comparison of *Fusarium verticilliodes* isolates from roots of the different maize varieties

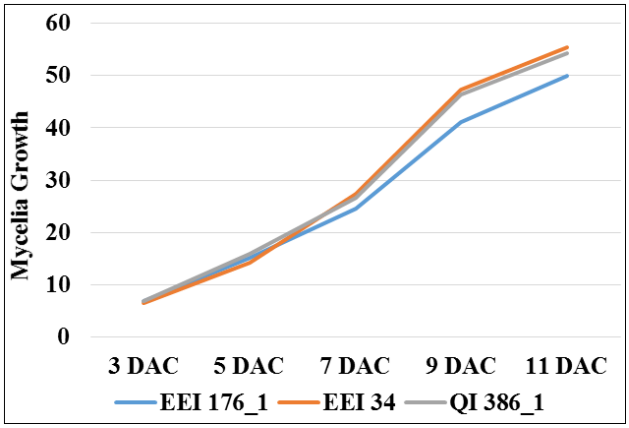

Figure 3: Growth comparison of *Fusarium oxysporum* isolated from the roots of the different maize varieties.

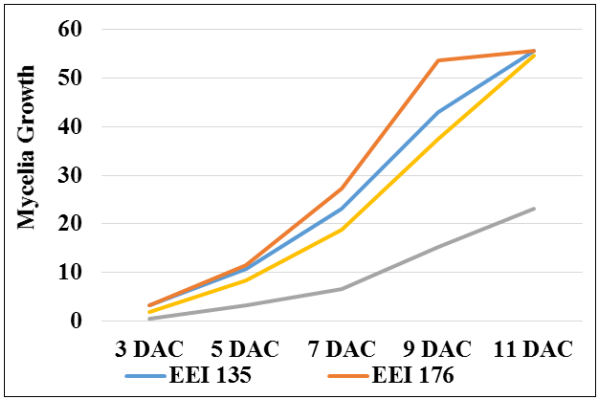

Figure 4: Growth comparison of *Fusarium fredkrugeri* isolated from the roots of the different maize varieties.

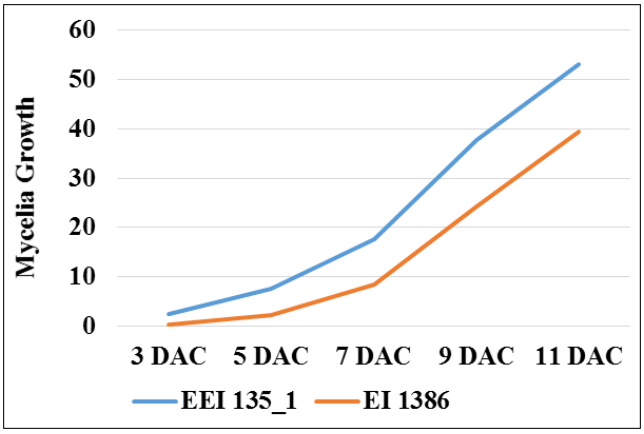

Figure 5: Growth comparison of *Fusarium solani* isolated from the roots of the different maize varieties.

Table 4: Macroconidia length and breadth of the *Fusarium* species

Sample ID	*Fusarium* species	Macroncidia Length (µm)	Macroconidia Breadth (µm)
EI 128 R3	*F. verticillioides*	2.93^{a}	0.39^{b}
EI 1386 R1	*F. verticillioides*	2.98^{a}	0.25^{d}
EI 135 R3	*F. solani*	3.24^{a}	0.39^{b}
EI 1386 R2	*F. solani*	2.50^{b}	0.39^{b}
DR 197 R3	*F. verticillioides*	2.30^{bc}	0.39^{b}
EEI 176 R2	*F. oxysporum*	2.15^{bcd}	0.39^{b}
QI 297 R1	*F. verticillioides*	2.20^{bcd}	0.28^{d}
QI 297 R3	*F. verticillioides*	2.22^{bcd}	0.39^{b}
EEI 34 R3	*F. verticillioides*	1.99^{cd}	0.39^{b}
EEI 81 R3	*F. verticillioides*	2.03^{cd}	0.59^{a}
EEI 29 R1	*F. verticillioides*	1.95^{cde}	0.39^{b}
DR 197 R1	*F. verticillioides*	1.91^{def}	0.39^{b}
STR 104 R3	*F. verticillioides*	1.60^{efg}	0.39^{b}
STR 105 R2	*F. verticillioides*	1.58^{efg}	0.325^{c}
EEI 34 R1	*F. oxysporum*	1.56^{fg}	0.39^{b}
QI 386 R1	*F. oxysporum*	1.25^{gh}	0.39^{b}
EI 135 R1	*F. fredkrugeri*	1.05^{h}	0.39^{b}
EEI 176 R3	*F. fredkrugeri*	0.90^{hi}	0.39^{b}
EI 1361 R2	*F. fredkrugeri*	0.65^{i}	0.25^{d}
QI 386 R2	*F. fredkrugeri*	0.53^{i}	0.25^{d}
LSD0.05		0.38	0.05
R-square		0.78	0.67

Means with different letters in the same column are significantly different ($p \leq 0.05$).

Table 5: Microconidia length and breadth of the *Fusarium* species

Sample ID	Species	Microconidia Length (µm)	Microconidia Breadth (µm)
EI 135 R3	*F. solani*	1.25^{a}	0.39^{a}
EI 1386 R2	*F. solani*	1.17^{a}	0.39^{a}
QI 297 R1	*F. verticillioides*	0.73^{b}	0.30^{b}
DR 197 R3	*F. verticillioides*	0.74^{b}	0.39^{a}
EI 128 R3	*F. verticillioides*	0.70^{bc}	0.39^{a}
EEI 176 R2	*F. oxysporum*	0.59^{bcd}	0.39^{a}
EEI 34 R1	*F. oxysporum*	0.59^{bcd}	0.39^{a}
EEI 29 R1	*F. verticillioides*	0.59^{bcd}	0.39^{a}
EEI 34 R3	*F. verticillioides*	0.64^{bcd}	0.39^{a}
EEI 81 R3	*F. verticillioides*	0.59^{bcd}	0.39^{a}
EI 1386 R1	*F. verticillioides*	0.55^{bcd}	0.25^{c}
QI 297 R3	*F. verticillioides*	0.55^{bcd}	0.39^{a}
STR 104 R3	*F. verticillioides*	0.59^{bcd}	0.39^{a}
STR 105 R2	*F. verticillioides*	0.58^{bcd}	0.28^{bc}
DR 197 R1	*F. verticillioides*	0.51^{cde}	0.39^{a}
QI 386 R1	*F. oxysporum*	0.47^{def}	0.39^{a}
QI 386 R2	*F. fredkrugeri*	0.47^{def}	0.39^{a}
EI 135 R1	*F. fredkrugeri*	0.33^{ef}	0.25^{c}
EEI 176 R3	*F. fredkrugeri*	0.30^{f}	0.25^{c}
EI 1361 R2	*F. fredkrugeri*	0.04^{g}	0.03^{d}

LSD0.05		0.2	0.03
R-square		0.6	0.92

Means with different letters in the same column are significantly different ($p \leq 0.05$).

Table 6: ANOVA table for microconidia and macroconidia of the *Fusarium* species

	Microconidia				Macroconidia			
	Length		Breadth		Length		Breadth	
	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F
Model	9.32	0.0001**	67.32	0.0001**	21.19	0.0001**	12.27	0.0001**
Fusarium spp.	13.42	0.0001**	98.81	0.0001**	30.44	0.0001**	17.64	0.0001**

**** = Highly significant, * = Significant**

Table 7a: Some key features of *Fusarium* species isolated from the roots of the different maize varieties

Sample ID	*Fusarium* Species	Type of mycelium	Clamydospore	Macroconidia Septation	Microconidia Septation
EI 135_1	*Fusarium fredkrugeri*	Aerial	present	0-1 septate	0-1 septate
EEI 176_3	*Fusarium fredkrugeri*	Aerial	present	0-1 septate	0-1 septate
EI 1361_2	*Fusarium fredkrugeri*	Aerial	present	0-1 septate	0-1 septate
QI 386 _2	*Fusarium fredkrugeri*	Cotton	present	0-1 septate	0-1 septate
EEI 176_2	*Fusarium oxysporum*	Aerial	present	1-3 septate	0-1 septate
EEI 34 _1	*Fusarium oxysporum*	Aerial	present	1-3 septate	0-1 septate
QI 386 _1	*Fusarium oxysporum*	Aerial	present	1-2 septate	0-1 septate
EI 135_3	*Fusarium solani*	Cotton	present	1-4 septate	0-1 septate
EI 1386_2	*Fusarium solani*	Cotton	present	1-3 septate	0-1 septate
EI 135_1	*Fusarium fredkrugeri*	Aerial	present	0-1 septate	0-1 septate
EEI 176_3	*Fusarium fredkrugeri*	Aerial	present	0-1 septate	0-1 septate

Table 7b: Some key features of *Fusarium* species isolated from the roots of the different maize varieties

Sample ID	*Fusarium* Species	Type of mycelium	Clamydospore	Macroconidia Septation	Microconidia Septation
EI 135_2	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
STR 105_1	*Fusarium verticillioides*	Aerial	Absent	1-2 septate	0-1 septate
STR 105_2	*Fusarium verticillioides*	Aerial	Absent	1-2 septate	0-1 septate
STR 105_3	*Fusarium verticillioides*	Aerial	Absent	1-2 septate	0-1 septate
EEI 128_1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 128_2	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 128_3	*Fusarium verticillioides*	Cotton	Absent	1-4 septate	0-1 septate
EEI 176_1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EI 1386_1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EI 1386_3	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate

Table 7 (c): Some key features of *Fusarium* species isolated from the roots of the different maize varieties

Sample ID	*Fusarium* Species	Type of mycelium	Clamydospore	Macroconidia Septation	Microconidia Septation
DR 197 _1	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
DR 197 _2	*Fusarium verticillioides*	Cotton	Absent	1-3 septate	0-1 septate
DR 197 _3	*Fusarium verticillioides*	Cotton	Absent	1-3 septate	0-1 septate
EI 1361_1	*Fusarium verticillioides*	Cotton	Absent	1-3 septate	0-1 septate
EI 1361_3	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
EEI 81_1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 81_2	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 81_3	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 29 _1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate

Table 7 (d): Some key features of *Fusarium* species isolated from the roots of the different maize varieties

Sample ID	*Fusarium* Species	Type of mycelium	Clamydospore	Macroconidia Septation	Microconidia Septation
EEI 29 _2	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
EEI 29 _3	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
STR 104 _1	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
STR 104 _2	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
STR 104 _3	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
QI 386 _3	*Fusarium verticillioides*	Aerial	Absent	1-3 septate	0-1 septate
QI 297_1	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
QI 297_2	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
QI 297_3	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 34 _2	*Fusarium verticillioides*	Aerial	Absent	1-4 septate	0-1 septate
EEI 34 _3	*Fusarium verticillioides*	Cotton	Absent	1-4 septate	0-1 septate

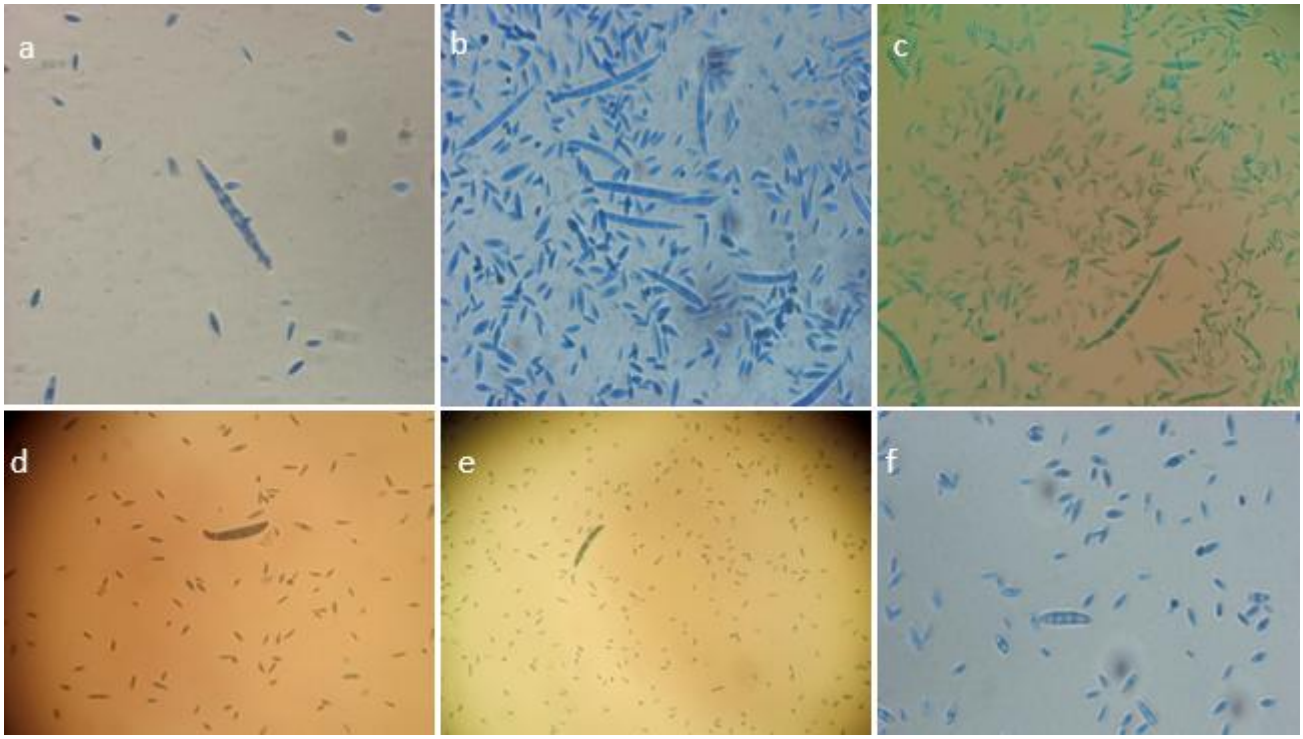

Plate 12: *Fusarium verticillioides* microscopic morphology (a-f) macroconidia and microconidia obtained from roots of different maize varieties

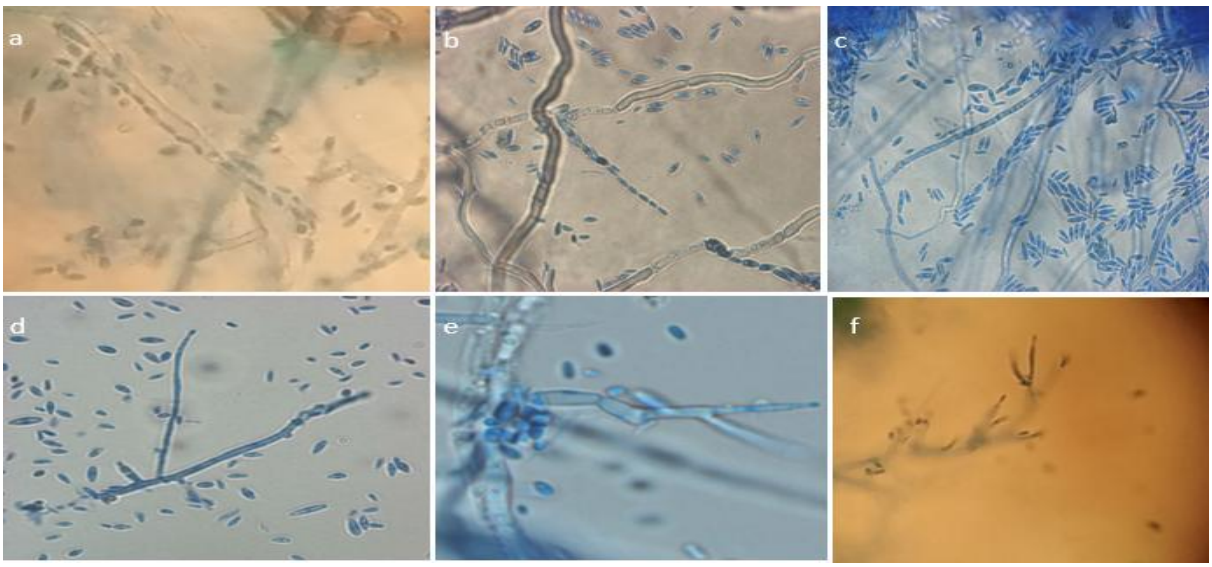

Plate 13: *Fusarium verticillioides* microscopic morphology (a-b) microconidia in chains (c) microconidia (d-f) monophialides.

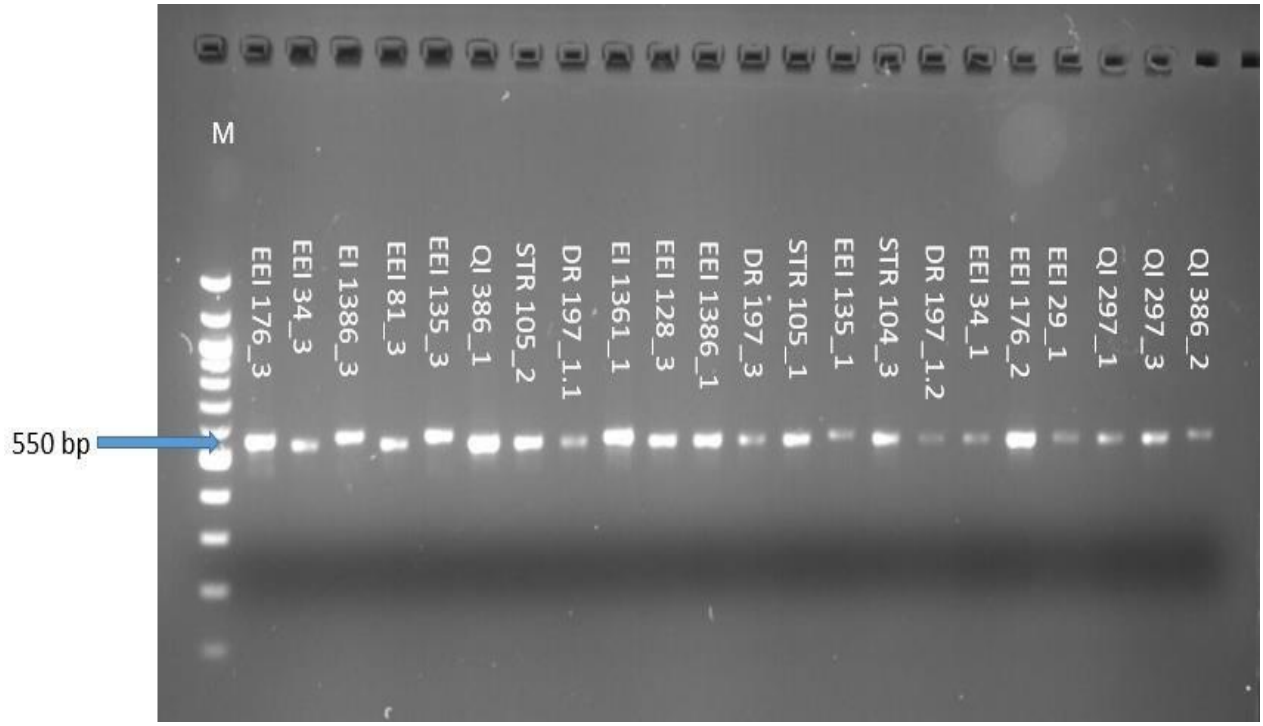

Plate 14: Gel electrophoresis of the PCR products of the regions ITS1 and ITS4 of the isolates of *Fusarium* species isolated from the roots of the different varieties of maize.

Lanes: (M) Molecular Marker 1000 bp.

Table 8: Homology and Gene Bank Accession Number of the *Fusarium* species used for phylogenetic analysis and the origin of the species

Sample ID	Origin	Homology (%)	Molecular Characterization	Gene Bank Accession number
EEI 176_3	Ibadan	99.22%	*Fusarium fredkrugeri*	X94177.1
EI 1361_2	Ibadan	98.46%	*Fusarium fredkrugeri*	MW016431.1
EI 135_1	Ibadan	99.61%	*Fusarium fredkrugeri*	X94177.1
QI 386_2	Ibadan	97.64%	*Fusarium fredkrugeri*	X94177.1
QI 386_1	Ibadan	100%	*Fusarium oxysporum*	MT453296.1
EEI 34_1	Ibadan	97.27%	*Fusarium oxysporum*	MK271275.1
EEI 176_2	Ibadan	99.80%	*Fusarium oxysporum*	KU527803.2
EI 1386_2	Ibadan	98.45%	*Fusarium solani*	MT251175.1
EI 135_3	Ibadan	99.43%	*Fusarium solani*	MN989028.1
EEI 34_3	Ibadan	99.80%	*Fusarium verticillioides*	MT505436.1
EEI 81_3	Ibadan	99.21%	*Fusarium verticillioides*	MT505436.1
STR 105_2	Ibadan	99.79%	*Fusarium verticillioides*	MT505436.1
DR 197_1	Ibadan	99.21%	*Fusarium verticillioides*	MT505436.1
EI 128_3	Ibadan	96.93%	*Fusarium verticillioides*	MT505436.1
EI 1386_1	Ibadan	99.21%	*Fusarium verticillioides*	MT505436.1
STR 105_1	Ibadan	99.41%	*Fusarium verticillioides*	MT505436.1
STR 104_3	Ibadan	99.20%	*Fusarium verticillioides*	MN429250.1
DR 197_2	Ibadan	99.80%	*Fusarium verticillioides*	MT505436.1
EEI 29_1	Ibadan	98.58%	*Fusarium verticillioides*	MT505436.1
QI 297_1	Ibadan	99.80%	*Fusarium verticillioides*	MN429250.1
QI 297_3	Ibadan	99.60%	*Fusarium verticillioides*	MK264336.1

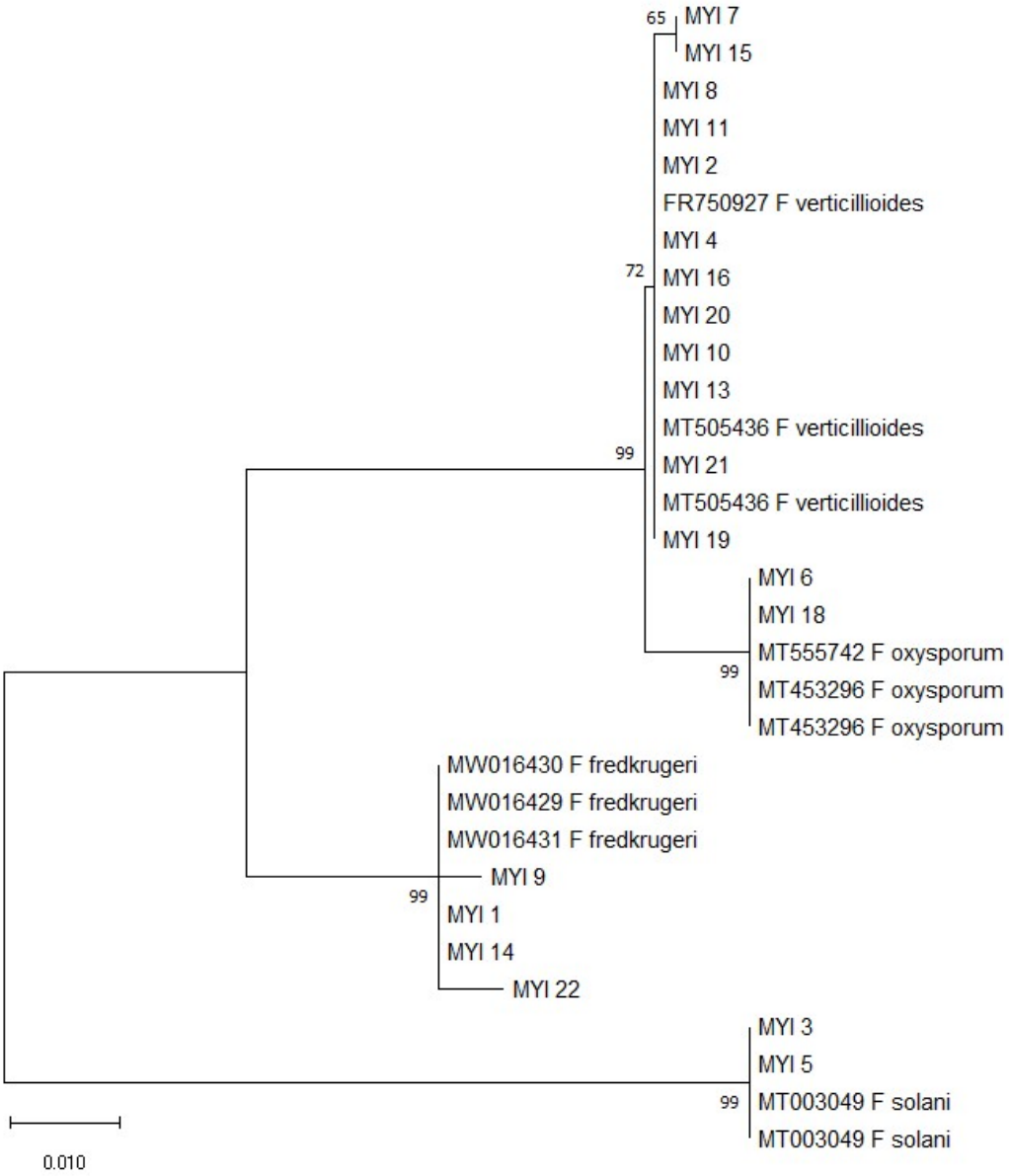

Figure 6: Phylogenetic tree of ITS sequence of the *Fusarium* species isolated from the roots of different maize varieties

4. Discussion

Fusarium oxysporum and *F. verticillioides* isolated from the root of maize varieties are in agreement with the findings of Campos *et al.* (2019) that also isolated *F. oxysporum* and *F. verticillioides* from maize roots. The isolation of *Fusarium solani* from the roots of the maize varieties corroborated the report of Okello and Mathew (2019), who isolated and identified *Fusarium acuminatum, F. equiseti, F. graminearum, F. oxysporum, F. proliferatum, F. solani*, and *F. subglutinans* in soybean and maize (corn). However, there is no information available on the isolation of *F. fredkrugeri* from maize roots, though it was reported by Sandoval-Denis *et al.* (2018) to have been isolated from soils collected in a catena landscape on a research supersite in the Kruger National Park, South Africa. However, it has been reported to be genetically closely related to *F. dlamini* (Sandoval-Denis *et al.*, 2018). To the best knowledge of the authors, this is the first report of *F. fredkrugeri* isolated from maize roots. The highest occurrence of *F. verticillioides* in the roots of the thirteen maize varieties agrees with the findings of Yates *et al.* (1997); Aguín *et al.* (2014) and Duan *et al.* (2016) who reported that *F. verticillioides* are the most prevalent fungus associated with maize.

The cultures of *Fusarium verticillioides* which was white or creamy cottony mycelium with a white to pink to purple pigmentation on Potato Dextrose Agar agrees with the work of Widiastuti *et al.* (2020) which reported that *Fusarium verticillioides* showed white mycelium with plum to violet pigment on PDA medium. *Fusarium oxysporum*, which had white to creamy aerial mycelium and a yellow to brown, purple and pink pigment corroborated the findings of Tirado-Ramirez *et al.* (2021) who also reported that *F. oxysporum* had a white or creamy cottony mycelium with a varying colour of yellow to brown to purple and pink at the reverse side of Potato Dextrose Agar (PDA) plate. The white and cottony mycelium on PDA exhibited by *Fusarium solani* agrees with the work of Mwangi *et al.* (2020) who reported that *F. solani* produced white to cream colour cultures. However, *Fusarium fredkrugeri* showed an abundance white aerial mycelium with a variation in colour

from greyish lilac to pale violet and a vinaceous buff at the bottom of the PDA plate.

The microscopic key features such as chlamydospores, shape and size of the conidia (microconidia and macroconidia) confirmed that the isolates were of *Fusarium* species (*F. fredkrugeri, F. oxysporum, Fusarium solani and Fusarium verticilliodes*) which agreed with the findings of Leslie and Summerell, (2006). The variability in the macroscopic and microscopic features may be indicative of the physiological difference among the conidia (Mwangi *et al.*, 2020). Results of the PCR analysis confirmed the results obtained from the morphological identification which were *Fusarium fredkrugeri, Fusarium oxysporum, Fusarium solani and Fusarium verticilliodes.* The study agrees with the work of Chehri *et al.* (2011) where PCR product of *Fusarium* species was amplified by using primer ITS1 and ITS4. The isolated *Fusarium* species produced approximately 550 bp band which supports the findings of Lee *et al.* (2000) and Chehri *et al.* (2011). The results of phylogenetic analysis of the *Fusarium* species, which had two groups, with *F. verticillioides* and *F. oxysporum* grouped closely together, agrees with the work of Mwangi *et al.* (2020) who reported the grouping together of *F. verticillioides* and *F. oxysporum*. The highly significant F value for model, regarding growth of the *Fusarium* species shows the appropriateness of the fitted model. The highly significant F value for *Fusarium* species shows the significantly different growth rate of the different *Fusarium* species at different days of incubation. The highly significant F values for microconidia and macroconidia shows the significant variability in the growth parameters of the microconidia and macroconidia of the different *Fusarium* species that were isolated.

5. Conclusion

In the thirteen varieties of maize considered, predominant *Fusarium* species were *F. verticillioides*, *F. fredkrugeri*, *F. oxysporum* and *F. solani* in that order. To the best of authors' knowledge, this might be the first reported case of *Fusarium fredkrugeri* isolated from maize roots.

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