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Characterization of Pathogenic Fungi Infecting *Citrullus lanatus* in Different Agroecological Regions of Embu County, Kenya

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ABSTRACT

Watermelon (*Citrullus lanatus*) fruit is nutritious and a profitable cash crop. Its production in Kenya however has dropped from 379.36K metric tonnes to 173.70K metric tonnes in the last five years; attributed to pests, diseases and unpredictable climatic conditions. Study done between July and November, 2018 isolated and characterized fungi associated with watermelon determining their diversity and occurence frequency in Embu County, Kenya. Necrotic fruits and leaves (n=160) were sampled from random farms (n=16). Fungi isolated and cultured on PDA.; characterized by morphological and molecular method. ANOVA was used to detect fungal prevalence differences among sites. *Fusarium oxysporum* was most prevelent with 73% frequency, *Aspergillus niger* (32%). *Penicilliun crustosum* and *Fusarium brachygibbosum* at 31% each. Least prevalent was *Trichoderma asperellum* (1%). Significant differences (F= 23.365, p=0.05, df=13), were observed among agroecological sites except for *Fusarium oxysporium* and *F. brachygibbosum*. Majority of fungi identified were of significant economic importance.

Keywords: *Citrullus lanatus, Pathogenic Fungi, Disease symptoms*

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a flowering vine-like plant in the order Violales of family Cucurbitaceae. Its centre of origin has been traced to both the Kalahari and Sahara deserts in Africa (Ufoegbune *et. al*., 2014) and these areas have been regarded as point of diversification to other parts of the world (Adekanye and Adelakun, 2017). It is a traditional food plant in Africa (Sousa and Raizada, 2020) with potential to improve nutrition, boost food security, foster rural development and support sustainable land use (Dube *et. al.,* 2020). There are over 1,200 varieties of watermelon worldwide, with varieties such as sukari F1, Zuri F1, sugarbaby, charleston grey and crimson sweet among others, recommended for Kenyan climate range (Ufoegbune *et. al*., 2014; Gichamu *et. al*., 2010). The watermelon fruit is composed of 93% water, trace amount of minerals, proteins, fats, carbohydrates, lycopenes and vitamins A and C (Naz *et. al.,* 2014). Its flesh is nutritious and medicinal rich in citrulline which promotes the dilation of blood vessel improving circulation; a source of arginine amino acid, which is a substrate for the synthesis of nitric oxide and is associated with cardiovascular and immune roles in humans. The seed contains phytochemicals such as carbohydrate, phenol, flavonoids, protein, fiber, phosphorus and iron which helps to relieve inflammation, and increases digestive ability of the body system (Manivannan *et. al.,* 2020). Watermelon is not only grown for local consumption, it is the second most important horticultural foreign exchange earner after tea (KNBS, 2019) being exported to other parts of the region, Europe and Middle East. In 2019, Kenya ranked 35th with a share of 0.24% in the global market watermelon production (Tridge, 2020).

Watermelon is a warm seasonal crop whose optimal growth is attained at temperatures above 38°C, with optimum temperature range for germination being 28-32°C. It requires hot dry climate and plenty of sunshine for better growth. The rainy season results in poor growth and continuous rainfall reduces the sugar content in the fruit (Lilly, 2013; Adojutelegan *et. al.,* 2015). In Kenya, watermelons flourish in dry plains and hot

coastal areas such as Machakos, Loitoktok, Kerio Valley, Garissa, Isiolo, Embu, Kirinyaga, Bura, Kitui, and parts of Meru (Greenlife, 2020). Dube *et. al.,* (2020) asserts that, watermelon grow best on well drained, sandy loam soil that is slightly acidic ranging between 6.0-6.8 in pH. When planted in poorly drained soils, the roots develop slowly. Smallholder farmers in the semi-arid eastern Kenya grow watermelon, mostly under rain conditions and to a lesser extent supplemental furrow irrigation (Ufoegbune *et. al.,* 2014).

Watermelon diseases are majorly caused by microorganisms (pathogens) that include fungi, bacteria and viruses (Damicone and Brandenberger, 2020). The most common ones are anthracnose, downy mildew, powdery mildew, damping off, watermelon mosaic, leaf spots, and fusarium wilt, gummy stem blight, bacterial fruit blotch, yellow vine, bacterial rind necrosis, cercospora leaf spot, angular leaf spot, alternaria leaf spot, Phytophthora blight, powdery mildew, downey mildew and viral diseases (Roberts and Kucharek, 2006; Said and Fatiha, 2018).

Diseases are frequent limit to watermelon quality and yield. They occur any time during the crop production cycle and can affect all plant parts from roots to fruit and are most severe when vines are attacked and prematurely killed. In addition, diseases that attack leaves (foliar diseases) reduce fruit quality by exposing melons to sun-scald. Consequently, fruit sweetness and normal ripening are dependent on healthy foliage. When disease affects fruit, they are generally rendered unmarketable and prone to decay, resulting in losses during shipment and transit (Damicone and Brandenberger, 2020)

Fungal blight caused by the *Didymella bryonaie*, damping off disease caused by *Pythium* spp. and *Rhizoctonia sp.* which affects young seedlings, anthracnose disease caused by *Colletotrichum lagenarium*, leaf-spotting caused by *Alternaria cucumerina*, fusarium wilt caused by *Fusarium sp*, and powdery mildew caused by the fungus *Erysiphe cucurbitarum* are some of the known watermelon fungal diseases in Kenya. (Gichimu *et. al.,* 2010; Shankar *et. al.,* 2014). The most common causative agents of fungal diseases in

watermelons worldwide include *Altenaria spp., Colletotrichum spp., Fusarium spp.* and *Cladosporium spp.*, (Kwon *et. al.*, 1999; Zhou & Everts, 2008).

Watermelon fruits are prone to pre harvest and post-harvest diseases caused by contamination with micro-organisms. Fruit rot in watermelon can be caused by a number fungi which includes, *Alternaria alternata, Botrytis cinerea, Choanephora cucurbitarum, Fusarium spp., Lasiodiplodia theobromae, Myrothecium roridum, Penicillium digitatum, Phomopsis cucurbitae, Phytophthora spp., Pythium spp., Rhizoctonia solani, Rhizopus stolonifer, Trichothecium roseum* and other fungi (Sharma *et. al.,* 2016). Symptoms vary depending on environmental conditions and the fungal fruit pathogen(s) present (Robet *et. al.,* 2008). Fruits are more likely to be infected when relative humidity is high or if the moisture is present on fruit surfaces (Zitter *et. al.,* 1996).

Watermelon farming is pivotal in ensuring food security and employment opportunities (Greenlife, 2020). An acre of land produces on average 15,000 fruits, each weighing between 5kg to 12kg or more, depending on the breed (Tuko,2018). Recently, the production of the crop has declined, from 379.36K in 2015, to 277.75K in 2017 to 173.70K metric tonnes in 2019 (Tridge, 2020). The observed decline in the main watermelon growing regions is attributed to unfavourable climatic conditions, pests and diseases (Horticultural Crops Directorate, 2016; Balliu and Sallaku, 2017).

Fungal infections are favored by poor production practices that includes excessive irrigation and nitrogen fertilization, lack of crop rotation and poor soil drainage, all of which can lead to total crop failure (Rahman *et. al.*, 2021; Wang *et.al.,* 2015). Leaf wetness duration, soil water tension and related water variables impact several aspects of plant disease cycle such as sporulation, survival of pathogen propagules, their dispersal to new hosts, germination and infection (Adalberto *et al.,* 2018; Gardening Know, 2020).

Fungi being saprophytic, have specialized mechanism of infection and penetration of the host organism. Phyto-pathogenic fungi have modified hyphae that initially adheres to the cuticle and direct growth of the germ tube on the plant surface. At the site of

penetration, appressoria are often formed to support the penetration process. The penetration hypha accumulates components of the cytoskeleton in the tip and secretes a variety of cell wall–degrading enzymes in a highly regulated fashion in order to penetrate the cuticle and the plant cell wall (Christian *et. al*., 2014; Mendgen *et. al.,* 2003). This study was undertaken to isolate and characterize fungi associated with watermelon and to determine their diversity and occurrence frequency in Embu County, Kenya. The research findings will assist in proper management of the diseases and reduction in the risk of crop failure resulting in economic loses.

MATERIALS AND METHODS

Study Site

A survey was conducted in selected watermelon farms from three agroecological zones that is, Karurumo at the highest altitude of between 1239-1193m ASL, Gachoka between 1208-1187m ASL and the lowest altitude zone, Ishiara at 979-791m ASL, within Embu County in Kenya (Figure 1). Embu County is located on the foothills of Mt. Kenya at an altitude of between 1406 m and 749m above sea level; at a latitude of 0° 31' 52.03" S and longitude of 37° 27' 2.20" E (Google maps, 2018). The climate in Embu is warm and temperate with rainfall throughout the year ranging from 640mm to 1,495 mm per annum with an average of about 1449 mm per year (Kenya Meteorological Department, 2016). It has a mean temperature of 21^{o}C with July being the coldest, up to 12^{o}C and March hitting highest temperatures of 31^{o}C (Embu County, 2019*).*

The County is characterized by highlands and lowlands. It slopes from North-West towards East and South-East with a few isolated hills such as Kiambere, Kianjiru and Kiang'ombe which rise above the general height and slope. It is served by six major rivers which includes Thũci, Tana, Kiĩ, Rũvingasĩ, Thiba and Ĩna river. All these rivers are perennial. The Southern part of the County is covered by Mwea plains (Embu County, 2019*).*

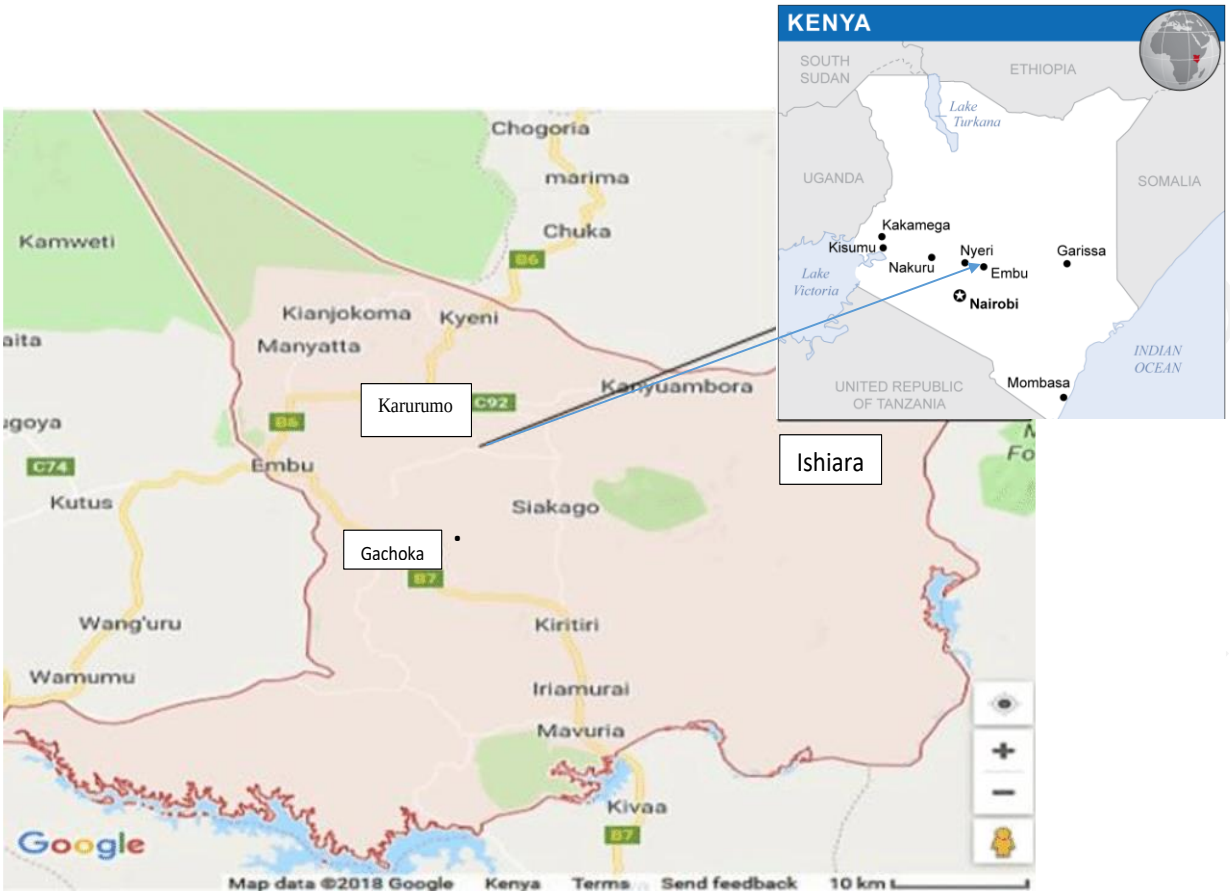


Figure 1: Map showing the Study Sites in Embu County, Kenya

Agriculture is the backbone and livelihood of the people of Embu County. The sector employs 70.1% of the population and 87.9% of the households are engaged in Agricultural activities (Embu County Government, 2018). Other than watermelon, Embu prides itself for cultivation of beans, sorghum, millet, sweet potatoes, irish potatoes and Maize. Tea, coffee and Macadamia nuts are grown in the upper zones of Embu as cash crops. Fruit crops grown in Embu County include: Avocado, bananas, loquat (macuca), tree tomato, passion fruit, mangoes etc. Khat (Muguka) is a common cash crop in the region that is gradually overtaking food crop cultivation (Business Daily, Tuesday Feb 16, 2021). The main types of vegetables grown in the county include cabbage, Kales, onions, carrots, tomatoes as well as indigenous vegetables that include: amaranth and cow peas leaves. Most commercial vegetables are grown under irrigation. Some of the

irrigation schemes already operational in Embu County include: Ena, Makengi, Karaba, Ishiara-Kathigi, Kibugu/Nguviu among others. Small scale group based and individual irrigators operate along the major rivers. In addition to crop farming, most farmers also keep local and exotic dairy cattle, goats, sheep, chicken and pigs (Embu County, 2019).

Survey and Sample collection

Necrotic water melon leaves and fruit samples were collected in the month of July and November 2018 from three ecological zones i.e. Karurumo, Gachoka and Ishiara in Embu County. Farmers were selected randomly based on their involvement in watermelon cultivation within the study sites. Two farms were selected per ecological site. Each farm was divided into plots of 25m by 10m, making a total of 16 plots. Ten samples of symptomatic leaves and fruits, were collected from each of the sixteen plots and transported in organic bags. The age of the water melon since planting was also recorded. The samples were then transported to Chiromo mycology laboratory for analysis.

Isolation of Fungal Pathogens

The blotter method was used to isolate the fungal pathogens from the freshly collected samples of leaves and fruits. Each plant section was washed under running tap water for approximately 1 minute. The surface of the sections were then disinfected in 5% solution of NaOCl for 5 min, rinsed thrice in sterilized water and air dried on a sterile paper towel. Each disinfected section was cut into small pieces of about 1 cm x 2 cm taken from the margins of the lesions and placed on well water-soaked three-layer blotters in Petri-dishes (ten replicates per plot). The samples were put in dark cabinets for 5 days at room temperature to allow the fungi to sporulate. The spores were asceptically inoculated on PDA media treated with 30 mg/l of streptomycin to suppress bacterial growth, and incubated at room temperature for upto 7 days (Mathur and Kongsdal, 2003; Nevalainen et. al., 2014). After the 5th day the media plates with different colonies exhibiting distinct colours were subcultured onto fresh PDA media so as to obtain pure cultures. The inoculated plates were then incubated in an inverted position at 25^{o}C (room temparature)

for 5 to 7 days in dark cabinets with plates sealed with parafilm. Pure colony of each sample were maintained on PDA slants at 4°C.

Identification of Fungi

The fungal isolates were identified using cultural and morphological features which included colony growth pattern, pigmentation and conidial morphology (Tafinta *et al*, 2013) as well as vegetative and reproductive structures (Oyeleke and Manga, 2008). Molecular characterization was done to further characterize the genomic identity of the species. DNA extraction was done through cetyltrimethylammonium bromide (CTAB) protocol (Umesha *et al*.2016) and quantity assessed by gel electrophoresis (Lee *et.al*., 2012) and shown to be of good quality.

The DNA was subjected to polymerase chain reaction (PCR) amplification, and sequencing of the internal transcribed spacers (ITS1–5.8S-ITS2 cluster) regions of the ribosomal DNA gene cluster. The sequences were trimmed, assembled and edited using CLC Main Workbench 8.0.3 and then compared by BLAST with the National Center for Biotechnology Information (NCBI) sequence database deposited in the GenBank (http://www.ncbi.nlm.nih.gov/).

Evolutionary Analysis by Maximum Likelihood Method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura K., 1992). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein , 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model

evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.5705)). This analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 492 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et. al.,* 2018).

Determination of Disease Prevalence

The prevalence of the detected fungi was calculated by counting the number of samples infected by each fungi species over total number of samples tested per location x 100%. The mean prevalence for each fungus was calculated by dividing the sum of values (prevalence) recorded from all locations by the total number of locations.

Statistical analysis

The data were analyzed using STATISTICA program (Stat Soft. Inc., 2007) version 8.0. To test whether there were any significant difference among means in prevalence and diversity of fungi in the three study sites, the data was subjected to one-way analysis of variance (ANOVA). Where data showed a significant difference in means, Turkeys test was applied to show the level of difference. Statistical tests were set at 95% level of confidence.

RESULTS

From the survey of farmers' fields, fungal diseases observed included vine wilt, leaf spots and fruit rot (Fig 2). Watermelon plant samples screened for the presence of pathogenic fungi yielded a variety of fungal species. Figure 4 provides pictures of the results on culture colony appearance and slide technique.

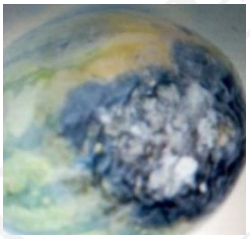
Fusarium wilt (2a) 	Fruit rot (2b) 
Necrotic leaf spot(2c) 	Gray mold fruit rot (2d) 

Figure 2: Disease Symptoms observed in watermelon fruits and leaves

Characterization of pure isolates from the infected watermelon fruits yielded five fungal species: *Penicillium crytosum. Aspergillus niger, Fusarium brachygibbosum, Trichoderma asperellum* and *Fusarium oxysporum*. Shown by cultural and microscopic characteristics (Figure 3) and confirmed through molecular characterization.

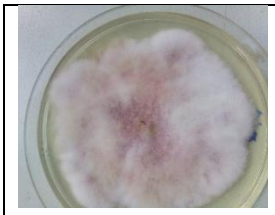
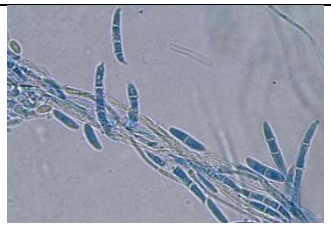
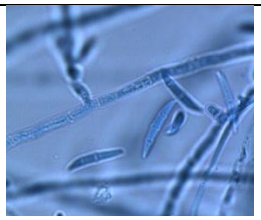
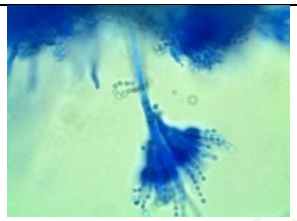
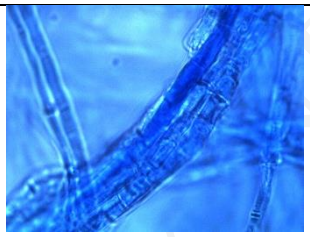
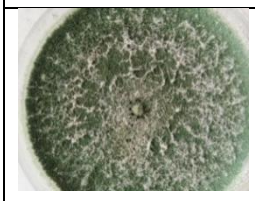
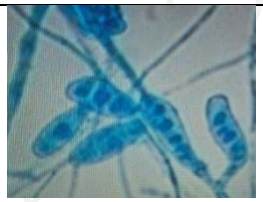
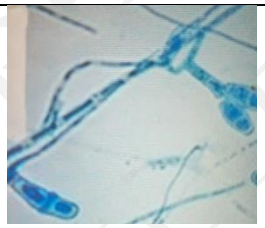
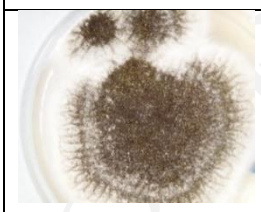
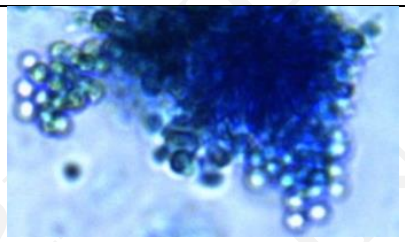
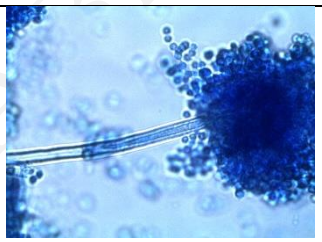
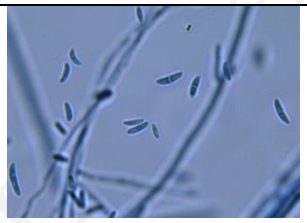
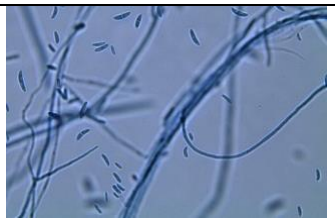
 *Fusarium oxysporium*	 *F. oxysporium* macroconidia	 *F. oxysporium* hyphae
 *Penicillium crustosum*	 *P. crustosum* phallides & spores	 *P.crustosum* septate hyphae
 *Trichoderma asperellum*	 *T. asperellum* spores	 *T. asperellum* hyphae
 *Aspergillus niger*	 *Aspergillus niger* spores	 *Aspergillus niger* hyphae
 *Fusarium brachygibbosum*	 *F. brachygibbosum* sickle spores	 *F. brachygibbosum* hyphae

Figure 3: Colony Pattern on PDA and Microscopy Photography of the Fungal Species Isolated

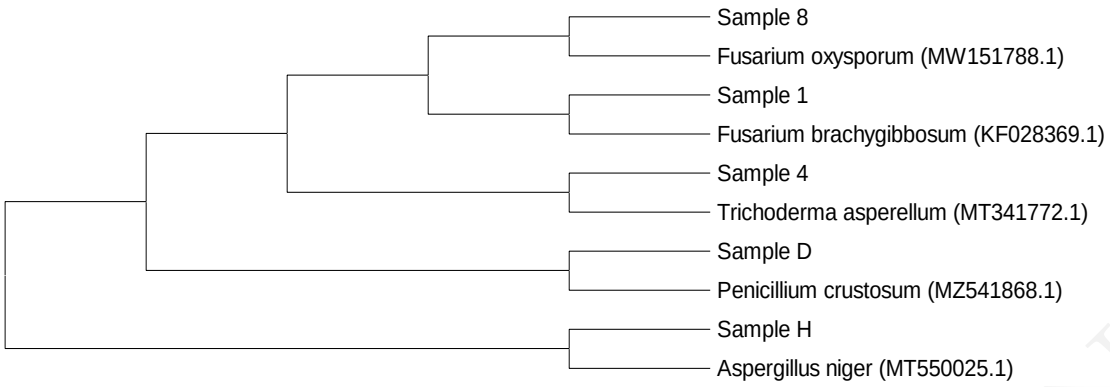


Figure 4. Phylogenetic Tree of Fungal Isolates Originating from *Citrus lanatus* plants using ITS1–5.8S-ITS2 cluster genes

The identities inferred from NCBI Database revealed *Fusarium oxysporum* showed 100% nucleotide similarity with GenBank accessory no. MW151788.1, *Trichoderma asperellum* 100% similarity with GenBank no. MT341772.1, *Fusarium brachygibbosum 100% with no.KF28369.1*, *Aspergillus niger 100% with no* MT550025.1 and *Penicillium crustosum 100%* with no. MZ541868.1.

The three agroecological zones showed variation in the prevalence of each species. In Karurumo zone, *Fusarium oxysporium* had prevalence of 80%, in Gachoka *Fusarium oxysporium* was at 82% whereas Ishiara, *penicillium crustosum* recorded the highest prevalence 77% (Figures 5).

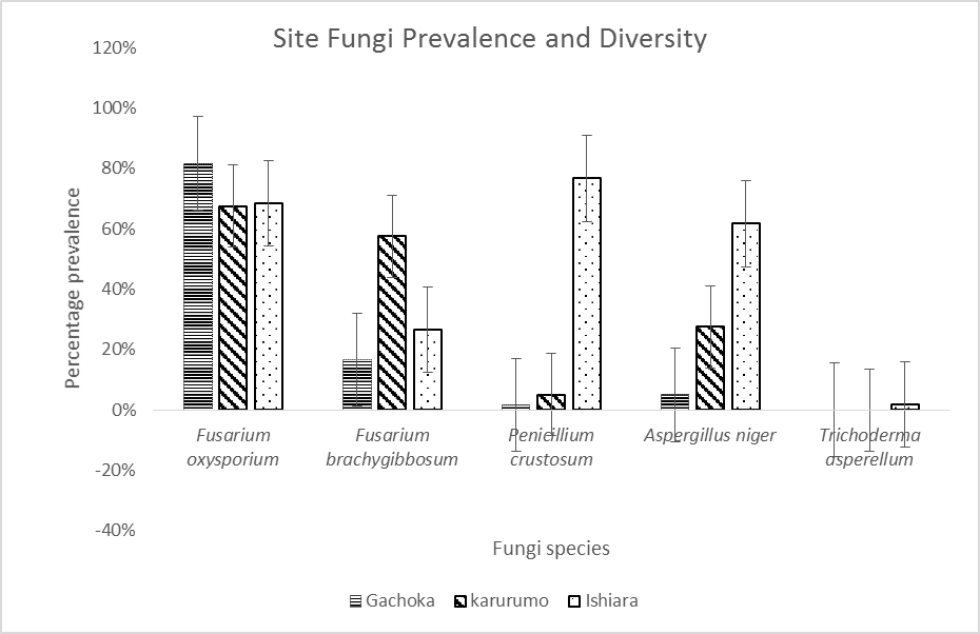


Figure 5: Frequency of Fungal Species Isolated from Watermelon Farms in the Three Agro Ecological Zones

Overall, *Fusarium oxysporium* (73%) recorded the highest mean prevalence, followed by *Aspergillus niger* (32%). *Penicillium crustosum* and *F. Brachygibbosum* that were detected in 31% of the isolates while *Trichoderma asperellum* had 1% prevalence (Figure 6). Significant differences were observed in fungal prevalence among the three agroecological sites ($F= 26.359$, $p= 0.0003$, $df=13$). Significant differences were also observed in *Penicillium crustosum* ($F= 118.61$, $p<0.05$, $df=13$) and *Aspergillus niger* ($F= 23.365$, $p< 0.001$ $df=13$) among the study sites. No significant differences were observed in *Trichoderma asperellum*, *Fusarium oxysporium* and *F. brachygibbosumi* among the sites (in all $p>0.05$, $df=13$).

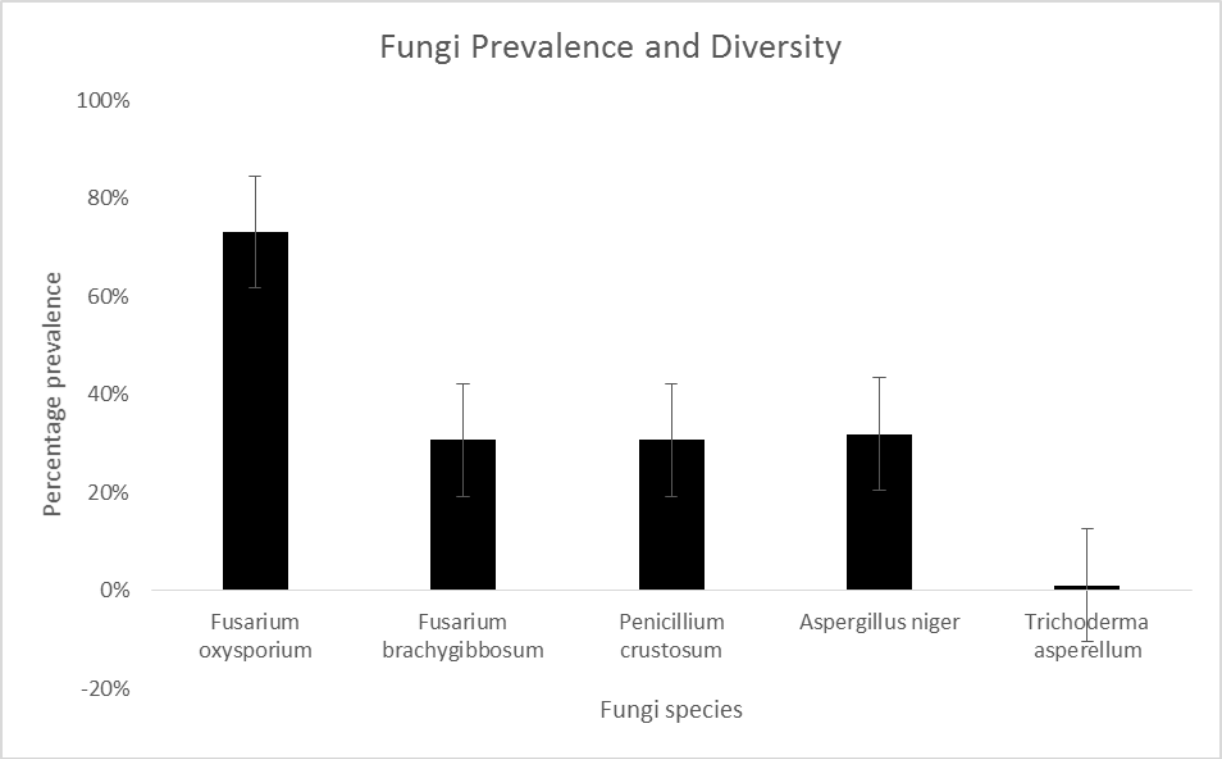


Figure 6: frequency of isolation of fungi from watermelon from Embu County

Discussion

This study identified an array of fungi species from the leaves and fruits of watermelon ranging from *Aspergillus niger, Fusarium oxysporium, Fusarium brachygibbosum*, *Penicillium crustosum,* and *Trichoderma aspellerum*. According to Gichimu *et al., (*2010) and Shankar *et al.,* (2014), fungal blights and fusarium wilts are some of the most common watermelon diseases in Kenya. The most prevalent from the three agro ecological sites was *Fusarium oxysporium* (73%) followed by *Aspergillus niger* at 32%, while *Penicillium crustosum.* and *F. brachygibbosum* were detected in 31% of the isolates. *Trichoderma aspellerum* was the least prevalent at 1%. Other than *Trichoderma aspellerum*, the other species are associated with watermelon diseases suggesting that they could be responsible for crop failure and reduced yields in the region. Similar observations were reported by Nabi *et al*. (2017) and Damicone and Brandenberger (2020), who assert that fungi are important plant

pathogens that cause large economic losses due to their ability to inflict diseases in various plant organs during pre-harvest and postharvest periods.

Fungal species diversity and prevalence in watermelon was analyzed along an agro ecological gradient and the findings showed the presence of vine wilt, necrotic leaf spots and fruit rot in the farmers' fields in the three locations in Embu County. There was no significant difference in fungal diversity among zones. However, there was a significant difference in species prevalence among the three agro ecological zones ($F= 23.365$, $p= 0.05$, $df =13$). According to Orłowska & Muszewska (2022), the ability of different fungal species to survive across several agro ecological zones shows levels of resilience and tolerance of fungi to a wide range of environmental conditions due to their high ecological plasticity. Their difference in prevalence shows the extent to which climatic conditions influence the populations of fungal species. The hotter Ishiara zone characterized by lower rainfall per annum exhibited higher populations of *Penicillium crustosum* and *Aspergillus niger* species compared to the higher *Fusarium oxysporium and Fusarium brachygibbosum*, species in the cooler Karurumo zone and the moderate region of Gachoka. In their study, Dhakar *et. al.* (2013), show that *Penicillium crustosum* can torelate high temperatures of up to 37^{o}C. Thermotorelant *A. niger* also thrives in high temperatures of up to 37^{o}C allowing it to be a more efficient opportunistic pathogen. On the other hand, *Fusarium* species thrives best at moderate temperatures of about 22^{0}C (Kaur *et. al*., 2022).

Fusarium wilt was observed with the following symptoms; stunting of the plant, yellowing of branches near the crown, elogated brown lesions on stem that extended as long, narrow, brown streaks and wilting of the entire plant. The affected area was soft and mushy with white mycelial growth on the affected area. The infections were found at different stages of development although it was most common in older plants, inline with reports from the work of González and others (González *et al*., 2020). Wilted leaves exhibited chlorosis and interveinal necrosis, while the mature infected plants with fruit

loads had collapsed (Fig. 2a). According to Okungbowa (2014), Fusarium species typically produce macro and microconidia, as well as mycelia and chlamydospores that serve as propagules in infecting host plants. Infection is characterized by stunting of the plant, dull, grey green appearance of leaves followed by yellowing of older leaves near the crown advancing outwards. The leaves then quickly wilts (Egel and Matryn, 2007; Park *et al.*, (1996). The fungus invades the vascular tissue through the root system, which causes wilting of the whole plant. According to Wei *et al.*, (1991), the fungus can survive in soil and cause early infection that may result in seedling damping-off. *Fusarium brachygibbosum* is a soil-borne filamentous fungus and a plant pathogen with opportunistic behaviour, characterised by a broad distribution worldwide. This fungus may remain cryptic and asymptomatic within the host and induce symptoms once plants are exposed to physiological stress. Nonetheless, asymptomatic, infected plant material may result in the development of the disease and high losses in production in the field (Marek et al., 2013; Punja et al., 2018). Severity of the disease is dependent on soil moisture and inoculum density (García-Jiménez *et al.,* 1997; Mehl and Epstein, 2007). Disease symptoms include lesions of variable size which are light brown colored at neck and root, causing canker and wilting of leaves or the whole plant (Renteria et. al., 2015)

Aspergillus niger was among the pathogenic fungal species isolated and characterized from different necrotic leaves and fruit samples. The lesions were soft and water soaked, yellowish at first and later covered with sooty black spores. *Aspergillus niger* is the causal pathogen of vine and leaf shrivel of watermelon and causes leaf blight and black mold rot of fruits (Kehinde, 2013). Previous studies have also isolated *Aspergillus* species from necrotic watermelon (Jidda and Adamu, 2017; Tizhe *et al.,* 2019). Bennett and Klich, (2003) found *Aspergillus sp.* to be a common contaminant in agriculture. Existing reports indicate that *A. niger*, produces potent mycotoxins called ochratoxins that can be harmful to human beings and animals when consumed as they are linked to kidney problems in both livestock and human populations (Cabañes *et al.,* 2010; Mailafia *et al.*, 2017). The presence

of *Aspergillus* species in watermelon fruit could also be attributed to the sugary environment in the fruit that favors their growth (Singh and Sharma, 2007). The temperature conditions of the study area might have been favorable to *Aspergillus* species. Dudareva and others (2004) attribute the spoilage of watermelon fruit and others to high temperature that favore fungal growth. This could explain the higher frequencies of *Aspergillus* sampled in Ishiara, a site that was generally warmer compared to the other two.

Penicillium crustosum isolated has been associated with production of *Penicillium* mycotoxin. It causes devastating rot due to pre- and postharvest infection of food crops as well as producing a diverse range of mycotoxins (Samson et al., 2007). *Penisillium crustosum* is a globally distributed foodborne fungus, known for its consistent production of several mycotoxins and other secondary metabolites such as penitrems, roquefortines, viridicatins, and terrestric acids (Sonjak *et al.,* 2007). These mycotoxins can cause serious health hazards that includes; cancerogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic effects as well as Kashin-Beck disease (Puel *et al.,* 2007 and Kehinde, 2013). It is a wound pathogen that uses bruises, punctures or rubs to penetrate in the fruit tissue and spores can live in the soil and organic material [including dead wood] (Yadav *et al.,* 2018). Studies have shown that, *Penicillium* fungi causes' serious damage to crops especially at post-harvest stage. Nevertheless, it can be present in the fruit upon harvest. Therefore, controlling *Penicillium* should start before fruit harvesting to reduce post-harvest decay.

Trichoderma asperellum, was isolated from leaves and fruits of watermelon but in contrast to the rest of the fungi present, it has been reported to be a successful biological control agent against wide range of plant pathogens (Marcello *et. al.,* 2010; Wu *et. al.,* 2017). *Trichoderma spp*. are known to produce a large number of antibiotics including; trichodermin, trichodermol, polyketides, peptaiboils, sesquiterpenes, and steroids. All these active compounds are known to promote plant growth besides having biocontrol

potential against pathogenic fungi (Harman *et. al.,* 2004; Müller *et. al.,* 2013). The genus *Trichoderma* is recognized for its biocontrol function against the fungal phyto-pathogens such as *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotium sclerotiorum*, *Pythium* spp., and *Fusarium* spp. (Contreras-Cornejo *et al.,* 2016; Veenstra *et al.,* 2019). *Trichoderma asperellum* has been identified to antagonize the pathogen *Fusarium oxysporum*, *Corynespora cassiicola*, and *Curvularia aeria* (Baiyee *et. al.,* 2019; Karuppiah *et. al.,* 2019).

Conclusions

Majority of the fungi identified in this study were phytopathogenic ranging from *Aspergillus niger*, *Fusarium brachygibbosum*, *F. oxysporium,* to *Penicillium crustosum* and had a wide distribution within the watermelon farms in the three agroecological zones in Embu County. *Fusarium species* were the most abundant fungi infecting watermelon crops across the region. The significant difference in species richness among the three agroecological zones indicate that climatic conditions have a great impact on the spatial distribution of fungal populations.

Recommendation

Since disease exclusion is the best approach in disease management, regular surveillance and laboratory testing plays a vital role in management of disease as identification of disease causative agent is key to its control. Accurate diagnostics is the best way to ensure that ?farmers we are aware of prevailing crop health status. Disease identification can be critical to choosing effective management options. The watermelon industry needs to remain vigilant and regular in crop surveillance for disease occurrence. Evaluation of disease prevalence in relation to watermelon variety should be augmented as this will go a long way in empowering disease management.

Authors' Contributions

Study was designed by the team led by MG. Sample collection and analysis was done by SK. Manuscript was drafted by SK, editing was done by SSI, SO and SSI reviewed the

manuscript. Financial support was provided by MG. All authors have read and approved the final version of manuscript for publication.

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Competing Interests

The authors declare that they have no conflict of interests.

References

Adalberto, C. Café-Filho, C., Alberto, L. & Maurício, R. (2018). Management of Plant Disease Epidemics with Irrigation Practices, Irrigation in Agroecosystems, Gabrijel Ondrašek, IntechOpen, DOI: 10.5772/intechopen.78253. https://www.intechopen.com/books/irrigation-in-agroecosystems/management-of-plant-disease-epidemics-with-irrigation-practice

Adekanye, T. A. & Adelakun O. J. (2017). Evaluation of a portable watermelon juice extracting machine. Agricultural Engineering International: CIGR Journal, 19(4): 219–223.

Baiyee, B., Ito, S. & Sunpapao, A. (2019). Trichoderma asperellum T1 mediated antifungal activity and induced defense response against leaf spot fungi in lettuce (Lactuca sativa L.). Physiological and Molecular Plant Pathology. 106. 96-101. 10.1016/j.pmpp.2018.12.009.

Balliu, A., & Sallaku, G. (2017). Early production of melon, watermelon and squashes in low tunnels. Good Agricultural Practices for greenhouse vegetable production in the South East European countries (pp. 341-351).

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical microbiology reviews*, *16*(3), 497–516. https://doi.org/10.1128/CMR.16.3.497-516.2003

Cabañes, F., Bragulat, M., & Castellá, G. (2010). Ochratoxin A Producing Species in the Genus Penicillium. Toxins. 2. 1111-20. 10.3390/toxins2051111

Christian, P., Trevor, L., & Louise, G. (2014). Plant Cell Wall-Degrading Enzymes and Their Secretion in Plant-Pathogenic Fungi. Annual Review of Phytopathology 2014 52:1, 427-451.

Contreras-Cornejo, H., Macias-Rodriguez, L., Del-Val, E., & Larsen, J. (2016). Ecological functions of Trichoderma spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiol. Ecol.* 92:fiw036. doi: 10.1093/femsec/fiw036

Damicone, J. & Brandenberge, L., (2020). Watermelon Diseases. Oklahoma State University.

Dhakar, K., Sharma, A., & Pandey, A. (2013). Cold, pH and salt tolerant Penicillium spp. inhabit the high altitude soils in Himalaya, India. World journal of microbiology & biotechnology. 30. 10.1007/s11274-013-1545-4.

Dube, J., Ddamulira, D. & Maphosa, M. (2020): Watermelon production in Africa: challenges and opportunities, International Journal of Vegetable Science, DOI: 10.1080/19315260.2020.1716128

Dudareva, N., Pichersky. E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. Plant Physiology. 135: 1893-1902.

Egel, D. and Martyn, R. (2007). Fusarium wilt of watermelon and other cucurbits. *The Plant Health Instructor.* Department of Botany and Plant Pathology Purdue University, West Lafayette, IN. DOI: 10.1094/PHI-I-2007-0122-01

Embu County (2019). Embu County Integrated Development Plan (CIDP) 2018-2022. Embu County Government. p. 3.

Embu County Government (2018). Agriculture, Livestock, Fisheries & Cooperative Development"

Felsenstein, J. (**1985**). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791.

García-Jiménez, J., Armengol, J., Moya, M., & Júnior, R. (1997). First Report of Fusarium solani f. sp. cucurbitae Race 1 in Spain. Plant Disease - PLANT DIS. 81. 1216-1216. 10.1094/PDIS.1997.81.10.1216A

Gardening Know How: Watermelon Disease Control: How to Treat Diseases of watermelon plant. https://www.gardeningknowhow.com/edible/fruits/watermelon/watermelon-disease-control.htm

Gichimu, B.M., Owuor, B.O. & Dida, M.M. (2010). Comparing the yield components of three most popular commercial watermelon cultivars in Kenya with one newly introduced cultivar and one landrace. Journal of Plant Breeding and Crop Science 1 (4): 65-71.www.academicjournals.org

González, V., Armijos, E. & Garcés-Claver, A. Fungal Endophytes as Biocontrol Agents against the Main Soil-Borne Diseases of Melon and Watermelon in Spain. *Agronomy* **2020**, *10*, 820. https://doi.org/10.3390/agronomy10060820

Google Maps (2018). https://www.google.co.ke

Greenlife. (2020). Retrieved from https://www.greenlife.co.ke.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. & Lorito, M. (2004). Trichoderma speciesopportunistic, avirulent plant symbionts. Nature Reviews Microbiology. 2: 43-56.

Horticultural Crops Directorate (HCD). (2016). *Validation report 2013-2014*. Nairobi: Kenya National Bureau of Statistics.

Jidda, M.B. & Adamu, M.I. (2017). Rot inducing fungi of watermelon (*Citrullus lanatus*) fruits in storage and fruit stalls in Maiduguri, Nigeria. International Journal of Advanced Academic Research – Sciences, Technology and Engineering, 3(5):11-18.

Karuppiah, V., Sun, J., Li, T., Vallikkannu, M. & Chen, J. (2019) Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 Causes Differential Gene Expression and Improvement in the Wheat Growth and Biocontrol Activity. *Front. Microbiol.* 10:1068. doi: 10.3389/fmicb.2019.01068

Kaur, S., Baraka, S., Kaur, J. & Epstein, L. The effect of Temperature and Disease severity and Growth of Fusarium oxysporium sp. Appi Races 2 and 4 in Celerye. Phytopathology. 2022 Feb;112(2):364-372. Doi:10.1094/PHYTO.11.20.0519.R.

Kehinde I.A. (2013). Characteristic Symptoms of Melon diseases caused by fungi in south western Nigeria. Department of Biological Sciences, University of Agriculture, Abeokuta, Ogun State, Nigeria. DOI: 10.5897/AJAR10.1108

Kenya Meteriological Department. (2016). *World weather information service – Embu*: World Meteorological Organization.

KNBS, Kenya National Bureau of Statistics - Economic Survey 2019. https://www.knbs.or.ke/download/economic-survey-2019/

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (**2018**). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**:1547-1549.

Kwon, M., Hong, J., Cho, B., Ki, U. & Kim, A. (1999). A scab disease caused by *Cladosporium cucumerinum* on watermelon seedlings. Plant Pathol. J. 15: 72-75.

Lee, P. Y., Costumbrado, J., Hsu, C. Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of visualized experiments: JoVE*, (62), 3923. https://doi.org/10.3791/3923

Lilly, V. (2013). Watermelon production in Tamilnadu-at a glance. Cultivation Patterns, Health Benefits, Watermelon: Indian Journal of Applied Research, 3(6)

Mailafia, S., Okoh, G. R., Olabode, H., & Osanupin, R. (2017). Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Veterinary world*, *10*(4), 393–397. https://doi.org/10.14202/vetworld.2017.393-397

Manivannan, A., Lee, E. S., Han, K., Lee, H. E., & Kim, D. S. (2020). Versatile Nutraceutical Potentials of Watermelon-A Modest Fruit Loaded with Pharmaceutically Valuable Phytochemicals. *Molecules (Basel, Switzerland)*, *25*(22), 5258. https://doi.org/10.3390/molecules25225258

Marcello, C. M., Steindorff, A. S., Da Silva, S. P., Silva Rdo, N., Mendes Bataus, L. A., and Ulhoa, C. J. (2010). Expression analysis of the exo-beta-1,3-glucanase from the mycoparasitic fungus *Trichoderma asperellum*. *Microbiol. Res.* 165, 75–81. doi: 10.1016/j.micres.2008.08.002

Marek S., Yaghmour M., & Bostock R. (2013). Fusarium ssp., Cylindrocarpon spp, and Environmental Stress in the Etiology of a Canker Disease of Cold-stored Fruit and Nut Tree Seedling in Carifornia. Plant Disease, 97, 259-2720

Mathur, S.B. & Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi.,Copenhagen Denmark: Danish Government Institute of Seed Pathology for Developing Countries, pp 1- 426.

Mehl, H. L., & Epstein, L. (2007). Identification of *Fusarium solani* f. sp. *cucurbitae* race 1 and race 2 with PCR and production of disease-free pumpkin seeds. Plant Dis. 91:1288-1292.

Mendgen, K., Hahn, M., & Deising, H. (2003). Morphogenesis and mechanisms of penetration by plant pathogenic fungi. First publ. in: Annual Review of Phytopathology 34 (1996), pp. 364-386. 34. 10.1146/annurev.phyto.34.1.367.

Müller, A., Faubert, P., Hagen, M., Zu-Castell, W., Polle, A., Schnitzler, J.P. & Rosenkranz, M. (2013). Volatile profiles of fungi chemotyping of species and ecological functions. Fungal Genetics and Biology. 54: 25-33.

Nabi, S.U., Raja, WH., Kumawat, K.L., Mir, J I., Sharma, O.C., & Singh, DB. (2017). Post harvest diseases of temperate fruits and their management strategies-a review. *Int. J. Pure Appl. Biosci.* 5, 885–898. doi: 10.18782/2320-7051.2981

Naz, A., Butt, M.S., Sultan, M.T., Qayyum, M.M., & Niaz, R.S. (2014). Watermelon lycopene and allied health claims. *EXCLI journal*, *13*, 650–660.

Nevalainen, H., Kautto, L. & Te'o, V. (2014). Methods for Isolation and Cultivation of Filamentous Fungi. Methods in molecular biology (Clifton, N.J.). 1096. 3-16. 10.1007/978-1-62703-712-9_1

Okungbowa, F. & Shittu, H. (2014). Fusarium Wilts: An Overview.

Orłowska, M., & Muszewska, A. (2022). In Silico Predictions of Ecological Plasticity Mediated by Protein Family Expansions in Early-Diverging Fungi. *Journal of Fungi*, *8*(1), 67. https://doi.org/10.3390/jof8010067

Oyeleke, A, & Manga, S.B. Essential of Laboratory Practice. 3rd ed. Minna, Niger state, Nigeria: Tobest Publisher; 2008. pp. 12–29.

Park, S., Kwon ,T., Lim Y., Jung, K. & Choic, B. (1996). Disease survey in melon, water-melon and cucumber with different successive cropping under viny/house conditions. Korean J. Plant Pathol. 12(4):428-431.

Puel, O., Tadrist, S,; Delaforge, M., Oswald, I.P., Lebrihi, A. The inability of *Byssochlamys fulva* to produce patulin is related to absence of 6-methylsalicylic acid synthase and isoepoxydon dehydrogenase genes. *Int. J. Food Microbiol.* **2007**, *115*, 131–139.

Punja, Z., Scott, C. & Chen, S., (2018). Root and Crown Rot Pathogens Causing Wilt Symptoms on Field-grown Marijuana (*Cannabis sativa L.*) Plants. Canadian Journal of Plant Pathology, 40. 528-541

Rahman, M., Ahmad, K., Siddiqui, Y., Saad, N., Hun, T., Mohd H., Rashed, O., Hossain M., Kutawa, A. First Report of Fusarium wilt disease on Watermelon Caused by Fusarium oxysporum f. sp. niveum (FON) in Malaysia. Plant Dis. 2021 May 27. doi: 10.1094/PDIS-04-21-0780-PDN. Epub ahead of print. PMID: 34042494

Renteria, M., Moller, A., Camacho, M., Tamayo, F., Ochoa-Meza, A., & Moreno, S. (2015). First Report of Watermelon Wilting Caused by Fusarium brachygibbosum in Sonora, Mexico. Plant Disease. 99. 729. 10.1094/PDIS-10-14-1073-PDN.

Roberts, P. & Kucharek, T. (2006). Watermelon; Specific Common Diseases. Florida Plant Disease Management Guide: Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. 3(55): 1-4.

Roberts, P., McGovern, R., Kucharek, T., & Mitchell, D. (2008). Vegetable Diseases Caused by Phytophthora capsici in Florida. University of Florida-IFAS, Cooperative Extension Service. SP159. 1-6.

Said, E., and Fatiha, H. 2018. Genotypic variability in fruits characters of Moroccan watermelon cultivars (Citrullus lanatus) cultivars under well and limited watered conditions. Hortic. Int. J. 2:378–381. doi: 10.15406/hij.2018.02.00080

Samson, R., & Varga J. *Aspergillus* Systematics in the Genomic Era. CBS Fungal Biodiversity Centre, Utrecht. 2007:206.

Shankar, R., Harsha, S. & Bhandary, R. (2014). A Practical Guide to Identification and Control Watermelon Diseases. Tropica Seeds PVT LTD; Basavangudi, Bangalore, INDIA

Sharma, A., Katoch, V. & Rana, C. (2016). Important Diseases of Cucurbitaceous Crops and Their Management.

Singh, D, & Sharma, R. (2007). Postharvest diseases of fruit and vegetables and their management. In:Prasad, D. (Ed.), sustainable pest management. Daya publishing house, New Delhi, India.

Sonjak, S., Frisvad, J.C. & Gunde-Cimerman, N. Genetic Variation Among *Penicillium crustosum* Isolates from Arctic and other Ecological Niches. *Microb Ecol* **54,** 298–305 (2007). https://doi.org/10.1007/s00248-006-9202-

Sousa, E. & Raizada, M. (2020). Contributions of African Crops to American Culture and Beyond: The Slave Trade and Other Journeys of Resilient Peoples and Crops. *Front. Sustain. Food Syst.* 4:586340. doi: 10.3389/fsufs.2020.586340

Tafinta, I.,Shehu, K., Abdulganiyyu, H., Rabe A., & Usman, A. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State. Niger. J. Basic Appl. Sci. 2013;21(3):193–196.

Tamura, K. (**1992**). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9**:678-687.

Tizhe, T., Yusuf, C., Zakawa , N., Kagana, D., & Abigail, E. (2019). Fungal Pathogens Associated with Watermelon (*Citrullus lanatus*) Fruit and Efficacy Determination of *Annona senegalensis* Leaf Extract against the Fungal Isolates. The Journal of Agricultural Science. 9. 309-314. 10.15580/GJAS.2019.3.072719146

Tridge. (2020). Seasonal market report. A comprehensive market update on agricultural products currently in the key harvest season. Watermelon, Kenya, April 2020. Retrieved from https://www.tridge.com/intelligences/watermelon/KE.

Tuko.co.ke (2018). Retrieved from https://www.tuko.co.ke/274710-areas-suitable-watermelon-farming-kenya.html

Ufoegbune, G., Fadipe, O., Belloo, N., Eruola, A., Makinde A., et al. (2014) Growth and Development of Watermelon in Response toSeasonal Variation of Rainfall. J Climatol Weather Forecasting 2: 117. doi:10.4172/2332-2594.1000117

Umesha, S., Manukumar, H., & Raghava, S. (2016). A rapid method for isolation of genomic DNA from food-borne fungal pathogens. *3 Biotech*, *6*(2), 123. https://doi.org/10.1007/s13205-016-0436-4

Veenstra, A., Rafudeen, M., and Murray, S. (2019). *Trichoderma asperellum* isolated from african maize seed directly inhibits *Fusarium verticillioides* growth in vitro. *Eur. J. Plant Pathol.* 153, 279–283

Wang, B., Li, B-H., Wang, C-x., Zhang, Z-F. (2015). Effects of Temperature, Wetness duration and Moisture on the Conidial Germination, infection and disease incubation period of Glomerella cingulata. Plant Disease;99(2):249-256

Wei, S., Zhong, Y., Ma, Z. & Jiang, H. (1991). A survey on water melon diseases in the Northern China. Chain Fruits 1:36-37.

Wu, Q., Zhang, L., Xia, H., Yu, C., Dou, K., Li, Y., *et al.* (2017). Omics for understanding synergistic action of validamycin a and *Trichoderma asperellum* GDFS1009 against maize sheath blight pathogen. *Sci. Rep.* 7:40140. doi: 10.1038/srep40140

Yadav, A., Verma, D., Kumar, V., Sangwan, P., Mishra, S., Panjiar, N., Gupta, V., Kumar N. & Saxena, A. (2018). Biodiversity of the Genus Penicillium in Different Habitats. 10.1016/B978-0-444-63501-3.00001-6.

Zhou, X. & Everts, K. (2008). First report of *Alternaria alternata* f. sp. Cucurbitae causing alternaria leaf spot of melon in the Mid- Atlantic Region of the United States. Disease. Notes 92(4): 652.

Zitter, A.T., Hopkins, D.L. &Thomas, C.E. eds. (1996). Compendium of cucurbit diseases. APS PRESS. The American Phytopathological Society, Minnesota.