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# Pathogenicity of *Alternaria* Species Causing Tomato Blight in Agro-Ecological Zones of Kirinyaga County in Kenya

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Tomato (*Lycopersicon esculentum* Mill) is a highly consumed and produced vegetable crop with an annual global production of over 185 million tonnes. High consumption and production are due to its nutritional and health benefits. However, tomato production is characterized by inconsistent quality and yields, partly emanating from biotic constraints attributed to fungal foliar diseases. In Kirinyaga County, where tomato production is a significant source of livelihood, knowledge gaps exist regarding the identity of *Alternaria* species causing tomato blight in different Agro-ecological zones (AEZs) and the susceptibility of locally grown tomato varieties to the *Alternaria* pathogens.

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To address these gaps, we conducted a study to determine the characteristics of an *Alternaria* pathogen isolated from tomato leaves from different AEZs and assess the susceptibility level of some tomato varieties grown in Kirinyaga County. Pathogen characterization was done using phenotypic and molecular approaches, and the susceptibility assay was evaluated by artificial inoculation. The molecular characterization involved BLASTIN analysis of Sanger sequenced DNA nucleotide amplicons, and the use of NCBI databases. The susceptibility assay was carried out in the greenhouse using a completely randomized design. Data on the fungal conidial lengths (µm) and the susceptibility (%) of tomato varieties to fungal isolates were subjected to Analysis of Variance (ANOVA) with significance means separated using LSD at $\alpha = 0.05$. The morphologies of the pathogens differed significantly ($p < 0.05$). Molecular characterization implicated *Alternaria solani*, *Alternaria cerealis*, *Alternaria arborescens*, and *Alternaria alternate* in causing tomato blight. Tomato varieties differed in their susceptibility to *Alternaria* pathogens ($p < 0.05$). Kilele $F_1$ (73.84%), and Terminator $F_1$ (56.48%) were the most and least susceptible tomato varieties, respectively. Thus, Terminator $F_1$ can serve as a source for the genetic improvement of tomato varieties in the Kirinyaga patho-system. Our study provides valuable information on the identification of Alternaria species causing tomato blight in different AEZs in Kirinyaga County and the susceptibility of locally grown tomato varieties to infection by Alternaria pathogen isolates. The findings can aid in the development of effective disease management strategies and the genetic improvement of tomato varieties in the area.

*Keywords: Alternaria solani; A. cerealis; A. arborescens; A. alternate; tomato; Kirinyaga.*

## 1. INTRODUCTION

Early blight is a destructive and widely spread fungus disease that may potentially cause tomato yield losses of up to 80% [1,2]. Necrotrophic fungi in the genus *Alternaria*, which include *Alternaria alternate*, *Alternaria solani,* and *Alternaria tomatophila,* have been vindicated as the causative agents of early blight diseases [1,2]. Plant diseases limit chlorophyll levels and enhance the build-up of injurious reactive oxygen species such as superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$) which damage biomolecules such as lipids and nucleic acids [3,4]. Pathogens such as *Alternaria solani* according to Sallam et al. [3], enhance the synthesis of ascorbic acid while limiting the synthesis of antioxidants (Glutathione and soluble phenolic compounds), salicylic acid, a stimulant, and catalysts such as polyphenol oxidase and phenylalanine ammonialyase, which are necessary in plant protection in times of stress.

Tomato production is practiced in different agro-ecological zones (AEZs) of Kirinyaga County, ranging from zone LM4 to UM2 [5]. These AEZs vary in climatic conditions [6] and tomato cultivation practices, such as the variety grown, among other factors [7]. Variations in microclimate, i.e., humidity, temperature, and light duration, are likely to influence pathogen selection, pathogen development, and disease progress [8,4]. Due to environmental fluctuations and their impact on pathogens' behavior, adaptations, and morphologies, the burden of disease control requires accurate identification of the causing pathogen [9]. Accurate pathogen identification is fundamental to farmers, as it links the disease-causing pathogen with the already available information necessary to effect management [10]. Traditionally, the identification of fungal pathogens entailed an elaborate process that included pathogen isolation and characterization [11,12,13]. Phenotypic characterization has been predominantly used in the identification of fungal pathogens, which uses conidial shape, color and septation among other traits [14,15,16].

However, phenotypic characterization provides variable results for different fungal phytopathogens. Kumar et al. [17] evaluated characteristics of *Alternaria solani* from different agro-climatic conditions and observed yellow, brown, black, and brownish-black pigments on potato dextrose agar medium. The conidiogenous hyphae varied in thickness from 1.17 to 9.56 µm. In the majority of the isolates, mycelial growth was smooth, with circular or irregular margins that did not form concentric rings. Regarding cultural traits, variation in *Alternaria solani* strains from the Jordan Valley was reported with a mycelial width range of 0.8 to 1.5 µm, conidial breadth of 35-75 µm, length of 10-20 µm, 2 to 7 transverse septa, and 1 to 4 longitudinal septa [18]. In Limpopo, Mphahlele [19] reported the variability of *Alternaria solani*

isolated from tomato leaves collected from four tomato production regions. Isolates were highly varied phenotypically with reference to the pigmentation of mycelial, conidia length, septa number, and diameter of the colonies.

In Kenya, despite reported cases of tomato early blight in Mwea [20], little work has been done on the characterization of the causative pathogens in different AEZs. Use of morphologies for identification of fungal phyto-pathogens is tedious, requires expertise, and is hindered by fungal inconsistency and phenotypic plasticity [21-24]. To resolve this inadequacy, there is a need for using advanced identification criteria, such as molecular techniques that allow comparative genome analyses to distinguish closely-related pathogens [1]. A combination of phenotypic and molecular based identification approaches can provide more comprehensive knowledge of the tomato foliar fungal pathogen in Kirinyaga County to facilitate management. Improved tomato varieties such as Terminator $F_1$, Kilele $F_1$, Hansal $F_1$ and Riotinto $F_1$ among others have been introduced to farmers in Kirinyaga County. However, cases of blight i.e., early blight and other fungal diseases have persisted [25,20,5]. Success in breeding for EB resistance has been slowed down by the limited availability of effective resistance genes, particularly among the cultivated tomato [26]. Therefore, susceptibility tests of tomato varieties to early blight disease may serve as guidelines for the breeding of disease-resistant cultivars, thereby improving tomato production and income for farmers. According to Odilbekov [27] and Adhikari et al. [1], a knowledge gap exists on the susceptibility of varieties of tomato grown by farmers to diseases that include early blight. Thus, screening of local tomato varieties against early blight diseases in different patho-systems such as the AEZ of Kirinyaga County is necessary to bridge the existing knowledge gap on their susceptibility to the pathogen in the area.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The fungal pathogens used in this study were isolated from diseased tomato leaves collected from farms in Kirinyaga County (Fig. 2). Kirinyaga County is located in the Southern side of the Mountains about 100 km North East of Nairobi [28] at latitudes of 0° 37'S and 0° 45'S and longitudes of 37° 14'E and 37° 26E, with altitude ranges of 1,100 m to 1,200 m above sea level. The average annual rainfall is 940 mm, with long and short rains occurring between April to May and October to November, respectively [6]. Temperatures range from 12°C to 26°C with an average of 20°C. The AEZ in Kirinyaga County are grouped from Tea Dairy Zone LH 1 at the base of Mount Kenya National Park, three coffee zones (UM 1, UM 2, and UM 3), and the marginal cotton zone in zones LM 3 and LM 4 (Table 1 [6]).

As shown in Table 1, soil types in Kirinyaga County differ within and across AEZs. For instance, whereas AEZ UM2 and UM3 are composed primarily of humic nitosols, LM4 is composed of three soil types, i.e., humic nitosols, eutric nitosols, and pellic vertisols [6]. Specifically, the diseased tomato leaves used in this study were collected in five tomato growing AEZs of Kirinyaga, namely LM 3, LM 4, UM 4, UM3, and UM 2 (Fig. 2).

### 2.2 Experimental Designs

To determine the susceptibility of tomato varieties to foliar fungal pathogens, a 5 × 5 factorial experiment was used, which was laid out in a complete randomized design (CRD) and replicated 6 times under greenhouse conditions. There were two factors, i.e., tomato varieties (Hansol $F_1$, Kilele $F_1$, Rambo $F_1$, Riotinto $F_1$ and Terminator $F_1$) and *Altenaria* pathogen isolates 5 levels (Isolates EB-KAM8, EB_WNG4, EB_NDM3, EB_KER7 and control). The control treatment consisted of an uninoculated tomato plant for each treatment.

#### 2.2.1 Isolation of blight pathogen from diseased leaf

A small piece of diseased leaf tissue (3 x 3 mm) at the margin of a blight lesion and a healthy leaf part were cut using a sterile blade. The cut lesion was surface disinfected with 0.3% sodium hypochlorite for 1 min, then rinsed in 3 changes of distilled water to remove the detergent. The excisions were surface sterilized in 70% ethanol for 30 seconds, rinsed with sterile distilled water three times, and then blot dried using sterilized Whatman filter paper. Water-free excised leaf parts were placed on PDA in the petri dishes and incubated for 1 week at 25°C to realize optimal fungal growth. Upon growth, the colonies were viewed under the microscope, and pure cultures were prepared.

**Table 1. Features of agro-ecological zones where diseased tomato leaves were collected in Kirinyaga County**

| *AEZs | Soil type | Altitude (m) | Temp (°C) | Subzone | Rainfall (mm) |
|---|---|---|---|---|---|
| UM2 | Humic Nitosols | 1400-1580 | 19.0-20.1 | m/l + m/s<br>m + s/m | 1220-1500<br>1200-1250 |
| UM3 | Humic Nitosols | 1340 - 1400 | 20.1-20.6 | m/s + s | 1100 - 1250 |
| UM4 | Humic Nitosols<br>Eutric Nitosols | 1280 - 1340 | 20.4-20.9 | s/m + s<br>s + s | 950 - 1200<br>350 - 960 |
| LM3 | Humic Nitosols | 1220 - 1280 | 20.9-21.2 | s /m+ s<br>s + s | 950 -1200<br>350 -960 |
| LM4 | Humic Nitosols,<br>Eutric Nitosols<br>Pellic Vertisols | 1090 - 1220 | 21.2-22.0 | s + s/vs | 850 - 950 |

**AEZ = Agro-Ecological Zones, in the subzones, m= medium rainfall, s= short rainfall, l= long rainfall, vs= very short rainfall, UM = Upper Midland (1, 2 and 3), LM = Lower Midland (3 and 4) Jaetzold et al [6]*

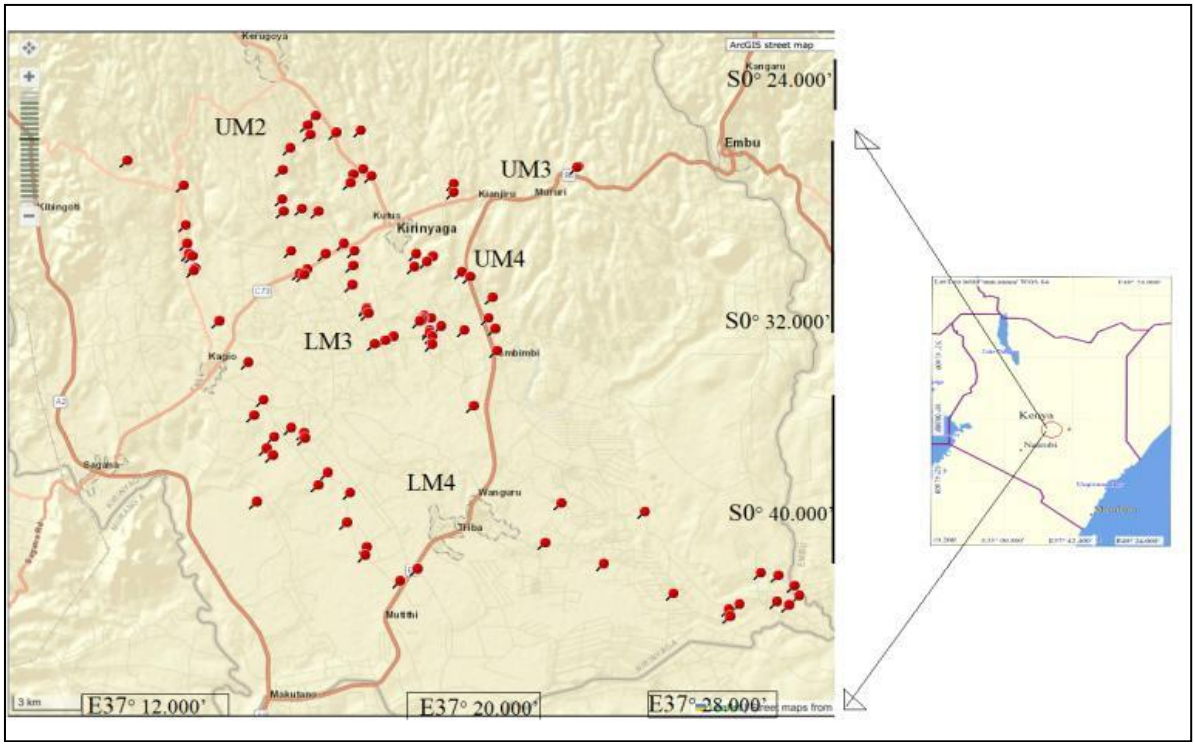


**Fig. 1. Map of Kirinyaga County showing agro-ecological zones (UM2, UM3, UM4, LM3 and LM4) surveyed for foliar diseases of tomato; where UM = Upper Midland (1, 2 and 3), LM = Lower Midland (3 and 4)**

### 2.2.2 Purification and identification of fungus

Pure fungal pathogens were obtained by subculturing in fresh PDA media. A flame sterilized scalpel was used to cut the culture media with grown mycelia at the periphery and transfer it into fresh medium, which were re-incubated for 2 weeks. After the pure mycelia had completely covered the plates, phenotypic characterization was done based on external cultural morphologies and by use of microscopy following the scotch tape method [29]. In culture plates, fungal phenotypic traits such as spore colour (SP), culture pigmentation, color of colony margins (M-col), growth rate (GR) and mycelial fluffiness [(MF) facilitate identification (Table 2). The Scotch tape method involved picking the spores using transparent adhesive tape where the spores were picked from the plates using the adhesive side of the tape, then placing the tape on the lactophenol cotton blue stain on the slide [29]. The slide was then observed under LCD

phase contrast light microscope (Model: OMAX 40X-400X) at × 40 objective lenses, microscopic details of the fungus were observed, measured, and the images photographed. Fungal characteristics assessed under the microscope were; conidial length in µm, number of septa, and the existence of beak and arrangement of conidia [(C-Arr) Table 2].

The reference materials used for identification of blight pathogen isolates included published material from related work [17,30,31,20]. A Pathogenicity test was carried out for selected pathogen isolates for further identification and characterization. All the conidial measurements were taken in micro-meter (µm).

### 2.2.3 Pathogenicity test and aggressiveness of early blight fungal pathogen isolates

A pathogenicity test was carried out as required by Koch's postulate [32] on disease-causing organisms in a pot experiment in the greenhouse at Chuka University. Spore suspension ($10^3$ spores/mL) prepared from two-week-old pure cultures of the blight disease pathogen The spores on the plates were scraped gently with a scalpel, suspended in 100 ml distilled water, and filtered through three-fold cheesecloth. A hemocytometer (30 x 70 x 4mm) was used to standardize the inoculum to $10^3$ spores/mL. A two-month-old Terminator $F_1$ tomato variety was used in the pathogenicity test (Koch's postulate experiment). The Terminator tomato variety was selected for the pathogenicity test because it is grown in most areas where the pathogens were obtained. The leaves of tomato plants were sprayed with respective fungal pathogen isolates and monitored for two weeks for the occurrence of blight-like disease symptoms.

To determine the aggressiveness of the blight pathogens, the detached leaf method described by Pryor and Michailides [33] and Tymon et al. [34] was used. Ten well-developed, mature, and disease-free tomato leaves from Terminator $F_1$ were aseptically collected from the green house. The leaves were transported to the laboratory in moist, sterile zip lock bags. At the laboratory, the leaves were rinsed under running tap water, surface-sterilized with 70% alcohol for 5 seconds, and rinsed again in three changes of sterile water to remove the alcohol. The water on the leaf surface was wiped out using sterile blotting paper. The five sets of leaves were pricked using a sterile needle and inoculated with a pathogen suspension (20 µl) of the respective disease pathogens. The suspension was dropped at the point of the lesion, and one leaf that served as the control was inoculated with 20 µl distilled water. Tomato leaves were then placed on a sterile, clean grid in a transparent glass desiccator (Diameter 300 mm height 240 mm). The desiccator had 250 ml of distilled water underneath to maintain humidity. The inoculated leaf area was monitored for five days for the development of lesions. The length (L) and width (l) of the lesion were measured for each leaflet inoculated. The lesion size (S in $mm^2$) was calculated using the formula below [35].

$$S = \frac{\pi(L+1)^2}{4}$$

### 2.2.4 Molecular identification of selected isolates

The molecular analysis of fungal pathogens isolated in this study was done at the University of Nairobi's Centre for Biotechnology and Bioinformatics. The DNA was extracted from 20 mg of mycelia harvested by scraping the colony surface. The mycelia were ground into fine pieces using a motor and pestle and placed into a 1.5 mL micro centrifuge tube containing 800 µL cetyltrimethyl-ammonium bromide (CTAB) extraction buffer (Final concentration: 2% (w/v) CTAB, 200 mM Tris–HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 MNaCl, 1% (w/v) polyvinylpyrrolidone, and 1% (v/v) b-mercaptoethanol [36]). The DNA extracts were dissolved in 50 µL of sterile distilled water and stored at 20°C for further use.

Polymerase chain reaction (PCR) was done using Thermocycler (Model C1000, Touch Biorad). The polymerase chain reaction was done in a 0.5 ml Eppendorf tube containing 10.5 µL $ddH_2O$, 12.5 µL Premix Ex Taq (Containing 0.625 U Taq DNA polymerase, 200 µM dNTP and 2 mM$MgCl_2$), 0.5 µL each of the two primers (10l M) and 1 µL DNA template (100 µgmL-1). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of the isolates was amplified using primer sets ITS1 [(50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) described by White et al. [37] and Lee and Taylor [36]. Amplification reactions were done with an initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 40 s, annealing at 58°C for 40 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. Polymerase chain reaction products were checked using gel electrophoresis on 1% agar rose gel stained with

ethidium bromide. The DNA amplicon (1µl) mixed with loading dye was assayed alongside a 1kb gene ruler plus ruler with an initial reading of 75kb. The DNA loading bands were viewed on a gel documenter imager (Bio- Rad). Positive DNA PCR products were cleaned using the Quick cleaning kit supplied by Gene Script Company in readiness for senger sequencing. The sequencing of the DNA amplicons was done at the International Livestock Research Institute in Nairobi, Kenya, using a Sanger sequencer (Model: ABI 3730 genetic analyzer) and The Big Dye Terminator v3.1.

#### 2.2.5 Susceptibility of tomato varieties to different fungal pathogen isolates

Five tomato varieties used for susceptibility screening were obtained from the Kenya Seed Company in Nairobi. The seedlings of the 5 tomato varieties (Kilele $F_1$, Hansol $F_1$, Rambo $F_1$, Riotinto $F_1$ and Terminator $F_1$) were germinated separately in sterile germination trays in the greenhouse at Chuka University. The trays were watered daily for three weeks before transplanting. Calcium Ammonium Nitrate (CAN) and Diammonium phosphate (DAP) were used to supplement tomato growth. The growth bags used for growing tomatoes were 45 x 30 x 30 cm in size. The containers were filled with oven sterilized soil made up of sand, silt, and compost (Ratio 3:1:1) [38]. The three-week-old tomato seedlings were individually transferred to the plant growth bags (one plant per container), with each treatment being replicated six times. The distance between each tomato plant and the next was 0.5 meter. Watering was done by sprinkler every morning and evening to maintain humidity. Three weeks after transplantation, each tomato variety was inoculated (by spraying) with 50 ml of the respective fungi pathogen culture containing $10^3$ CFU/ml as determined by hemocytometer.

After inoculation, the tomato plants were watered on alternate days and inspected for the development of disease symptoms. At the end of 28 days after inoculation, the disease symptoms were scored using a rating scale of 1 – 6 [39]. The scores for different pathogens were used to calculate disease severity that represented the susceptibility of individual tomato variety using the formula below as used by Negesa and Ayana [40]:

$$\text{Percent Disease Severity} = \frac{\text{Number of individual ratings}}{\text{Total number of leaves assessed}} x \frac{100}{\text{Maximum scale}}$$

## 2.3 Data Analysis

#### 2.3.1 Characterization of *Alternaria* fungal pathogens causing foliar diseases of tomato in different agro ecological regions of Kirinyaga County in Kenya

Cluster analysis of data collected on the phenotypic traits was done using factoextra R package in R studio version 4.0.3. The normality test was evaluated using the Shapiro-Wilk test. Given that the normality test for conidial length and breadth gave non-significance ($p$ > 0.05) Shapiro-Wilk test results, their means were subjected to the ANOVA using the general linear model and significance means were separated using LSD in SAS version 9.4 at α= 0.05.

The PCR fragment ITS rDNA sequences were compared with the record present in the NCBI/Gene Bank database (www.ncbi.nlm.nih.gov/blast/). The obtained fungal species sequences from the database were subjected to multiple sequence alignment using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) program method available in Mega X version 11 [41]. A phylogenetic tree of the pathogen sequences and those of related fungal species downloaded from the gene bank was generated using the unweighted pair group method with arithmetic mean (UPGMA) program and bootstrapping performed with 10,000 replications to estimate the stability of the branches [42]. The evolutionary distances of the species in the phylogenetic tree were computed using the p-distance method, and the output was presented in units of the number of base substitutions per site [43].

#### 2.3.2 Susceptibility test of tomato varieties to different foliar fungal disease pathogens

The skewness value for the data on susceptibility, presented as percentages of symptom severity, was calculated to be 0.0119. Additionally, the results of the Shapiro-Wilk normality test indicated that the data followed a normal distribution with a p-value greater than 0.05. Consequently, the susceptibility data was analyzed using the general linear model procedure in SAS version 9.4, with the significance of the means being determined through the least significant difference (LSD) test at an alpha level of 0.05.

## 3. RESULTS

### 3.1 Phenotypic Characterization of Early Blight Pathogen

A total of 30 Alternaria pathogen isolates were obtained from diseases tomato leaves from different AEZ. Most on the isolates on potato dextrose agar were not different in terms of spore's colour on PDA. The isolates had green-dark grey spores and had dark pigmentation. The cultures had either regular or irregular margins (Table 3).

The study observed that the conidiophores of *Alternaria* spp isolates were either solitary or grouped and exhibited straight or flexuous morphologies. The conidia displayed variations in breadth, length, and the presence of beaks. The shape of the conidia was either straight or slightly curved, with some displaying muriform or ellipsoid morphology. The conidia tapered towards one end and were observed to be olivaceous brown in colour (Plate 1). Notably, the transverse and longitudinal septa of the conidia were found to vary, ranging from 1 to 6 and 0 to 2, respectively. The conidia appeared in chains or as single units, and some exhibited short, long, or no beaks (Plate 1).

A total of 30 early blight pathogen isolates from five distinct Agro-Ecological Zones (AEZ) were subjected to cluster analysis based on their phenotypic characteristics, resulting in the formation of three clusters. The first and third clusters consisted of 13 isolates each, which were further subdivided into two sub-clusters. The smallest cluster comprised of isolates EB_KBU3 (from AEZ), EB_LM4, and EB_KD09 (both from AEZ UM2), isolate EB_KER2 and EB_KER7 (both from AEZ LM3 and AEZ LM4), and isolate NDM7 (from AEZ UM4), as depicted in Table 3 and Fig. 2. It is worth noting that isolates EB_KD09 and EB_KER7 had similar distances while isolate EB_KBU3 formed a separate sub-sub clade. Isolates EB_KD09 and EB_KER7 exhibited similar growth rates, beak morphology, colony margins, conidia arrangement, and the number of transverse septa, as outlined in Table 3.

There was a significant ($F$ (29, 210) =8.25, $p$ <0.0001) difference observed in the conidial lengths of pathogen isolates from infected tomato leaves collected from different Agro-Ecological Zones (AEZ). Within AEZ LM4, the lengths of pathogen isolates were significantly ($F$ (5, 42) =22.31, $p$ <0.0001) different, with the minimum and maximum conidial lengths being 52.8 and 194.2 µm, respectively (Table 4). Isolate EB_ND4 had longer conidia (148.37 µm), while isolate EB_KBU3 had the smallest length of conidia at 78.52 µm (Table 4). In AEZ LM3, the lengths of the conidia of the pathogen did not differ significantly ($F$ (5, 42) =2.05, $p$ <0.0909); however, the conidia lengths ranged from 95.60 to 204.6 µm, respectively. In AEZ UM4, the lengths of the conidia of the pathogen were significantly ($F$ (5, 42) =3.93, $p$ <0.0051) different, with the minimum and maximum conidia lengths measured being 85.2 and 184.2 µm, respectively. Isolate EB_NJI2 had longer conidial length (131.17 µm), while isolate EB_NDM7 had shorter conidial length (101.17 µm). The length of conidia for the early blight pathogen was significantly ($F$ (5, 42) =5.30, $p$ <0.0007) different in UM3, where the minimal and maximum conidia lengths measured were 64.2 and 159.4 µm, respectively. Isolate EB_KAM3 had longer conidial length (123.62 µm), while isolate EB_GTH5 had a shorter conidial length [(92.75 µm) Table 4]. Conidia lengths in AEZ UM2 differed significantly ($F$ (5, 42) =2.59, $p$ <0.0398), with the minimum and maximum lengths being 64.6 µm and 138.4 µm, respectively. Isolate EB_KER7 was significantly longer, measuring 112.37 µm (Table 4).

**Table 2. Aspects of blight pathogen isolates used for phenotypic characteristic**

| Cultural characteristic blight pathogen isolates | | |
|---|---|---|
| **Growth rate (GR)** | **Colony margin (M-col)** | **Spore colour (SP)** |
| 1= Slow | 1= Brownish | 1= Greenish |
| 2= Medium | 2= Greyish | 2= Greyish |
| 3= Rapid | 3= Whitish | 3= Dark |
| **Presence of Beak (PB)** | **No. transverse septor (T-Spt)** | **No. longitudinal septa (L-Spt)** |
| 1= Beak present<br>2= Beak absent | | |
| Conidia arrangement (C-Arr) | 1=Single<br>2 = Chains | |

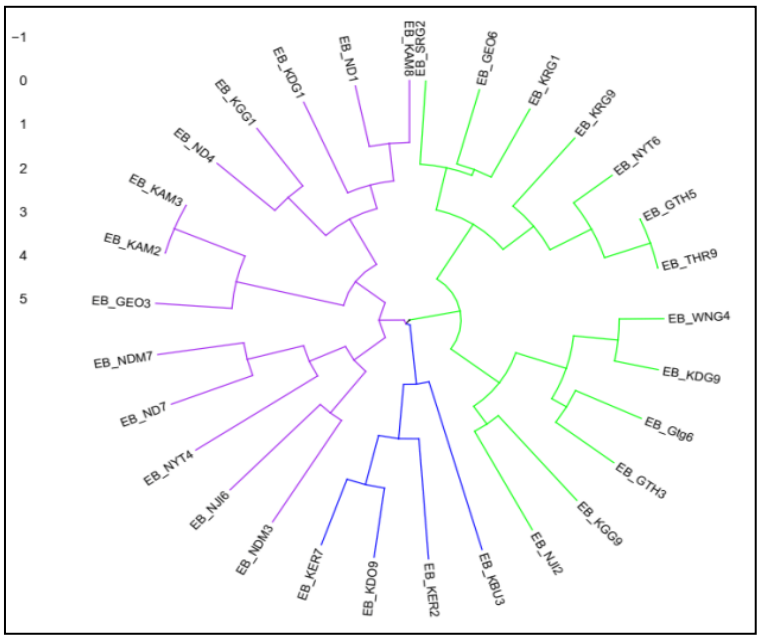


**Fig. 2. Cluster membership based on phenotypic traits of early blight pathogens isolated from tomato leaves from different agro-ecological zones of Kirinyaga County**

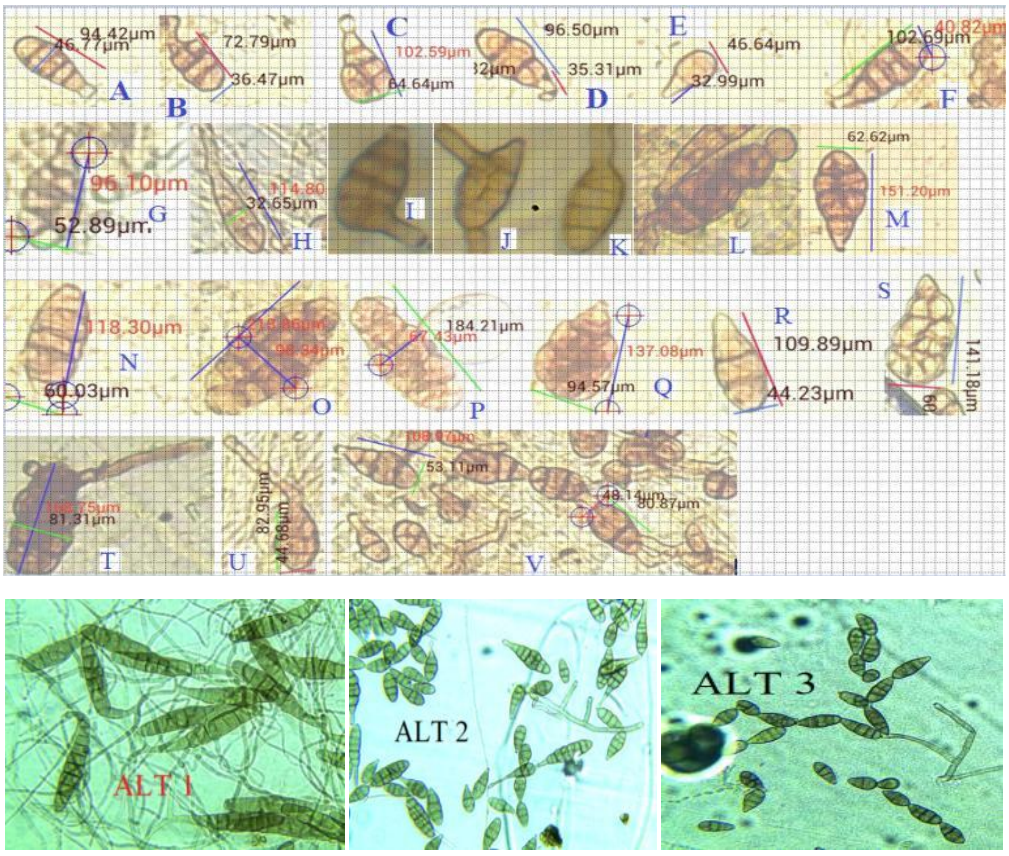


**Plate 1. Selected conidia morphologies for the early blight pathogen (*Alternaria* spp) isolates from tomato leaves from Kirinyaga County, Kenya**

*NOTE: Muriform shape (A, C, F, R, S), Pyriform (I, E, O, P), Cuneate shape (Q), Conidia with short beak (A, C, D, E), Medium curved beak (B and D), long beak (H and U) no beak (M, N, O, P, Q, R, S), ALT 1= Isolate EB-KAM8, ALT2= Isolate EB-NJ12-, ALT3= Isolate EB-ND4*

**Table 3. Phenotypic characteristics of alternaria pathogen isolated from different agro-ecological zones of Kirinyaga County**

| AEZ [1] | Isolate | GR[2] | SP[3] | PB[4] | Mcol[5] | L-Spt[6] | T-Spt[7] | C-Arr[8] |
|---|---|---|---|---|---|---|---|---|
| LM4 | EB _Gtg6 | 3 | 1 | 2 | 1 | 3 | 4 | 1 |
| | EB _KBU3 | 2 | 3 | 1 | 2 | 0 | 1 | 1 |
| | EB _ND1 | 2 | 2 | 1 | 1 | 0 | 5 | 2 |
| | EB _ND4 | 3 | 3 | 1 | 1 | 2 | 4 | 2 |
| | EB _ND7 | 2 | 3 | 1 | 2 | 0 | 3 | 1 |
| | EB _WNG4 | 3 | 1 | 2 | 1 | 2 | 4 | 1 |
| LM3 | EB _KDG1 | 2 | 2 | 1 | 2 | 2 | 5 | 2 |
| | EB _KDG9 | 3 | 1 | 2 | 1 | 1 | 4 | 1 |
| | EB _KDO9 | 3 | 2 | 1 | 1 | 2 | 3 | 1 |
| | EB _NYT4 | 2 | 3 | 1 | 2 | 0 | 6 | 1 |
| | EB _NYT6 | 1 | 2 | 2 | 2 | 2 | 4 | 1 |
| | EB _SRG2 | 1 | 2 | 2 | 2 | 1 | 5 | 1 |
| UM4 | EB _KGG1 | 2 | 2 | 1 | 1 | 2 | 3 | 2 |
| | EB _KGG9 | 2 | 1 | 2 | 1 | 0 | 3 | 1 |
| | EB _NDM3 | 3 | 3 | 1 | 3 | 3 | 5 | 1 |
| | EB _NDM7 | 1 | 3 | 1 | 3 | 1 | 4 | 1 |
| | EB _NJI2 | 2 | 1 | 2 | 1 | 1 | 4 | 2 |
| | EB _NJI6 | 1 | 2 | 1 | 1 | 3 | 5 | 1 |
| UM3 | EB _GTH3 | 1 | 1 | 2 | 2 | 2 | 4 | 1 |
| | EB _GTH5 | 1 | 2 | 2 | 2 | 2 | 3 | 1 |
| | EB _KAM2 | 2 | 1 | 1 | 3 | 0 | 4 | 2 |
| | EB _KAM3 | 2 | 1 | 1 | 3 | 0 | 4 | 2 |
| | I EB _KAM8 | 3 | 2 | 1 | 1 | 1 | 4 | 2 |
| | EB _THR9 | 1 | 2 | 2 | 2 | 2 | 3 | 1 |
| UM2 | EB _GEO3 | 2 | 2 | 1 | 3 | 0 | 3 | 2 |
| | EB _GEO6 | 2 | 3 | 2 | 2 | 0 | 4 | 1 |
| | EB _KER2 | 2 | 1 | 1 | 3 | 2 | 3 | 1 |
| | EB _KER7 | 3 | 1 | 1 | 1 | 1 | 3 | 1 |
| | EB _KRG1 | 1 | 2 | 2 | 3 | 0 | 4 | 1 |
| | EB _KRG9 | 3 | 3 | 2 | 3 | 2 | 3 | 1 |

*[1]Agro-ecological zones: UM = Upper midland, LM = Lower midland, [2]Growth rate (1= Slow, 2= Medium, 3 =Rapid), [3]Spore colour (1= Greenish, 2= Greyish, 3= Dark), [4]Presence of beak (1= Beak present, 2= Beak absent), [5] Margin colour (1= Brownish, 2= Greyish, 3= whitish), [6]Number of longitudinal septa, [7] Number of Transverse septa, [8] Conidia arrangement (1=Single, 2 = Chains),*

Analysis of the length of conidia of the Alternaria pathogen isolated in the five agro-ecological zones (AEZs) revealed a statistically significant difference ($F$ (4, 235) =8.25, $p$ <0.0001). Specifically, conidia length of the isolates in AEZ LM3 were significantly longer (130.5 µm), while those from AEZ UM2 were significantly shorter, measuring 101 µm (Table 5).

In the pathogenicity test using isolate EB_WNG4, typical symptoms similar to those observed in tomato leaves from farmers' fields were observed, characterized by concentric rings visible in the lesion. Re-isolation of the pathogens from the lesions showed similar phenotypic features to the initial pathogen isolates, including spore colour and conidia shape. In the detached leaf experiment, inoculation of tomato leaves with selected inoculation of tomato leaves with selected 10 isolates showed a significant difference in lesion size formed by the isolates ($F$ (13, 36) =12.48, $p$ <0.0001), as presented in Table 6. Isolate EB_KAM8 demonstrated high aggressiveness with a lesion size of 122.17mm$^2$, followed by isolate EB_NDM3 with a lesion size of 110.17mm$^2$. Isolate EB_KDG1 exhibited a smaller lesion size of 17.10 mm$^2$, followed by isolate EB_ND4 with a lesion size of 20.09 cm$^2$ (Table 6).

## 3.2 Molecular Characterization of Alternaria Pathogen Isolates from infected tomato leaves

Agarose gel electrophoresis of DNA amplicons (ITS rDNA) from Alternaria pathogens isolated

from tomato leaves with early blight symptoms showed distinct bands ranging from 500 to 700 bp, as visualized using a 1kb gene ruler plus ladder (Plate 2).

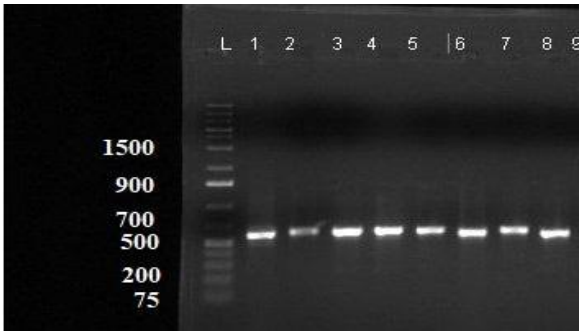


**Plate 2. The agar rose gel analysis of DNA amplicon PCR products (ITS rDNA) of tomato foliar pathogen isolates isolated from diseases leaf samples from Kirinyaga County**

*where L= ladder (1kb gene ruler plus), 2= KRG-F02, 3= EB-KAM8., 4= EB-WNG4, 5= KRG-F05, 6= EB_NDM3., 7= KRG-F07, 8= KRG-F08, 9= EB-KD09, 10= KRG-F13, 11= EB-NJ12, G1= KRG-G01. 12= EB_KER7, 13= KRG-F06, 14= KRG-F08, 15= EB_KER7, 16= EB-KAM8; Isolates EB_KER7, EB-KAM8 were assayed twice*

The analysis of DNA amplicon Sanger sequenced nucleotides, specifically ITS rDNA sequences, using the BLASTn tool, identified multiple pathogens involved in causing blight-like symptoms. Notably, *Alternaria solani* and *Alternaria alternata* were found to be among the causative agents (Table 7). Sequence similarities between the pathogen isolates and fungal species in the NCBI GenBank ranged from 97% for *Alternaria solani* to 100% for *Alternaria alternata* and *Alternaria cerealis* (Fig. 3).

### 3.3 Susceptibility of Tomato Varieties grown in Kirinyaga County to Early Blight Foliar Fungal Disease Pathogen Isolates

The susceptibility of different tomato varieties to Alternaria pathogen isolates was found to differ significantly [($F$ (19, 100) = 4.73, $p$ <.0001)]. Kilele $F_1$ exhibited the highest severity of blight symptoms (78.24%) when inoculated with isolate EB-KAM8, followed by Riotinto $F_1$ (72.22%). Conversely, Terminator $F_1$ exhibited the least severity at 58.33%. Inoculation with isolate EB_KER7 resulted in severe symptoms in Kilele $F_1$ (69.91%) and Rambo $F_1$ (67.59%), while Terminator $F_1$ exhibited the least severity (54.17%). Similarly, isolate EB_NDM3 induced severe symptoms in Kilele $F_1$ (70.83%) and Riotinto F1 (68.06%), while Terminator $F_1$ exhibited the least severe blight symptom (58.33%). Additionally, inoculation with isolate EB_WNG4 resulted in severe symptoms in Kilele $F_1$ (76.39%) and Riotinto $F_1$ (62.2%), while Hansol $F_1$ exhibited the least severity [(58.33%) Table 8].

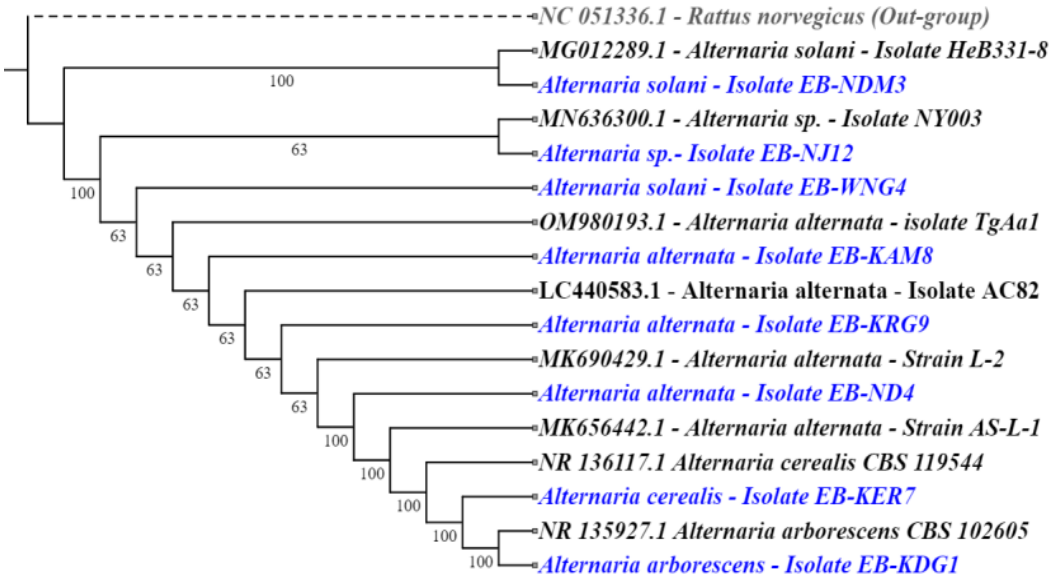


**Fig. 3. Phylogenetic analysis of sequences of some Alternaria pathogens isolated from tomato foliar and similar sequences in the gene bank using MEGA X 11 software, UPGMA method and 10000 boot-strapped replications. The species in blue font are the fungal pathogens species isolated in the current study**

**Table 4. Conidia length and breadth of alternaria pathogens isolated from diseased tomato leaves collected from different agro-ecological zones of Kirinyaga County**

| AEZ [a] | Isolate | N | Length (µm) | Breadth (µm) | Length (µm) | | Length |
|---|---|---|---|---|---|---|---|
| | | | | | Min[b] | Max[c] | Anova Statistics |
| LM4 | EB_Gtg6 | 8 | 123.72 [edf] | 39 [h] | 99.00 | 149.00 | Cv = 17.86 |
| | EB _KBU3 | 8 | 78.52 [m] | 38.7 [h] | 66.00 | 94.40 | Mean= 115.71 |
| | EB _ND1 | 8 | 83.95 [km] | 48.83 [de] | 52.80 | 109.00 | $p < 0.0001$ |
| | EB _ND4 | 8 | 148.37 [abc] | 42.65 [efgh] | 104.20 | 194.20 | LSD = 20.85 |
| | EB _ND7 | 8 | 97.97 [hijkl] | 42.68 [efgh] | 84.40 | 114.80 | |
| | EB _WNG4 | 8 | 161.72 [a] | 45.6 [efgh] | 133.20 | 207.20 | |
| LM3 | EB _KDG1 | 8 | 139.85 [abcd] | 41.1 [fgh] | 104.80 | 180.40 | Cv = 20.89 |
| | EB _KDG9 | 8 | 138.50 [abcd] | 41.77 [fgh] | 108.40 | 174.60 | Mean= 130.49 |
| | EB _KDO9 | 8 | 150.3 [ab] | 41.3 [fgh] | 96.60 | 204.60 | $p = 0.0246$ |
| | EB _NYT4 | 8 | 124.5 [edf] | 40.5 [fgh] | 95.60 | 156.20 | LSD = 27.52 |
| | EB _NYT6 | 8 | 126.77 [cdef] | 40.58 [fgh] | 89.40 | 171.80 | |
| | EB _SRG2 | 8 | 103.05 [ghijh] | 44.15 [efgh] | 81.60 | 137.80 | |
| UM4 | EB _KGG1 | 8 | 102.65 [ghij] | 47.05 [def] | 88.40 | 141.60 | Cv = 17.33 |
| | EB _KGG9 | 8 | 102.72 [ghij] | 46.23 [efg] | 85.20 | 130.20 | Mean= 114.58 |
| | EB _NDM3 | 8 | 121.8 [edfg] | 62.43 [b] | 89.80 | 161.00 | $p = 0.0051$ |
| | EB _NDM7 | 8 | 101.17 [hijk] | 46.78 [edf] | 89.80 | 112.60 | LSD = 20.04 |
| | EB _NJI2 | 8 | 131.17 [bcde] | 71.1 [a] | 91.80 | 184.20 | |
| | EB _NJI6 | 8 | 128.0 [bcde] | 58.75 [bc] | 114.60 | 167.80 | |
| UM3 | EB _GTH3 | 8 | 99.2 [ijkl] | 45.07 [efgh] | 77.80 | 159.40 | CV= 15.28 |
| | EB _GTH5 | 8 | 92.75 [jklm] | 40.65 [fgh] | 64.20 | 111.00 | Mean= 107.93 |
| | EB _KAM2 | 8 | 107.0 [fghij] | 45.43 [efgh] | 95.80 | 125.20 | $p = 0.0007$ |
| | EB _KAM3 | 8 | 124.9 [bcdef] | 60.58 [b] | 109.40 | 158.20 | LSD = 16.65 |
| | EB _KAM8 | 8 | 123.62 [cdef] | 69.97 [a] | 113.20 | 152.80 | |
| | EB _THR9 | 8 | 100.15 [hijk] | 40.83 [fgh] | 85.20 | 128.60 | |
| UM2 | EB _GEO3 | 8 | 100.07 [hijk] | 44.58 [efgh] | 80.80 | 138.40 | CV= 14.93 |
| | EB _GEO6 | 8 | 91.5 [klm] | 39.43 [gh] | 64.60 | 132.80 | Mean= 101.16 |
| | EB _KER2 | 8 | 94.0 [ijkl] | 44.43 [efgh] | 84.60 | 100.60 | $p = 0.0398$ |
| | EB _KER7 | 8 | 112.37 [efgh] | 52.98 [cd] | 93.20 | 126.40 | LSD 15.24 |
| | EB _KRG1 | 8 | 98.4 [hijk] | 44.8 [efgh] | 84.80 | 121.60 | |
| | EB _KRG9 | 8 | 110.62 [efghi] | 40.23 [fgh] | 78.20 | 124.60 | |
| Means (µm) | | | 113.97 | 46.91 | | | |
| LSD ($p < 0.05$) | | | 20.034 | 7.02 | | | |
| Cv (%) | | | 17.832 | 15.18 | | | |

*[a]Agro-ecological zones: UM= Upper midland, LM = Lower midland, [b] Minimum conidia lengths, [c] Maximum conidia lengths, [d] Means followed by the same letter in columns is not significantly different at α = 0.05*

**Table 5. Overall lengths of conidia of Alternaria pathogens isolated in different agro-ecological zones of Kirinyaga County**

| AEZ [a] | N | Length (µm) | Minimum (µm) | Maximum (µm) |
|---|---|---|---|---|
| LM3 | 48 | 130.5 [a] | 81.60 | 204.60 |
| LM4 | 48 | 115.71 [b] | 52.80 | 207.20 |
| UM2 | 48 | 101.16 [c] | 64.60 | 138.40 |
| UM3 | 48 | 107.94 [bc] | 64.20 | 159.40 |
| UM4 | 48 | 114.59 [b] | 85.20 | 184.20 |
| Means (µm) | | 113.97 | | |
| LSD ($p < 0.05$) | | 10.59 | | |
| Cv (%) | | 23.11 | | |

*[a]Agro-ecological zones, UM = Upper midland, LM = Lower midland, N = Sample size,*
*[b] Mean followed by the same letter in columns is not significantly different at α = 0.05*

**Table 6. Aggressiveness of alternaria pathogen isolates from tomato leaves in Kirinyaga county**

| Isolate | N | Aggressiveness ($mm^2$) |
|---|---|---|
| EB-KAM8 | 5 | 12.27 [a] |
| EB_NDM3 | 5 | 110.17 [a] |
| EB_KER7 | 5 | 49.85 [b] |
| EB_WNG4 | 5 | 46.01 [b] |
| EB_KAM3 | 5 | 45.15 [b] |
| EB_KRG9 | 5 | 43.20 [b] |
| EB_KDO9 | 5 | 37.31 [b] |
| EB_NJI2 | 5 | 20.73 [c] |
| EB_ND4 | 5 | 20.09 [c] |
| EB_KDG1 | 5 | 17.10 [c] |
| Mean ($cm^2$) | | 41.27 |
| LSD ($p < 0.05$) | | 1.67 |
| Cv (%) | | 24.33 |

[a] *Means followed by the same letters are not significantly different at α = 0.05*

The results of the experiment showed that the Rambo $F_1$ tomato variety did not demonstrate a significant difference in susceptibility towards Alternaria pathogen isolates, as indicated by the statistical analysis ($F$ (3, 20) = 0.56, $p$ = 0.649). In contrast, the Riotinto $F_1$ variety showed a significant difference in susceptibility towards pathogen isolates ($F$ (3, 20) = 4.36, $p$ = 0.0162), with isolate EB_NDM3 resulting in a higher severity mean of 55.86%, while isolate EB_KER7 resulted in a lower severity mean of 50.31%. However, the Hansol $F_1$ and Kilele $F_1$ varieties did not exhibit a significant difference in susceptibility towards pathogen isolates, as indicated by the statistical analysis ($F$ (3, 20) = 1.57, $p$ = 0.229 and $F$ (3, 20) = 2.16, $p$ = 0.124, respectively). Similarly, the Terminator $F_1$ variety did not demonstrate a significant difference in susceptibility towards pathogen isolates ($F$ (3, 20) = 1.36, $p$ = 0.283) Results are presented in Table 9.

## 4. DISCUSSION

### 4.1 Characterization of Early Blight Pathogen

Fungal morphology, biochemistry, and metabolites are essential in the identification and diagnosis of plant pathogenic fungi [11,14,13]. Phenotypic characteristics such as conidial shape, colour, and septation are key features in the identification of fungal pathogens [14,15,16]. In this study, the conidia of Alternaria blight pathogen isolates appeared greyish to greenish in colour and darkened as the culture aged. The margins of the cultures were either regular or irregular in pattern, which is consistent with previous study conducted in Mwea in Kirinyaga County, Kenya [20]. Pigmentation and colony texture of the Alternaria blight pathogens in this study were similar to previous reports where greenish, dark, and brownish pigmentation have been observed [17,30,20]. The surface texture of the colonies exhibited slight variations with zonation at the margins, which were similar to those observed in other studies involving Alternaria blight pathogens [17,30,31].

Microscopic observation revealed variation in the conidia morphology of the pathogen isolates, which is consistent with the findings of Mugao et al. [20] where variability of the tomato early blight pathogen in Mwea was reported. Furthermore, the observations on the morphological traits of the conidia are in agreement with other studies where conidia have differed in breadth, length, and beak and have been reported to be straight, slightly curved, muriform or ellipsoid [44-48]. The conidial transverse and longitudinal septa varied ranging from 1 to 6 and from 0 to 2, respectively, which was in tandem with other studies [45,46,47,49]. The length of conidia of these Alternaria pathogen isolates ranged from 78.52 µm to 161.72 µm. The observed conidia lengths in this study were within the range of 105-220 µm reported earlier by Nolla [50]. However, the conidia lengths were longer than those reported earlier by Neergaard [51] and Abubakar and Ado [52] ranging from 10.26 - 77.52 µm. These findings suggest that the pathogen populations in each AEZ are distinct and may have different virulence and pathogenicity levels.

Due to the intricate nature of Alternaria species isolates, molecular characterization was deemed essential for accurate identification. The analysis of Internal Transcribed Spacer (ITS) rDNA sequences revealed that the causal agents of tomato Alternaria blight in Kirinyaga County in Kenya are *Alternaria solani*, *Alternaria alternata*, *Alternaria cerealis*, and *Alternaria arborescens*. While there is limited documentation of *Alternaria cerealis* and *Alternaria arborescens* as tomato pathogens in Kenya, these pathogens have been reported in tomato crops in other regions. For instance, *Alternaria arborescens* has been documented as a tomato pathogen in Egypt, Algeria, Iraq, and Iran, causing leaf blight, stem canker, tomato collar rot, and fruit rot [53-57].

Similarly, *Alternaria cerealis* has been reported as a tomato pathogen in Yemen [58,59]. It is possible that our study may constitute the inaugural documentation of *Alternaria cerealis* and *Alternaria arborescens* as a pathogenic organism for tomato crops within the geographical confines of Kenya. Basing on these results, it is essential to consider the variability of pathogen populations in the management of Alternaria pathogens in tomato crops. Our results also highlight the importance of conducting regular surveillance and monitoring of the Alternaria pathogen populations in tomato crops in different AEZs. This information can be used to develop appropriate control strategies and management practices that are tailored to specific pathogen populations. Further, it is a need for further studies to investigate the genetic and molecular basis of the observed differences in conidial lengths among the pathogen isolates.

**Table 7. Molecular identification of selected Alternaria pathogens isolated from tomato from Kirinyaga Country in Kenya**

| Isolate | Place of isolation | Close match | GenBank Accession No. | % Sim[a] | County |
|---|---|---|---|---|---|
| EB_KER7 | Kidaruni | *Alternaria cerealis* | NR_136117.1 | 100 | New Zealand |
| EB_KDG1 | Kiangunga | *Alternaria arborescens* | NR_135927.1 | 99.62 | USA |
| EB_WNG4 | Wanguru | *Alternaria solani* | KT354939.1 | 87.98 | Egypt |
| EB-KAM8 | Kiumbu | *Alternaria solani* | MG012289.1 | 99.82 | China |
| EB_NDM3 | Thumaita | *Alternaria alternata* | LC440583.1 | 99.64 | Japan |
| EB_KRG9 | Kerigo | *Alternaria alternata* | MK690429.1 | 99.33 | China |
| EB-NJ12 | Thumaita | *Alternaria sp,* | MN636300.1 | 99.82 | Kenya |
| EB-ND4 | Ndindiruki | *Alternaria alternata* | MK656442.1 | 100 | China |

[a] *Percentage similarity*

**Table 8. Susceptibility of selected tomato varieties to Alternaria pathogen isolated from tomato leaves in Kirinyaga County**

| Isolates | Varieties | N | Severity (%) | Lsd ($p < 0.05$) | Cv (%) | Mean (%) |
|---|---|---|---|---|---|---|
| EB-KAM8 | Hansol $F_1$ | 6 | 64.81 [bc] | | | |
| | Kilele $F_1$ | 6 | 78.24 [a] | | | |
| | Rambo $F_1$ | 6 | 66.20 [bc] | 8.43 | 10.44 | 67.96 |
| | Riotinto $F_1$ | 6 | 72.22 [ab] | | | |
| | Terminator $F_1$ | 6 | 58.33 [c] | | | |
| EB_KER7 | Hansol $F_1$ | 6 | 64.81 [a] | | | |
| | Kilele $F_1$ | 6 | 69.91 [a] | | | |
| | Rambo $F_1$ | 6 | 67.59 [a] | 8.57 | 11.18 | 64.44 |
| | Riotinto $F_1$ | 6 | 65.74 [a] | | | |
| | Terminator $F_1$ | 6 | 54.17 [b] | | | |
| EB_NDM3 | Hansol $F_1$ | 6 | 61.11 [bc] | | | |
| | Kilele $F_1$ | 6 | 70.83 [a] | | | |
| | Rambo $F_1$ | 6 | 66.67 [abc] | 8.49 | 10.98 | 65 |
| | Riotinto $F_1$ | 6 | 68.06 [ab] | | | |
| | Terminator $F_1$ | 6 | 58.33 [c] | | | |
| EB_WNG4 | Hansol $F_1$ | 6 | 58.33 [c] | | | |
| | Kilele $F_1$ | 6 | 76.39 [a] | | | |
| | Rambo $F_1$ | 6 | 61.57 [bc] | 7.57 | 9.83 | 64.72 |
| | Riotinto $F_1$ | 6 | 66.20 [b] | | | |
| | Terminator $F_1$ | 6 | 61.11 [bc] | | | |
| Mean (%) | | | 65.53 | | | |
| LSD ($p < 0.05$) | | | 3.99 | | | |
| Cv (%) | | | 10.62 | | | |

[a] *Means followed by the same letters are not significantly different at α = 0.05*

**Table 9. Susceptibility of selected tomato varieties isolates of Alternaria pathogen isolated from tomato leaves in Kirinyaga County**

| | Susceptibility level (%) | | | | |
|---|---|---|---|---|---|
| **Isolate** | **Riotinto $F_1$** | **Hansol $F_1$** | **Rambo $F_1$** | **Kilele $F_1$** | **Terminator $F_1$** |
| EB-KAM8 | 51.23 [bc] | 64.82 | 66.20 | 78.24 | 34.87 |
| EB_WNG4 | 54.32 [ab] | 58.33 | 61.57 | 76.39 | 37.96 |
| EB_NDM3 | 55.86 [a] | 61.11 | 66.67 | 70.83 | 40.12 |
| EB_KER7 | 50.31 [c] | 64.81 | 67.59 | 69.91 | 37.96 |
| Mean (%) | 68.06 | 62.26 | 65.51 | 73.84 | 57.98 |
| LSD ($p < 0.05$) | 3.67 | 7.43 | 10.60 | 8.22 | 7.23 |
| Cv (%) | 9.73 | 9.91 | 13.44 | 9.24 | 10.35 |

[a] *Means followed by the same letters in column are not significantly different at α = 0.05*

#### 4.1.1 Evaluation of aggressiveness of *Alternaria* fungal pathogens of tomato

Understanding the aggressiveness level of different pathogen isolates is critical for assessing the potential crop damage caused by individual isolates [60]. In this study, the blight pathogen isolates displayed significant differences in their aggressiveness level, which could be attributed to environmental conditions, farming practices, and the occurrence of different pathogen strains. Molecular analysis revealed the involvement of four Alternaria species in this study. According to reports, environmental factors such as temperature and moisture variations, as well as cropping systems, can influence the aggressiveness of early blight pathogens such as *Alternaria solani* [61,34,62]. For instance, monoculture or mixed cropping can impact pathogen structure and affect aggressiveness. Variation in the aggressiveness of isolates may indicate the existence of different pathogen races, which can result in synergistic interactions and increased crop damage [63,64]. These findings are consistent with those of van der Waals et al. [65]. Differences in the aggressiveness of blight pathogen isolates may influence the susceptibility of crop varieties in the region [54]. Further studies are needed to investigate the factors that influence the aggressiveness of Alternaria blight pathogens in the study area.

### 4.2 Susceptibility of Tomato Varieties to Foliar Fungal Disease Pathogen Isolates from Kirinyaga County

Cultivation of crop varieties that are tolerant or resistant to diseases can decrease the cost of disease management and protect the environment from the continuous use of harmful fungicides. To identify and select resistant varieties that can be adopted by farmers in order to lower the high production costs associated with fungicide use, screening of tomatoes against disease pathogens in different niches may be helpful. The tomato varieties screened against various Alternaria disease pathogens in this study showed significant variation in their susceptibility level. Kilele $F_1$ variety was found to be the most susceptible to Alternaria pathogen isolates (73.84%), while Terminator $F_1$ was the least susceptible (57.99%). The differences in the susceptibility of the tested tomato varieties suggest variation in their genetic makeup. Varieties with a high susceptibility rating may lack genes that confer resistance to early blight infection [66,67]. These results can be compared to the findings of Mphahlele et al. [66-71], who observed variation in the susceptibility of Money-maker and Rodade tomato varieties to early blight isolates in South Africa. However, there was no significant difference in the effect of isolates on the susceptibility of individual tomato varieties screened in this study. The findings of this study imply that tomato varieties screened do not have resistance towards Alternaria pathogens in Kirinyaga County. However, Terminator $F_1$ variety displayed low susceptibility when compared to the rest of the varieties screened. The results deviate from the findings of Mphahlele et al. [19] in Limpopo Province, where a significant effect of early blight isolates on the susceptibility of individual tomato varieties was observed. Furthermore, the results of this study differed from those of Bessadat et al. [60] and Ragupathi et al. [48], which reported variation in the susceptibility of tomato varieties to early blights in Algeria and India, respectively.

## 5. CONCLUSION

Cultural analysis of Alternaria pathogen isolates from tomato plants with early blight-like symptoms revealed the presence of different

species, including *A. solani*, *A. alternata*, and *A. cerealis*. The morphological analysis of the isolates showed variations in colony colour, texture, and spore morphology. Microscopic examination of the spores revealed distinct morphological features such as the shape, size, and septation pattern. Molecular analysis revealed involvement of different Alternaria pathogen in causing tomato blight in Kirinyaga County. In terms of susceptibility, the study found that different tomato varieties had varying levels of susceptibility to Alternaria pathogen isolates. Kilele $F_1$ variety expressed the most severe symptoms of early blight to isolate EB-KAM8, while Terminator $F_1$ had the least severe symptoms. In conclusion, this study highlights the importance of using a combination of cultural, morphological, microscopic, and molecular techniques in identifying and characterizing Alternaria pathogen isolates from tomato plants. It also underscores the need to select tomato varieties that exhibit resistance to Alternaria pathogen isolates, such as Terminator $F_1$.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Adhikari P, Yeonyee O, Panthee DR. Current status of early blight resistance in tomato: an update. International Journal of Molecular Sciences. 2017;18(10)
2. Awan ZA, A Shoaib. Combating early blight infection by employing *Bacillus subtilis* in combination with plant fertilizers. Current Plant Biology. 2019;100125.
3. Sallam N, AbdElfatah HAS, Dawood MF, Hassan EA, Mohamed MS, Khalil Bagy HM. Physiological and histopathological assessments of the susceptibility of different tomato (*Solanum lycopersicum*) cultivars to early blight disease. European Journal of Plant Pathology. 2021;160: 541–556.
4. Gulzar N, Kamili AN, Shah MA. Tomato early blight (*Alternaria solani*), pathogen disease development and defense response phytohormone signaling, International Research Journal of Plant Science. 2021;12(4).
5. Ogolla FO, Muraya MM, Onyango BO. Occurrence of fungal foliar diseases of tomato in different agro-ecological zones of Kirinyaga County, Kenya. Fundamental and Applied Agriculture. 2022a;7(1):31–46.
6. Jaetzold R, Schmidt H, Hornetz B, Shisanya C. Farm management handbook of Kenya: part C, East Kenya. 2nd ed., Nairobi: Ministry of Agriculture. 2007;2.
7. Ogolla FO, Onyango BO, Muraya MM. Tomato cultivation and farmers' knowledge on selected foliar fungal diseases in agro-ecological zones of Kirinyaga county, Kenya. Asian Journal of Agricultural and Horticultural Research. 2022b;9(4):66-80.
8. Dillon WW, Meentemeyer RK. Direct and indirect effects of forest microclimate on pathogen spillover. Ecology. 2019;100(5): e02686.
9. Francisco CS, Ma X, Zwyssig MM, McDonald BA, Palma Guerrero J, Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. Scientific Reports. 2019;9(1):9642.
10. Bhunjun CS, Phillips AJL, Jayawardena RS, Promputtha I, Hyde KD. importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on koch's postulates. Pathogens. 2021;10(9):1096
11. Simmons E. Alternaria, an identification manual. CBC Biodiversity Series. 2007;6: 1–775.
12. Tan DHS, Sigler L, Gibas CFC, Fong IW, Disseminated fungal infection in a renal trans- plant recipient involving *Macrophomina phaseolina* and *Scytalidium dimidiatum*: Case report and review of taxonomic changes among medically important members of the *Botryosphaeriaceae*. Medical Mycology. 2008;46:285–292
13. Lawrence DP, Rotondo F, Gannibal PB. Biodiversity and taxonomy of the pleomorphic genus Alternaria. Mycological Progress. 2016;15(3).
14. Meena P, Chattopadhyay C, Kumar V, Meena R, Rana U. Spore behaviour in atmosphere and trends in variability of *Alternaria brassicae* population. Indian Journal of Mycology and Plant Pathology. 2005;35:511.
15. Kaur L. Multiple disease resistant sources of mungbean. Acta Horticulturae. 2007; 752:423–426
16. Ramjegathesh R, Ebenezar E. Morphological and Physiological Characters of *Alternaria alternata* Causing

Leaf Blight Disease of Onion, International Journal of Plant Pathology. 2012;3:34-44.
17. Kumar V, Haldar S, Pandey K, Singh R, Singh A, Singh P. Cultural, morphological, pathogenic, and molecular variability amongst tomato isolates of *Alternaria solani* in India. World Journal of Microbiology and Biotechnology. 2008;24 (7):1003-1009
18. Alhussaen KM. Morphological and physiological characterization of *Alternaria solani* isolated from tomato in Jordan valley. Research Journal of Biological Sciences. 2012;7:316–319.
19. Mphahlele GH. Characterisation and aggressiveness of tomato early blight fungus (*Alternaria solani*) in Limpopo province. Msc, university of Limpopo, South Africa; 2017.
20. Mugao L, Muturi P, Gichimu B, Kamiri A. Morphological and molecular characterization of *Alternaria solani* and *Phytophthora infestans* Isolates from Tomato Farms in Kenya. Plant Pathology Journal. 2021;20:29-40.
21. McCartney HA, FSJ, Fraaije BA, Ward E. Molecular diagnostics for fungal plant pathogens. Pest Management Science. 2003;59(2):129–142.
22. Toumatia O, Yekkour A, Goudjal Y, Riba A, Coppel Y, Mathieu F, Sabaou N, Zitouni A. Antifungal properties of an actinomycin D-producing strain, *Streptomyces* sp. IA1, isolated from a Saharan soil. Journal of Basic Microbiology. 2015;55(2):221–8.
23. Naroei K, Salari M. Reliable detection of the fungal pathogen, molecular detection and identification *Fusarium oxysporum*, Biological Forum – An International Journal. 2015;7(2):473-492
24. Luchi N, Ioos R, Santini A. Fast and reliable molecular methods to detect fungal pathogens in woody plants. Applied Microbiology Biotechnology. 2020;104: 2453–2468
25. Nuwamanya A, Mwangi M, Runo S, Prevalence and farmers' management of major tomato fungal diseases in Kiambu, Kirinyaga and Kajiado Counties, Kenya, in 3rd Phytosanitary Conference, Nairobi, Kenya; 2021.
26. Chaerani R. Early blight resistance in tomato: screening and genetic study, Ph. D (Doctoral dissertation, thesis, Wageningen University. Wageningen); 2006.
27. Odilbekov F. Resistance to early blight in potato and genetic structure of the pathogen population in Southeast Sweden. Thesis Swedish University of Agricultural Science. 2015;46.
28. Serede IJ, Mutua BM, Raude JM Calibration of channel roughness coefficient for thiba main canal reach in mwea irrigation scheme, Kenya. Hydrology. 2015;3(6):55-65.
29. Kali A, Srirangaraj S, Pravin CM. A modified fungal slide culture technique. Indian Journal of Pathologists and Microbiologists. 2014;57:356-7.
30. Loganathan M, Venkataravanappa V, Saha S, Rai AB, Tripathi S, Rai RK, Pandey AK, Chowdappa P. Morphological, pathogenic and molecular characterizations of *Alternaria* Species causing early blight of tomato in northern India, proceedings of the National Academy of Sciences, India - Section B: Biological Sciences. 2016;86(2):325-330.
31. Kaur T, Yadav AN, Sharma S, Singh N. Diversity of fungal isolates associated with early blight disease of tomato from mid Himalayan region of India. Archives of Phytopathology and Plant Protection. 2020;53(4):1-13
32. Agrios N. Plant pathology, 5th Edition ed., Amsterdam, London: Elsevier-Academic Press; 2005.
33. Pryor B, Michailides T. Morphological, pathogenic, and molecular characterization of Alternaria isolates associated with Alternaria late blight of pistachio. Phytopathology. 2002;92:406–16.
34. Tymon LS, Cummings TF, Johnson DA. Pathogenicity and Aggressiveness of Three Alternaria spp. on Potato Foliage in the U.S. Northwest. The American Phytopathological Society. 2015;100(4): 797-801.
35. Goufo P, Fontem DA Ngnokam D. Evaluation of plant extracts for tomato late blight control in Cameroon. New Zealand Journal of Crop and Horticultural Science. 2010;38(3):171-176.
36. Lee S, Taylor J. Isolation of DNA from fungal mycelia and single spores. in PCR protocols: A guide to methods and applications, Innis M, Gelfand D, Sninsky J, White T, Eds., San Diego, CA, USA, Academic Press. 1990;282–287.
37. White T, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to methods and applications. Innis MA,

Gelfand DH, Sninsky JJ, White TJ, Eds., San Diego, USA: Academic Press, 1990;315-322.

38. Aslam S, Tahir A, Aslam MF, Alam MW, Shedayi AA, Sadia S. Recent advances in molecular techniques for the identification of phytopathogenic fungi – a mini review. Journal of Plant Interactions. 2017;12(1): 493-504.
39. Desta M, Yesuf M. Efficacy and economics of fungicides and their application schedule for early blight (*Alternaria solani*) management and yield of tomato at South Tigray, Ethiopia. Journal of Plant Pathology and Microbiology. 2015;6(5):1000268.
40. Negesa H, Ayana G. Spatial distribution of tomato early blight and reaction of some tomato varieties to the disease in Southern Tigray, Ethiopia. Journal of Plant Pathology & Microbiology. 2021;12(11): 1- 7.
41. Tamura K, Stecher G, Kumar S. MEGA 11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution; 2021.
42. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987;4:406-425.
43. Tamura K, Qiqing T, Kumar S. Theoretical foundation of the reltime method for estimating divergence times from variable evolutionary rates. Molecular Biology and Evolution. 2018;35:1770-1782.
44. Sharma M, Deep S, Bhati DS, Chowdappa P, Selvamani R, Sharma P. Morphological, cultural, pathogenic and molecular studies of *Alternaria* brassicae infecting cauliflower and mustard in India. African Journal of Microbiology Research. 2013;7(26):3351-3363.
45. Rotem J. The genus *Alternaria*: biology, epidemiology and pathogenicity, Minnesota: APS Press, St Paul; 1994.
46. Singh D, Singh R, Singh H, Yadav R, Yadav N, Barbetti M, Salisbury P, Nimbal S, Chattopadhyay C, Kumar A. Cultural and morphological variability in *Alternaria brassicae* isolates of Indian mustard (*Brassica juncea L.* Czern and Coss). in Proceeding of the 12th International Rapeseed Congress, 26-30 March , Wuhan, China; 2007.
47. Blanchard D. Tomato diseases: identification, biology and control: Colour handbook, 2nd ed., Manson Publishing. 2012;688.
48. Ragupathi KP, Renganayaki P, Sundareswaran S, Kumar S, Kamalakannan A. Characterization of *Alternaria* species causing early blight of tomato. International Journal of Current Microbiology and Applied Sciences. 2020;9(12):2603-2609.
49. Ismael JHs, Omer SA. Diagnosis and in vitro control of tomato leaf spot caused by *Alternaria alternata* In Sulaimani governorate. Journal of Zankoy Sulaimani – Special Issue, 2ndInt. Conference of Agricultural Sciences. 2018;637-650.
50. Nolla J. A new Alternaria Disease of onions (Allium cepa),. Phytopathology. 1927;17:115-137.
51. Neergaard P, Danish species of *Alternaria* and *Stemphylium*. taxonomy, parasitism, economic significance, Copenhagen: Einar Munksgaard; 1945.
52. Abubakar L, Ado S. Genotype x environment interaction for resistance to purple blotch (*Alternaria porri L. (Ellis) Cif*.) in onion (*Allium cepa L.*) in Nigeria. Asian Journal Of Crop Science. 2009:1(1):15-25.
53. Gobashy SFEl, Mikhail WZ, Ismail AM, Zekry A, Moretti A, Susca A, Soliman AS. Phylogenetic, toxigenic and virulence profiles of *Alternaria* species causing leaf blight of tomato in Egypt. Mycological Progress. 2018;17:1269-1282.
54. Bessadat N, Benichou S, Kihal M, Henni DE. Aggressiveness and morphological variability of small spore *Alternaria* species isolated from Algeria. Journal of Experimental Biology and Agricultural Sciences. 2014;2(2S).
55. Ramezani Y, Taheri P, Mamarabadi M. Identification of *Alternaria spp.* associated with tomato early blight in Iran and investigating some of their virulence factors. Journal of Plant Pathology. 2019;101:647-659.
56. Bosqueiro AS, Bizarria Jr R, Rosa Magri MM. Biocontrol of post-harvest tomato rot caused by *Alternaria arborescens* using *Torulaspora indica*. Biocontrol Science and Technology. 2023;23(2):1-18.
57. Simmons E. Alternaria themes and variations. 2000;244-1-115.
58. Mahmoud GAE, Abdel Sater MA, Al Amery E, Hussein NA. Controlling *Alternaria cerealis* MT808477 tomato phytopathogen by *Trichoderma harzianum* and tracking

the plant physiological changes. Plants. 2021;10(9):1846.
59. Razak NJ, Abass MH. First report of *Cladosporium cladosporioides*, *C. oxysporum*, and *C. uredinicola* as potential pathogens on tomato shoots system in Iraq. Applied Nanoscience. 2023;13(2): 1065-1072
60. Bessadat N, Benichou S, Kihal M, Henni D. Aggressiveness and morphological variability of small spore *Alternaria* species isolated from Algeria. Journal of Experimental Biology and Agricultural Sciences. 2014;2(2S):265-278.
61. B. Tsedaley. Review on early blight (*Alternaria* spp.) of potato disease and its management options. Journal of Biology, Agriculture and Healthcare. 2014;4: 2224–3208.
62. Hussain A, Ali S, Abbas H, Ali H, Hussain A, Khan SW. Spatial distribution of early blight disease on tomato, climatic factors and bioefficacy of plant extracts against *Alternaria solani*. Acta Scientiarum Polonorum Hortorum Cultus. 2019;18(6): 29–38.
63. Bonde R. Physiological strains of *Alternaria solani*. Phytopathology. 1929; 19:533–548.
64. Bessadat N, Berruyer R, Hamon B, Bataille Simoneau N, Benichou S, Kihal M, Henni D, Simoneau P. *Alternaria* species associated with early blight epidemics on tomato and other *Solanaceae* crops in northwestern Algeria. European Journal of Plant Pathology. 2017;148(1):181-197.
65. Van Der Waals J, Korsten L, Slippers B. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. Plant Disease. 2004;88:959-964.
66. Chaerani R, Voorrips RE. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. Journal of General Plant Pathology. 2006;72:335–347.
67. Ramathani I, Ddamulira G, Kangire A, Wasswa P, Tusiime A. Evaluation of tomato genotypes for tolerance to major diseases in Uganda. African Crop Science Journal. 2021;29(2):241–258.
68. Wang J, Xiao S, Zheng L, Pan Y, Zhao D, Zhang D, Li Q, Zhu J, Yang Z. Multiomic approaches reveal novel lineage-specific effectors in the potato and tomato early blight pathogen *Alternaria solani*. Phytopathology Research. 2022;4(1).
69. Amini J, Kazemi M, Abdollahzadeh J, Darvishnia M. Identification of *Fusarium* spp. and their pathogenicity associated with root rot of tomato, Marvdasht. Iranian Journal of Plant Protection Science. 2013;44(1):Pe71-Pe80.
70. Micah RI, et al. Occurrence, Identification, and Pathogenicity of Fusarium spp. Associated with Tomato Wilt in Mexico. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2018;46(2): 484-493.
71. Mphahlele GH, Kena MA, Manyevere A. Evaluation of aggressiveness of *Alternaria solani* isolates to commercial tomato cultivars. Archives of Phytopathology and Plant Protection. 2020;53(11-12).



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