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Identification of fungal pathogens causing postharvest rot of bulb onions (*Allium cepa* L.) in selected major growing regions of Kenya

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Abstract

Rot is a major cause of bulb onion losses in Kenya, accounting for about 14 % of total postharvest losses. In Kenya, the fungi associated with bulb onion postharvest rot of onion postharvest rots are not well known. Therefore, this study aimed at identifying the fungal pathogens contributing to bulb onion postharvest rot in major growing regions of Kenya. Bulb onion samples were collected from seven major markets and isolates were obtained by cutting 3 mm tissue segments from the edges of rotten lesions. These were cultured in water agar followed by incubation for seven days at room temperature (23 ± 3°C). After seven days, mycelia plugs from the growing edge of each colony were sub-cultured in potato dextrose agar and incubated for ten days. A total of fifty fungal isolates were obtained from the isolations and in vitro pathogenicity test was done on bulb onions. Eighteen fungal isolates that turned out to be pathogenic were inoculated in bulb onions to assess their level of virulence by measuring lesion size after 21 days of incubation at room temperature (23 ± 3 °). The fungal isolates caused statistically ($P < 0.001$) different sized lesions, ranging from 0.4 ± 0.1 cm to 2.6 ± 0.5 cm. Based on morphological characteristics the eighteen fungal pathogenic isolates were identified as *Fusarium* spp. and were grouped into three clusters. Molecular technique confirmed the three *Fusarium* spp. clusters as *Fusarium oxysporum* f.sp. *cepae* (55 %), *F. acutatum* (17 %) and *F. solani* (28 %). *F. oxysporum* f.sp. *cepae* was predominantly isolated from bulb onions collected in Bungoma County, while *F. solani* was mainly obtained from samples in Kajiado County and *F. acutatum* on bulb onions from Meru County. This study indicates that these three *Fusarium* species are the main fungal species causing postharvest rot in the major bulb onion growing regions of Kenya. Application of appropriate postharvest technology such as curing before storage would minimize postharvest rot in bulb onion.

Keywords: *Fusarium*, molecular, morphological, pathogenic, postharvest losses

1 Introduction

Bulb onion (*Allium cepa* L.) is widely cultivated in different climatic zones ranging from tropical to temperate zones (Brewster, 2008). It belongs to the amaryllis family (Amaryllidaceae) and the genus Allium (Grubben *et al*., 2004). The vegetative growth and good bulb maturation is obtained under dry and cool conditions with day temperatures ranging between 20-28 °C and a soil pH above 5.6 (Currah, 1990; Grubben *et al*., 2004). According to FAOSTAT (2020), in 2018 bulb onions were produced on a total area of 50.4 million ha worldwide, with a production volume of 96.8 million tonnes. Globally, China is the leading producer with over 18 million tons of bulb onions in 2018, followed by India (11 million tons) and USA (3.2 million tons) (FAOSTAT ,2020). The largest bulb onion producers in Africa are Egypt (1.4 million tons), Sudan (1.3 million tons), Nigeria (0.93 million tons) and Uganda (0.22 million tons) (Hanci, 2018).

In Kenya, bulb onion is grown from low altitude to the highest altitude areas of about 2000 m above sea level. In 2018, bulb onion ranked first in terms of area covered, quantity produced and income generated among aromatic crops including spring onions, garlic, chives, leeks and coriander in Kenya (HCD, 2018). During the same year, bulb onions covered an area of 7005 ha, and 105,585 tons of the produce was harvested with a value of USD $ 40 million. In 2018, the major bulb onion producing Counties in Kenya were Kajiado

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(25,233 tons), Bungoma (13,805 tons) and Meru (9,721 tons) (HCD, 2018). Bulb onion production in Kenya has gained popularity in recent years due to increased adoption of irrigated agriculture (HCD,2018). However, postharvest losses remain one of the major challenges for bulb onion productivity. In a recent study, Gathambiri *et al*. (2021) reported that 5-30 % postharvest losses occur during postharvest handling of bulb onion in Kenya.

Postharvest diseases are the main contributor to bulb onion losses during postharvest handling. In Asia 20-30 % losses occurs due to postharvest diseases while in Serbia 20-50 % losses are often reported (Duduk *et al*., 2017; Gupta, 2013). In addition in Kenya, bulb onion postharvest rot contributes 14 % of the total postharvest losses at market level (Gathambiri *et al*., 2021). Bulb onion rot can be associated with several pre-and postharvest factors such as variety, soil properties, climatic conditions, agronomic management practices and storage conditions (Yurgel *et al*., 2018). Postharvest diseases in bulb onion mostly begin in the field and become severe during handling. Rasiukevičiute *et al*., (2016) reported that *Aspergillus* spp., *Botrytis* spp. and *Fusarium* spp. which are known to be seed-borne fungi can contribute to rot during postharvest handling.

To properly manage postharvest diseases in bulb onions, it is essential to identify their causal fungal pathogens. According to Yurgel *et al*. (2018), *Aspergillus, Penicillium, Alternaria*, *Fusarium*, *Rhizopus, Colletotrichum, Pseudomonas, Lactobacillus* and *Botrytis* spp. are some of the fungal pathogens contributing to postharvest diseases of bulb onion. Various countries have reported diverse fungal pathogens as causative agents of postharvest rot. In Ghana, Adongo *et al*., (2015) highlighted *Aspergillus niger, A. flavus, Penicillium* sp., *Rhizopus solonifer* and *Fusarium oxysporum* while in Iran *Fusarium oxysporum* f.sp. *cepae*, *F. solani*, *F. redolens* and *F. proliferatum* were identified (Ghanbarzadeh *et al*., 2014). In Sudan, *Aspergillus niger* was reported to contribute to 80 % of bulb onion postharvest rot (El-Nagerabi *et al*., 2003).

Although postharvest rot is a major constraint in enhancing bulb onion productivity in Kenya, information on pathogenic fungi associated with postharvest diseases is limited. Therefore, this study was carried out to identify the pathogenic fungi contributing to postharvest bulb onion rot in the major growing regions of Kenya using morphological and molecular techniques.

2 Materials and methods

2.1 2.1 Study sites and bulb onion samples collection

Onion samples were collected from major markets in Kajiado and Kitengela (Kajiado County), Meru and Nkubu (Meru County), and Kimilili, Cheptais and Chwele markets (Bungoma County) (Fig. 1).

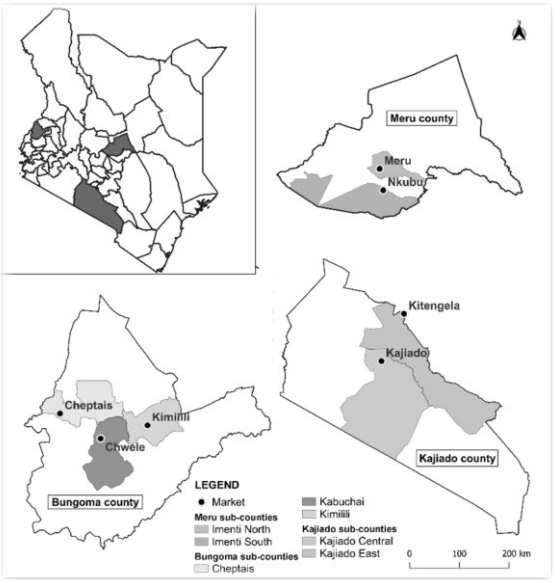

Fig. 1: *Map indicating the seven markets where bulb onion samples were collected in Bungoma, Meru and Kajiado Counties.*

In each market, a list of all bulb onion wholesalers was compiled and a simple random sampling procedure was used to select three traders selling at least 100 kg or more per week were selected. A formula to determine the number of bulb onion samples to be taken per wholesaler was based on 15 % of the total postharvest losses occurring at market level and 10 % of the total losses caused by rot (Sharma, 2016).

$$\text{Number of samples} = 0.15\,\text{QT}\ x\ 0.01$$

where, QT is quantity sold per week.

Onion bulb samples that were soft and gave in slightly under finger pressure at the base and neck were collected from the markets. In total 524 samples were collected from the seven markets (Annex 1). Bulb onion samples were collected in brown bags, placed in cool boxes and transported to the Kenya Agricultural and Livestock Research Organization (KALRO) Kandara postharvest laboratory. They were placed on the laboratory bench at room temperature (23 ± 3 °) and after one week, bulb onions with visible signs of rot were used for fungal isolation. The samples were

coded based on which market they were collected; KJ (Kajiado market), KT (Kitengela market), MR (Meru Market), NK (Nkubu market), KM (Kimilili market), CP (Cheptais market) and CL (Chwele markets).

2.2 Fungal isolation from bulb onions

The bulb onion samples with clear symptoms of rot (300) were prepared for fungal isolation by stripping the outer dry scales and washing under running water to remove soils and any debris. The onion bulbs were sterilised in 70 % alcohol for 60 seconds as described by Li *et al*. (2017). They were rinsed three times in a row with sterile distilled water and blot-dried with cotton wool. Using a sterile scalpel blade, the bulb onions were cut open at the neck to expose the infected inner scales. Two 3 mm segments per bulb onion sample were cut out from the edges of the lesions with a sterile scalpel. Each segment was plated in Petri dishes containing water agar (Oxoid LP0013 Agar Technical No. 3), and placed in a laboratory growth chamber at 23 ± 3 °C under natural light for seven days. After this period mycelia plug from the growing edge of each colony were sub-cultured in Potato Dextrose Agar (PDA) (Oxoid CMO-139). The Petri dishes were sealed with Para-film® and incubated at room temperature (23 ± 3 °C) for ten days to enhance sporulation. From each culture some mycelia were cut-off and observed under microscope (B-350 Optika) to confirm sporulation.

2.3 Isolation of single spore cultures

To obtain pure single spore isolates, the ten-day-old fungal colonies were soaked in 5 ml sterilised water and carefully spread with a sterile L-shaped plastic rod. The fungal suspension was sieved into sterile 30 ml universal plastic tubes using a sterile muslin cloth, to remove mycelia. From each isolate, 10μl of spore suspension was placed into each of the four haemocytometer (Marienfeld, German) chambers (A, B, C and D). The spores in each chamber were counted and the average number of spores per isolate was calculated. The spores per ml was calculated using the formula of Gilchrist-Saavedra *et al*. (2006):

$$\text{Spores ml}^{-1} = (\text{n})\ x\ 10^4$$

Where: n = the average number of spores counted.

The spore suspension concentration was adjusted to the desired concentration of 1.0×10^5 spores ml^{-1} using sterilised water. One millilitre of 1.0×10^5 spores ml^{-1} was drawn from the spore suspension, then spread on water agar using a sterile plastic L-shaped plastic rod. The plates were left open for 20 minutes under laminar flow hood to allow drying. They were then sealed with Para-film® and incubated at 23 ± 3 °C for 24 hours. A single strand of one day old mycelia observed under a microscope was cut at the edge and placed on PDA covered with small pieces of sterile filter papers (Whatman medium fast qualitative circle 90 mm). This was incubated at room temperature (23 ± 3 °C) under natural light for seven days. Seven-day old isolates grown on pieces of sterile filter papers were peeled off, placed in clean Petri dishes and dried in silicon dioxide (SiO2) (silica gel self-indicating-course blue) for seven days (Fong *et al*.,2000). Fifty dried single spore isolates on sterile filter papers were placed in small brown envelopes and stored at 4 °C in a refrigerator.

2.4 Fungal isolates pathogenicity evaluation on bulb onions

Fifty preserved single spore isolates obtained from bulb onion samples were evaluated for pathogenicity *in vitro*. Three millimetre of filter paper containing pure preserved fungal isolates were placed on PDA and incubated for 10 days at room temperature (23 ± 3 °C) under natural light. Spores were obtained by flooding the colonies with two millilitre gelatin solution (0.2 %) and spread carefully using a sterile L-shaped plastic rod. Using a muslin cloth the fungal suspension was sieved in 30 ml plastic universal tube to remove mycelia. The spore suspension concentration was calculated as previously described and then adjusted to 1.0×10^5 spores ml^{-1} using sterile water.

Using a sterile cork-borer, a wound of about 5 mm in diameter and 3 mm deep was punctured in healthy bulb onions after surface sterilization with 70 % ethanol. The punctured bulb onions were inoculated with 40 μl of 1.0×10^5 spores ml^{-1} suspension. Each isolate was inoculated in six bulb onions and another six bulb onions inoculated with 2 % gelatin solution were used as control. An experiment of three bulb onions per replicate in two replications for each isolate was set in a completely randomized design (CRD). After 21 days of storage at room temperatures (23 ± 3 °C), the infected bulb onions were dissected with a sterile scalpel blade through the point of inoculation. Rotting symptoms (where present) were compared to those of bulb onions originally collected from markets. One three-millimetre bulb onion segment was cut from rotten lesion, plated on PDA and incubated at room temperature (23 ± 3 °C) for seven days. Fungal colony colour of re-isolated fungi were noted and compared to the original fungal isolate.

2.5 Virulence evaluation of pathogenic fungal isolates on bulb onions

Eighteen pathogenic fungal isolates were assessed for their virulence on healthy bulb onions purchased from a

commercial farm in Kajiado County. Spore suspension of the isolates was prepared as previously described. Using a sterile cork-borer a wound of about 5 mm in diameter and 3 mm deep was punctured in healthy bulb onions. Forty microliter of 1.0×10^5 spores ml^{-1} suspension was inoculated in the wound. Nine bulb onions per isolate were inoculated while nine bulb onions were inoculated with 2 % gelatin solution and used as control. Three replications experiment with three inoculated onions bulbs per isolate for each replication was set in CRD. The inoculated bulb onions and control samples were randomly placed on trays and stored at room temperatures (23 ± 3 °C) for 21 days. Using a sterile blade the bulbs were dissected into half and from the point of inoculation the size of the lesions was measured using a ruler.

2.6 Fungal pathogen morphological identification

2.6.1 Assessment of fungal isolates colony radial growth rate

Colony radial growth rate was determined by tabulating Radial Growth Rate (RGR) for each isolate according to Miyashira *et al.* (2010) and Pal *et al.*, (2019)) with slight modification. Eighteen isolates found to be pathogenic were grown on PDA at room temperature (23 ± 3 °C) in triplicates. From the point of initial inoculum, two diameter readings perpendicular to each other (Plate 1) were recorded from the underside of the petri-dish on day four after inoculation.

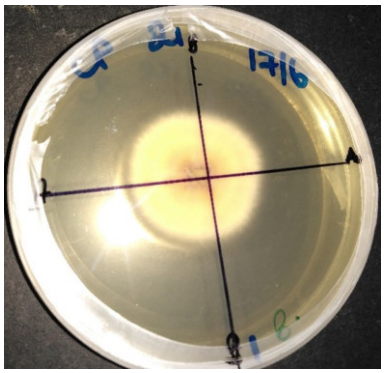

Plate 1: *Perpendicular lines A and B on the underside of the petri-dish indicating where the diameter of the fungal colony was measured.*

Colony radial growth rate (cm/day) of each isolate was calculated using the formula below:

$$\text{RGR (cm/day)} = (D/2)/\ d$$

where: RGR is Radial Growth Rate per day (cm/day), D = Average colony diameter, and d = Number of days incubated.

2.6.2 Colony and conidia description

For morphological identification, eighteen pathogenic isolates were grown on Carnation Leaf-Piece Agar (CLA) for 10 days at room temperature (23 ± 3 °C). Carnation Leaf-Piece Agar was prepared by placing sterile carnation leaf pieces into a petri dish and adding 2 % water agar (20 g agar in 1 L of water). Culture characteristics of each isolate were identified by describing their colony appearance and pigmentation and conidia structures (Duduk *et al.*, 2017; Manoj *et al.*, 2016). Microscopic photos were taken using Olympus BX51 powered microscope with DIC Nomarski view and a DP72 digital camera, Japan. In addition, 10 micro and macro-conidia of each isolate were randomly selected, and their sizes measured from stained slides using the microscope.

2.7 Molecular identification of pathogenic fungal isolates

2.7.1 Fungal isolates DNA extraction

DNA extraction was done on 18 pathogenic isolates as described by Aamir (2018) and Cenis (1992). Five-day old culture mycelia of each isolate grown on PDA were scraped using a sterile scalpel and placed in sterilised Eppendorf micro-centrifuge tubes containing ceramic bead after which 500 μl of extraction buffer (1M Tris-HCL, 5M NaCl, 0.5M EDTA, 0.5 % SDS) was added and the mixture placed in faststep® −24 genogrinder at 4 $m\,s^{-1}$ for 1 minute to grind the mycelia. A 200 μl of 3M sodium acetate pH 5.5 was added and the samples kept at −20 °C for 10 minutes and then centrifuged at 13,000 rpm for five minutes. About 70 μl supernatant were pipetted into sterilised tubes and an equal amount of iso-propanol was added and left at room temperature (23 ± 3 °C) for five minutes. The samples were centrifuged at 13,000 rpm for 10 minutes and the supernatant was poured out to obtain the DNA pellets. A 500 μl of 70 % ethanol was added to wash the DNA pellets and centrifuged at 13,000 rpm for five minutes. Ethanol as supernatant was decanted and the samples were air dried under the laminar flow hood for 30 minutes. A 40 μl MH2O/TE was added to resuspend the DNA pellets and left at room temperature for 30 minutes. The quality of DNA was assessed using a Thermo scientific Nano Drop 2000c spectrophotometer, USA.

2.7.2 Fungal isolates DNA amplification

Amplification of the translation elongation factor (TEF) 1α gene and internal transcribed space (ITS) regions was done using primer pair of ef1 (5'ATGGGTAAGGA(A/G)GACAAGAC-3' and ef2 (5'GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') (Chehri *et al.*, 2012), and ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (White *et*

al., 1990) respectively. ITS are the universal fungi primers while ef1 primers are *Fusarium* species-specific (Kalman *et al*., 2020). DNA amplification was carried out according to Karlsson *et al*. (2016) with slight modification. It was done using GeneAMP 9700 DNA Thermal Cycler (Perkin-Elmaer) with a reaction volume of 20 μl containing: 10 μl of one-taq quick load 2X master mix with standard buffer, 0.4 μl of 10 μM of each primer, 2 μl of 50 ng ml^{-1} template DNA and 7.2 μl nuclease free water. The amplification process involved initial denaturing step at 94 °C for 30 s, followed by 35 cycles at 94 °C for 30 s and annealing process for ITS4 and ITS5 primers at 56 for 45 s while ef1 and ef2 primers were done at 60 °C for 45 s. Extension was done at 68 °C for 1 min and the final extension at 68 °C for 5 min for both primer pairs. The quality of PCR products was confirmed on 1.5 % agarose gel and visualised under UV light using EDURO™ GDS, UK. The amplified rDNA was submitted for Sangar sequencing with ef1 and ef2 forward and reverse primers at Inqaba Africa Genomic platform, South Africa. The 18 obtained sequences were subjected to Basic Local Alignment Search Tool (BLAST) in *Fusarium*-ID database to enable identification of eighteen pathogenic isolates. The 18 sequences (Accession Numbers OL631163 to OL631180) were submitted to National Centre for Biotechnology Information (NCBI) databases.

2.8 Statistical data analysis

Mean of colony radial growth rate and lesion size were calculated and then subjected to one-way ANOVA using Genstat program 15th edition. The means were separated using Tukey's test at 5 % probability level. The mean spore size for each isolate was calculated using Microsoft excel program.

3 Results

3.1 Isolation of fungal microorganism and pathogenicity assessment

Out of 524 bulb onion samples collected from major markets in Bungoma, Kajiado and Meru Counties, 300 bulb onion developed symptoms that were clearly visible in inner scales (Plate 2). They included 8 % bulb onions from Kitengela market, 12 % from Kajiado market, and 17 % from Meru market, 13 % from Nkubu market, 25 % from Chwele market, 16 % from Cheptais market and 9 % from Kimilili market. A total of fifty fungal isolates were obtained from 300 diseased bulb onions. Out of fifty fungal microorganisms inoculated in healthy bulb onions, eighteen isolates caused symptoms similar to those originally observed from

Plate 2: *Onion bulb from Bungoma County showing symptoms of rot in the inner scales.*

infected bulb onion collected from different markets. The symptoms included brown discolorations extending to the neck or base in the inner scales of the bulb onion (Plate 3) and were identified to satisfy Koch's postulates. Disease symptoms were not observed in control treatments. The re-isolated fungal isolates showed similar colony characteristics on PDA as observed for original fungi isolated from bulb onions collected from different markets. Out of the eight-

Plate 3: *Bulb onion indicating rot symptoms after inoculating with a fungal isolate and incubated at room temperature (23 ± 3 °C) for 21 days.*

een pathogenic isolates 67 % were obtained from Bungoma County samples, 22 % from Meru County and 11 % from Kajiado County.

3.2 Evaluation of virulence on pathogenic fungal isolates

The eighteen isolates inoculated in bulb onion caused mean lesion size ranging from 0.4 ± 0.1 to 2.6 ± 0.5 cm (Fig. 2). KTA isolate caused the largest mean lesion size (2.6 ± 0.5 cm) while KM26 caused the smallest (0.4 ± 0.1 cm) lesions on bulb onion. Mean lesion size (2.6 ± 0.5 cm) formed by KTA isolate was significantly ($P < 0.001$)) larger compared to lesions caused by KTB (1.1 ± 0.23 cm), NK (1.0 ± 0.36 cm), CP110 (1.0 ± 0.19 cm), CP19 (0.8 ± 0.23 cm), CL110 (1.0 ± 0.12 cm), CP12 (0.8 ± 0.19 cm) and KM26 (0.4 ± 0.10 cm) on bulb onions. Fungal isolate KM26 caused significantly ($P < 0.001$) smaller lesion size (0.4 ± 0.1cm) compared to lesions caused by CL14 (1.5 ± 0.13 cm), CPF2 (1.9 ± 0.33 cm),

FMR2 (1.9 ± 0.03 cm), MR31 (2.0 ± 0.19 cm), FMR1 (2.2 ± 0.08 cm) and KTA (2.6 ± 0.53 cm) isolates on bulb onion. Fungal isolates; FMR1 (2.2 ± 0.08 cm), MR31 (2.0 ± 0.19 cm), FMR2 (1.9 ± 0.03 cm) and NK (1.0 ± 0.36 cm) collected from Meru County developed lesions on bulb onions that were not significantly ($P \geq 0.05$) different from each other in size. Among the isolates collected from Bungoma County, CPF2 isolate formed significantly ($P < 0.001$) larger lesion (1.9 ± 0.3 cm) compared to lesion (0.4 ± 0.1 cm) produced by KM26 isolate collected from the same County. In addition, CPF2 isolate caused lesion that was not significantly different ($P \geq 0.05$) in size compared to those formed by CL41 (1.5 ± 0.13cm), CL13 (1.3 ± 0.20 cm), KM29 (1.3 ± 0.42 cm), KM28 (1.3 ± 0.11 cm), CP17 (1.2 ± 0.41 cm), CP21 (1.2 ± 0.31 cm), CP110 (1.0 ± 0.19 cm), CP19 (0.8 ± 0.23 cm), CL110 (0.8 ± 0.12 cm) and CP12 (0.8 ± 0.19 cm), isolates collected from the same County of Bungoma. KTA fungal isolate obtained from Kajiado County caused statistically ($P < 0.001$)) larger lesion (2.6 ± 0.53) compared to lesion (1.1 ± 0.23 cm) caused by KTB isolate from the same County.

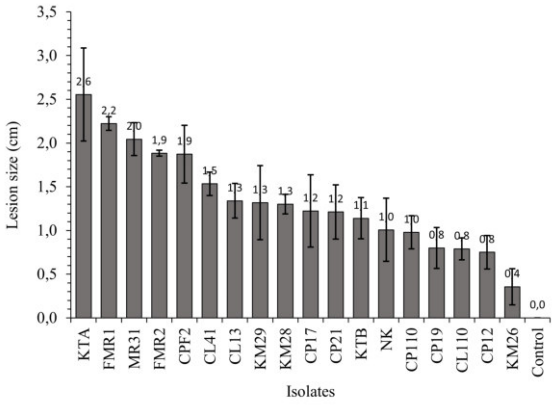

Fig. 2: *Mean lesion size (cm) on bulb onion 21 days after inoculation with different fungal isolates. Error bars represents standard error (SE) of the mean.*
The isolates codes relate to the market where bulb onion samples were collected; KJ (Kajiado market), KT (Kitengela market), MR (Meru Market), NK (Nkubu market), KM (Kimilili market), CP (Cheptais market) and CL (Chwele markets).

3.3 Fungal isolates cultural and morphological characteristics

3.3.1 Fungal colony growth rate evaluation

Colony radial growth rate for the eighteen fungal pathogenic isolates ranged from 0.6 ± 0.078 to 1.0 ± 0.004 cm/day. An isolate collected from Meru market (FMR2) had the highest colony growth rate (1.0 ± 0.004 cm/day) while KTB isolate collected from Kajiado market had the least colony growth rate of 0.6 ± 0.078 cm/day (Fig. 3). FMR2 isolate grew significantly ($P < 0.001$) faster (1.0 ± 0.004 cm/day) compared to FMR1 (0.6 ± 0.005 cm/day), CP110 (0.8 ± 0.002 cm/day), KTA (0.8 ± 0.044 cm/day), CL13 (0.8 ± 0.046 cm/day), CP19 (0.7 ± 0.108 cm/day), KM26 (0.7 ± 0.057 cm/day), CP17(0.7 ± 0.073 cm/day), CP21(0.7 ± 0.068 cm/day), CL110 (0.7 ± 0.022 cm/day) and KTB (0.6 ± 0.078 cm/day), isolates (Fig. 3). Though FMR2 had the highest colony growth rate among the isolates (1.0 ± 0.004 cm/day), the growth rate was not significantly ($P \geq 0.05$) different compared to that of CL41 (0.9 ± 0.017 cm/day), NK (0.9 ± 0.019 cm/day), KM28 (0.9 ± 0.045 cm/day), MR31 (0.9 ± 0.031 cm/day), CP12 (0.9 ± 0.047 cm/day), CPF2 (0.9 ± 0.072 cm/day) and KM29 (0.9 ± 0.042 cm/day) isolates. In addition, KTB isolate had the lowest colony growth rate (0.6 ± 0.08 cm/day) but it was not statistically ($P \geq 0.05$) different to growth rates of CL110 (0.7 ± 0.022 cm/day), CP21 (0.7 ± 0.068 cm/day), CP17 (0.7 ± 0.073 cm/day), KM26 (0.7 ± 0.057 cm/day), CP19 (0.7 ± 0.108 cm/day), CL13 (0.8 ± 0.046 cm/day) and KTA (0.8 ± 0.044 cm/day), fungal isolates (Fig. 3). Colony growth rate of FMR2 (1.0 ± 0.004 cm/day), NK (0.9 ± 0.02 cm/day) and MR31 (0.9 ± 0.03 cm/day) isolates obtained from Meru County were not significantly different ($P \geq 0.05$) from each other. However, FMR2 (1.0 ± 0.004 cm/day) grew significantly ($P < 0.001$) faster compared to FMR1 (0.8 ± 0.005 cm/day) isolate obtained from the same County of Meru. Isolates from Kajiado County; KTA (0.8 ± 0.044 cm/day) and KTB (0.6 ± 0.08 cm/day) exhibited growth rate that were not statistically ($P \geq 0.05$) different from each other. Among the isolates from Bungoma County, CL41 isolate exhibited statistically ($P < 0.001$) higher colony growth rate (1.0 ± 0.004 cm/day) compared to CP110 (0.8 ± 0.002 cm/day), CL13 (0.8 ± 0.046 cm/day), CP19 (0.7 ± 0.108 cm/day), KM26 (0.7 ± 0.057 cm/day), CP17 (0.7 ± 0.073cm/day), CP21 (0.7 ± 0.068 cm/day) and CL110 (0.7 ± 0.022 cm/day) isolates from the same County of Bungoma. However, CL41 colony growth rate (0.9 ± 0.017 cm/day) was not statistically ($P \geq 0.05$) different from that of KM28 (0.9 ± 0.045 cm/day), CP12 (0.9 ± 0.045 cm/day), CPF2 (0.9 ± 0.072 cm/day), KM29 (0.9 ± 0.042 cm/day) fungal isolates collected from the same Bungoma County.

3.3.2 Colony and conidia characteristics of fungal isolates

The eighteen isolates showed varied characteristics on the upper side of the colony that included white dense to sparse aerial mycelia. The pigmentation on PDA varied from white, violet, brown while on CLA conidia were categor-

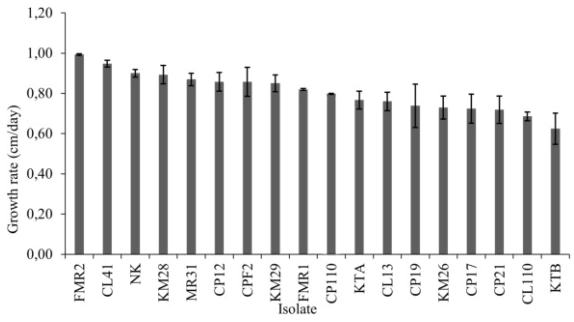

Fig. 3: *Colony growth rate (cm/day) of pathogenic fungal isolates obtained from bulb onions collected from different markets. The isolate codes relates to markets where bulb onions were collected, see Fig. 2 for explanation.*

ized as oval, straight, and curved in shape and were either aseptate or septate. The length of microconidia ranged from $0.50 \pm 0.41\,\mu$m to $0.84 \pm 0.61\,\mu$m while that of macroconidia was between $19.48 \pm 1.43\,\mu$m to $34.60 \pm 1.57\,\mu$m (Annex 2). Consequently, based on colony and conidial features the isolates were grouped into three clusters as follows; cluster one (CL13, CPF2, CP17, CL41, CP12, NK, KM29, KM28, CP110 and CP21), cluster two (KM26, FMR2, MR31, FMR1 and CL110) and cluster three (CP19, KTB and KTA). On PDA, cluster one isolates developed colonies with dense white cottony mycelia on the upper side and on reverse side there was brown colour at the centre of the colony (Plate 4). On CLA, macroconidia were observed which had falcate to almost straight shape, and pointed at the end with three to four septa (Plate 5). They were short to moderate in length ranging from 27.8 ± 2.72 to $34.7 \pm 1.82\,\mu$m. Cluster one isolates formed microconidia that were oval or kidney shaped (Plate 5).

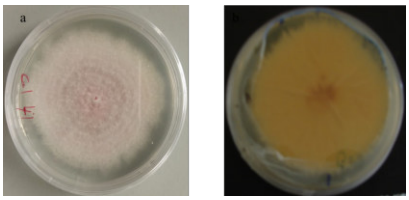

Plate 4: *Representative colony of cluster one isolates; (a) Top side and (b) Underside.*

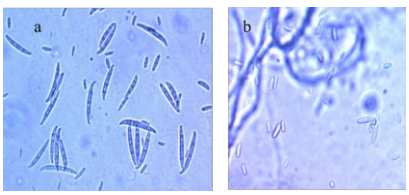

Plate 5: *Spores of cluster one isolates; (a) Macroconidia and (b) Microconidia.*

Cluster two isolates formed sparsely white colony on the upper side while on reverse side the colony was white with pale yellow pigmentation spreading throughout the colony area. On CLA, macroconidia were sparsely produced, had bent apical and were foot shaped with three septa. The cluster two isolates had numerous microconidia that were oval in shape and aseptate (Plate 6). On PDA, cluster three

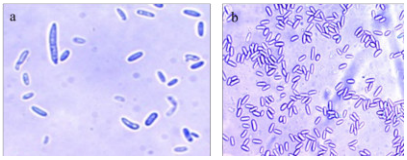

Plate 6: *Fungal spores of cluster two isolates; (a) Macroconidia and (b) Microconidia.*

fungal isolates formed white to violet, sparse floccose mycelia on the colony upper side and the reverse side had violet pigmentation (Plate 7). On CLA, the cluster three isolates produced long (28.1 ± 2.13 to $34.6 \pm 1.57\,\mu$m) macroconidia that were slightly curved with relatively wide three to five septate (Plate 8). Microconidia were formed in false heads on long monophialides, oval in shape and two septate.

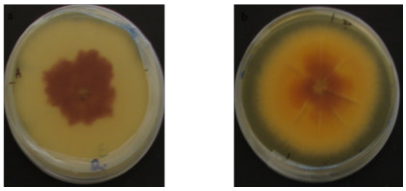

Plate 7: *Representative colony of cluster three isolates (a) Top side and (b) Underside.*

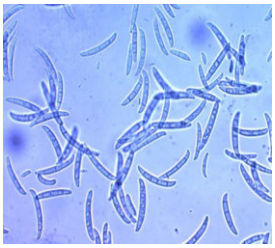

Plate 8: *Macroconidia spores of cluster three fungal isolates.*

3.4 Molecular identification of pathogenic isolates from bulb onion

Molecular identification technique was used to confirm pathogenic isolates described using morphological characterisation. Three *Fusarium* species were identified and clustered as follows: cluster one; *F. oxysporum* (OL631180, OL631166, OL631165, OL631169, OL631174, OL631175,

OL631173, OL631170, OL631179, and OL631167), cluster two; *F. acutatum* (OL631178, OL631171, OL631172, OL631164 and OL631177) and cluster three; *F. solani* (OL631176, OL631163 and OL631168) (Fig. 4).

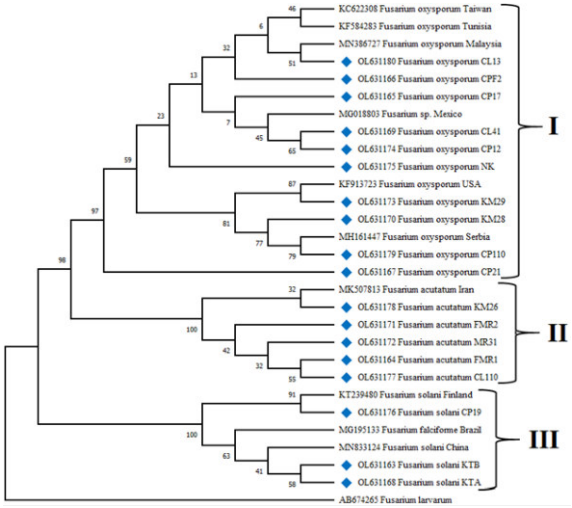

Fig. 4: *Phylogenetic tree of pathogenic Fusarium species isolated from bulb onions collected from different markets.*

The percentage query coverage aligned to isolates sequences from this study and those obtained from the *Fusarium*_ID Gene bank had a range of 99 to 100 percent (Table 1). Fifty five percent of isolates were *F. oxysporum*, 28 % were *F. acutatum*, while *F. solani* were 17 %. *Fusarium* solani was mainly isolated from Kajiado County, *F. acutatum* from Meru while *F. oxysporum* was predominately from Bungoma County.

4 Discussion

Rot caused about 14 % losses during bulb onions postharvest handling in Kenya (Gathambiri *et al*., 2021),though fungal pathogens causing the decay have not been identified. However, globally some fungal genera are associated with bulb onion rot, namely Aspergillus, Penicillium, Alternaria, *Fusarium*, Rhizopus, Colletotrichum, and Botrytis (Yurgel *et al*., 2018). To successfully control postharvest rot in bulb onions, it is important to identify the pathogens contributing to the rot (Klokocar-Smit *et al*., 2008).

Out of 300 diseased bulb onion samples collected from various markets, fifty fungal isolates were obtained hence indicating that some of the diseased bulb onion samples could have been infected with other microorganisms. As Ghanbarzadeh *et al*. (2014) indicated postharvest bulb onion rot might be caused by various fungal and bacterial pathogens.

From this study eighteen fungal isolates developed similar symptoms to those observed in diseased bulb onions collected from study sites of Bungoma, Kajiado and Meru Counties, therefore the pathogens were found to cause bulb onions postharvest rot. Consequently, fungal pathogens had been reported to cause postharvest rot in tomato and avocado fruits in Kenya (Mugao and Birgen, 2021; Wanjiku *et al*., 2020). However, this is the first report which indicated that fungal pathogens contribute to bulb onion postharvest rot in Kenya. Higher number of pathogenic isolates were obtained from Bungoma County compared to Meru and Kajiado Counties, though Kajiado County leads in production levels (HCD, 2020). Postharvest bulb onions rot caused by fungi is more dominant in Bungoma County compared to Meru and Kajiado Counties. According to Tischner *et al*. (2022) climatic conditions have an effect on fungal pathogens prevalence and distributions and in addition postharvest practices employed by bulb onion value chain actors affect occurrence of postharvest rot (Gathambiri *et al*., 2021).The pathogenic fungal isolates were able to cause rotting in bulb onions 21 days after inoculation at room temperature (23 ± 3 °C). Similar results were reported by Bektast & Kusek (2019) who indicated that *F. oxysporum* f.sp. cepae inoculated in bulb onion developed symptoms 21 days after inoculation at 24 ± 3 °C.

The eighteen pathogenic isolates were grouped into three clusters based on colony and conidia characteristics. The colony and conidia characteristics described in this study for the three clusters defined *Fusarium* spp.as described by Keith, (1996). In addition, the results corroborates with other researchers who observed similar colony and conidia features; Wanjiku *et al*. (2020) described cluster one isolates' features, Chehri *et al*. (2015) observed cluster two isolates and Kalman *et al*.(2020) stated cluster three isolates. Consequently, molecular identification method confirmed the three *Fusarium* spp. clusters which were identified as *F. oxysporum* (cluster one), *F. acutatum* cluster two) and *F. solani* (cluster three). Therefore, *F. oxysporum* f.sp. *cepae*, *F. acutatum* and *F. solani* were identified as fungal causative agents of postharvest rot in bulb onion in Bungoma, Meru and Kajiado Counties of Kenya. Majority of pathogenic fungal isolates (55 %) obtained during this study were identified as *F. oxysporum* f.sp. *cepae*. Thus bulb onion postharvest rot in major growing regions of Kenya is mainly caused by *Fusarium oxysporum* f.sp. *cepae*. In addition, from the results it was observed that *F. oxysporum* f.sp. *cepae* was mainly isolated from Bungoma, *F. solani* from Kajiado and *F. acutatum* from Meru, this indicated that factors such as soil and environmental conditions and plant host may affect the distribution of fungal pathogens (Le *et al*., 2021).

Eighteen pathogenic fungal isolates obtained from study sites of Bungoma, Meru and Kajiado Counties caused differ-

Table 1: *Molecular identification of Fusarium species isolated from bulb onions collected from different markets.*

			Genebank accession			
isolate code	*Cluster*	*Fusarium spp.*	*number*	*best match*	*query coverage**	*similarity**
CL13	1	*Fusarium oxysporum*	OL631180	MN386727	100	98.34
CPF2	1	*Fusarium oxysporum*	OL631166	CP052043	100	99.02
CP17	1	*Fusarium oxysporum*	OL631165	KP964904	100	92.73
CL41	1	*Fusarium oxysporum*	OL631169	KP964890	100	99.15
CP12	1	*Fusarium oxysporum*	OL631174	KP964890	99	98.88
NK	1	*Fusarium oxysporum*	OL631175	KP964904	99	99.72
KM29	1	*Fusarium oxysporum*	OL631173	MT305189	100	99.12
KM28	1	*Fusarium oxysporum*	OL631170	MH161447	100	98.89
CP110	1	*Fusarium oxysporum*	OL631179	KP964881	100	96.16
CP21	1	*Fusarium oxysporum*	OL631167	MK172059	100	99.15
KM26	2	*Fusarium acutatum*	OL631178	MT010989	100	98.88
FMR2	2	*Fusarium acutatum*	OL631171	MT010989	100	99.02
MR31	2	*Fusarium acutatum*	OL631172	MT01098	100	98.88
FMR1	2	*Fusarium acutatum*	OL631164	MT010989	100	99.16
CL110	2	*Fusarium acutatum*	OL631177	MK507814	100	98.92
CP19	3	*Fusarium solani*	OL631176	KT313615	99	99.05
KTB	3	*Fusarium solani*	OL631163	MN833124	99	99.17
KTA	3	*Fusarium solani*	OL631168	MN833124	100	99.04

* in percentage. The samples were coded based on which market they were collected; KJ (Kajiado market), KT (Kitengela market), MR (Meru Market), NK (Nkubu market), KM (Kimilili market), CP (Cheptais market) and CL (Chwele markets)

ent lesion sizes after inoculation in bulb onions. The study findings indicated that *Fusarium* solani was most virulent, followed by *Fusarium* acutatum and *Fusarium oxysporum* f.sp. *cepae*. The results did not corroborate with Ghanbarzadeh *et al*. (2014) who reported that *Fusarium oxysporum* f.sp. *cepae* was more virulent than *F. solani* on bulb onion. However, the results of this study were in agreement with Kalman *et al*.(2020) who reported that *Fusarium* acutatum was more virulent compared to *F. oxysporum* f.sp. *cepae* on bulb onion. In addition, results showed that *Fusarium* acutatum had higher colony growth rate compared to *Fusarium oxysporum* f.sp. *cepae*, this could be an indication that colony growth rate and virulence of fungal isolates may have a positive correlation.

The isolated *Fusarium* species in this study caused *Fusarium* basal rot (FBR) disease in bulb onions (Le *et al*., 2021). The disease can be caused by single *Fusarium* spp.; *F. falciforme* (Tirado-Ramírez *et al*., 2018) or a complex of different *Fusarium* spp.; *F. solani*, *F. acuminatum*, *F. oxysporum*, *F. verticilliodes and F. proliferatum* (Delgado-Ortiz *et al*., 2016). *Fusarium* basal rot has been reported in Asia to contribute about 30 to 40 % bulb onion loss during storage (Gupta and Gupta, 2013). The infection starts in the field where the symptoms include damping-off, stunting, chlorotic leaves, roots, bulb discoloration and eventually death of the roots. However, the infected onions do not always exhibit external disease symptoms in the field, the infection remains latent until storage where the signs of rotting are observed thus causing postharvest rotting (Lager, 2011). Postharvest rots occur when the infection happens late in the season during bulb onion growing (Le *et al*., 2021). Therefore, FBR disease may contribute to postharvest loss of bulb onion during postharvest handling in Kenya.

5 Conclusion

This study indicates that fungal pathogens associated with postharvest rots in major growing regions of Bungoma, Kajiado and Meru Counties of Kenya were *Fusarium* species namely; *Fusarium oxysporum* f.sp. *cepae*, *Fusarium solani* and *Fusarium acutatum*. The isolated *Fusarium* species were mainly region specific, thus future studies should be done on effect of geographical variations in bulb onion postharvest *Fusarium* rot. *Fusarium solani* was the most virulent among the three pathogens, however *F. oxysporum* f.sp. *cepae* was frequently isolated. All the three *Fusarium* species isolated are associated with FBR which is an economically important disease of bulb onion postharvest rot. This is the first report of *Fusarium* spp. which contributes to bulb onion postharvest loss in Kenya. Pre- and postharvest management practices such as use of pesticides, biological methods and curing of bulb onions can be employed to manage postharvest rot caused by *Fusarium oxysporum* f.sp. *cepae*, *Fusarium solani* and *Fusarium acutatum*.

Data availability

All data supporting the conclusion are included in this paper.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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