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The Occurrence and Characterization of *Alternaria alternata* Causing Leaf Spots Disease of Spinach in Nandi and Uasin Gishu Counties, Kenya

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Abstract

Spinach (Spinacia oleracea) is an important vegetable crop in Kenya cultivated by small-scale farmers for domestic use and commercial production. Alternaria leaf spot caused by Alternaria alternata is among the economically significant diseases of spinach causing low yield and poor-quality crop. There is little information available on occurrence and the nature of Alternaria leaf spot disease of spinach in Kenya. The aim of the present study was to establish the occurrence and characterize the causal pathogen in the laboratory and in farmers' fields. An extensive survey was conducted in seven selected Sub-counties namely; Mosop, Chesumei, Aldai, Nandi Hills, Emgwen of Nandi County and Kapseret, Moiben of Uasin Gishu county. In each farm the disease incidence was determined and diseased samples collected for isolation, identification and characterization in the laboratory. The study revealed that the disease occurred in all the Sub-counties surveyed. Out of the 40 farms surveyed 23 (57.5%) had leaf spot disease with a mean incidence of 21.37 %. The highest disease incidence was observed in Mosop Sub-county (41.31%). Similarly, when isolates were studied in the laboratory, their morphological characteristics varied from fairly compact to luxuriant mycelial growth, texture varied from feathery to cottony. The growth rate was between 2.98 and 4.05 mm/day, and the spore count after 12 days was between 4.68×10^4 and 7.4×10^4 conidia/ml. This is the first report on the occurrence and incidence of Alternaria leaf spot caused by Alternaria alternata of spinach in Kenya. This therefore necessitates the development of management practices to reduce crop loss due to the pathogen.

Keywords: Spinach, *Alternaria alternata*, Incidence, Colony Characterization

INTRODUCTION

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae and is native to central and western Asia. It is one of the most common vegetable cultivated worldwide (Sato *et al.*, 2010, Wachira *et al.*, 2014). In Kenya spinach is grown majorly by small scale holders for home consumption and for the market. The crop favours well fertile and well drained sandy loam soil. The rising demand of spinach has resulted to an increment in cultivation of this vegetable in Kenya (MoA, 2016). The total area under spinach production in Kenya was 5,615 hectares producing 75,563 tonnes in 2016 (FAOSTAT, 2016). Spinach has health benefits that include lowering the risk of cancer, enhancing bone health, lowering blood pressure, enhancing blood glucose control in people with diabetes and reducing the risk of developing asthma.

Spinach like other vegetables is infested by pests and diseases. Among the major diseases infecting spinach are bacterial soft rot, cucumber mosaic virus, downy mildew,

fusarium wilt, white rust and alternaria leaf spots which cause huge losses (MOA, 2006). Thomma (2003) noted that the genus *Alternaria* is a significant phytopathogen infecting crops in the fields, causing foliage diseases and is further credited for post-harvest spoilage of crops and other plant products. In addition, Kuna *et al*. (2011) noted that the sensitivity to *Alternaria* sp. is a key factor in inducing asthma and allergic rhinitis on immune-depressed patients. The sum losses caused by *Alternaria* sp., rank among the highest caused by any plant pathogen (Agrios, 2005). Leaf spot disease on spinach caused by *Alternaria* sp. has been documented to cause vast economic losses in Saudi Arabia (Marraiki *et al*., 2012), Poland (Czajka *et al.,* 2015) and Pakistan (Aslam *et al.,* 2019) where the mortality due to the disease was found to range between 20-80%**.** The main aim of this study was to establish the occurrence of leaf spot and characterize the causal pathogen.

MATERIALS AND METHODS

Determination of Incidences and Collection of Leaf Spot Diseased Leaves of Spinach

Field surveys were conducted in September 2018, during the rainy season in the different agro ecological zones of Nandi and Uasin Gishu counties of Kenya. Forty farmers' fields at approximately five kilometers apart having spinach at different stages of growth and development were sampled. The leaf spot incidence on each farm surveyed was recorded by drawing three (3) quadrants of $1m^2$. Spinach plants showing symptoms such as small, circular dark black coloured spots along the margins encircling the necrotic spots with concentric ring were counted and used to determine the percentage incidence over total spinach plants in the quadrant. The incidence from each farm was calculated as described by Bdliya and Gwio-Kura (2007).

$$\text{Disease Incidence (\%)} = \frac{\text{Total plants infected}}{\text{Total plants in the quadrant}} \times 100$$

Altitude of the surveyed area was recorded as read from Geographical Position System (GPS) equipment. Collection of diseased leaves was randomly sampled from infected spinach in the fields during survey. The collected leaves were then kept in a keep cool box containing ice (4^{o}C) and brought to the Microbiology laboratory at the University of Eldoret for further studies.

Data Analysis

The Alternaria leaf spot incidence per farm was first calculated into percentage per quadrant and the mean of the three quadrants in each was calculated and taken as the disease incidence of the farm. The obtained percentage incidence was then entered into excel and were analysed by Analysis of Variance (ANOVA) procedure using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 0.05.

Isolation of Alternaria Species

One centimeter portion of the diseased section with early levels of infection was cut from diseased leaves and washed in running tap water for 30 seconds and thereafter surface sterilized in 1% sodium hypochlorite for 3 minutes. They were then rinsed in three changes of sterile distilled water and plated onto potato dextrose agar (PDA) media in a 90mm diameter petri plate under aseptic conditions. The inoculated petri dishes were maintained at room temperature (25 ± 2^{o}C) and observations made from 24 hours onwards. After 5 days, hyphal tip transfer was made onto sterilized PDA. Pure cultures of sporulating fungi were obtained by single spore isolation technique as described by Noman *et al.* (2018) and grown in PDA medium. The isolates were designed as follows: NM01, NM02 … to NM09 for isolates from Mosop sub-county of Nandi, NC01, NC02…to NC06 for isolates from Chesumei Sub-county of Nandi, NA01, NA02…to NA05 for isolates from Aldai Sub-county of Nandi, NE01, NE02…to

NE05 for isolates from Emgwen sub-county of Nandi, NH01, NH02…to NH05 for isolates from Nandi Hills Sub-county of Nandi, UK01, UK02… to UK06 for isolates from Kapseret Sub-county of Uasin Gishu, UM01, UM02…to UM04 for isolates from Moiben Sub-county of Uasin Gishu. The pathogen, *Alternaria alternata*, were identified using taxonomic keys, cultural and morphological reference as illustrated by Ellis (1971) and Simmons (2007) and confirmed by pathogenicity tests to fulfill Koch's postulates.

Cultural and Morphological Features

A disc of 10 mm in diameter was cut with a cork borer from 10-day old single spore cultures grown on PDA and moved onto the middle of freshly prepared PDA plates. Three replications for each of the twenty three isolates were maintained. The petri plates were incubated at 25^{o}C in a 24-hour dark cycle for ten days. The experimental design was a completely randomized design. The experiment was repeated twice. The cultures in the petri plates were then used to study the nature of aerial mycelium development, pigmentation (substrate), conidial size, growth rate and to later establish spore quantities in each isolate.

Data Collection and Data Analysis

The pigmentation on the mycelium and the substrate was studied using mycological colour chart as described by Rayner (1970) while the nature of aerial mycelium was studied by visual observation. The growth rate of each of the isolates was taken after every 24 hours and further the growth calculated by taking the mean on a 90mm diameter petri plate divided by 14 (number of days the fungus grew). The data on growth rate were analysed by ANOVA procedures using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 0.05. The conidial measurement was done by taking a block of 0.5 cm of agar from ten days old PDA culture and mounted directly on a slide with a drop of in lactophenol cotton blue stain and covered with a cover slip and viewed under a light microscope. Morphology of conidia was described and measurements made in micrometres (μm). Fifteen conidia per isolate were measured to ascertain the length and the width using a light microscope fitted with a micrometre. Determination of the breadth was done by measuring the widest part of the conidia.

Determination of Sporulation and Spores in *Alternaria alternata*

To estimate sporulation, conidial suspension was prepared from each culture plate using a modification of Jeff and Janet (2012) method. Ten-day old single spore cultures grown on PDA media were flooded with ten milliliters of sterile distilled water, gently rubbed with a sterilized glass rod and suspended in sterile universal bottles and put in a rotary shaker at 150 revolutions per minute (rpm) for 20 minutes. The spore suspension was then filtered through two layers of sterile cheese cloth and the conidial suspension calculated using haemocytometer. A mean of five counts was taken per colony.

Data Analysis

The number of spores obtained from 5 counts on the haemocytometer was taken as the mean number of spores produced by each isolate in 3 replicates. The data from these 3 replicates was then analysed by ANOVA procedures using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 0.05. Pearson correlation coefficient was used to determine between the growth rate and sporulation.

RESULTS AND DISCUSSION

Disease Incidence and Field Symptoms of Alternaria Leaf Spot

Out of 40 farms surveyed, 23 (57.50%) farms confirmed the presence of spinach infected with Alternaria leaf spot (Plate 1). The Alternaria leaf spot disease incidence ranged from 25.79% in Chesumei sub-county to 50.51% in Mosop sub-county among the farmers visited, with a mean of 21.37% across all the areas surveyed (Table 1). The occurrence of the disease did not differ significantly ($P \leq 0.05$) among the various sites surveyed except at Kapkong'ony and Kabiyet.

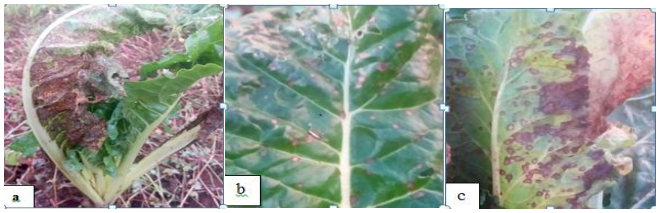

Plate 1: Diseased Spinach Plants Showing Leaf Spot Symptoms (a), Diseased Leaf Showing the Characteristic Black Spots on the Front Side (b), Characteristic Black Spots on the Back Side (c) of a Spinach Plant Leaf.

Table 1: Incidence of Alternaria Leaf Spot of Spinach from Various Sites of Nandi and Uasin Gishu Counties during 2018

County	Sub-county	Site	Altitude(m.a.s.l)	*A. alternata* isolate	Mean incidence (%)
Nandi	Chesumei	Chepterit	2038.67	NC05	0a
Nandi	Chesumei	Lelboinet	2018.84	NC06	0a
Nandi	Aldai	Kibwareng	1784.34	NA04	0a
Nandi	Aldai	Mugundoi	1925.67	NA05	0a
Nandi	N/Hills	Samoei	2041.65	NH02	0a
Nandi	N/Hills	Chekunyuk	2103.45	NH03	0a
Nandi	N/Hills	Chemase	1538.81	NH04	0a
Nandi	N/Hills	Kapsimatwo	1918.22	NH05	0a
Nandi	Emgwen	Kipsigak	2016.72	NE02	0a
Nandi	Emgwen	Tulon	1976.54	NE03	0a
Nandi	Emgwen	Meswo	2001.16	NE04	0a
Nandi	Emgwen	Kilibwoni	1998.68	NE05	0a
U/Gishu	Kapseret	Kipkenyo	1996.56	UK05	0a
U/Gishu	Kapseret	Simat	1992.67	UK06	0a
U/Gishu	Moiben	Kimumu	2156.63	UM02	0a
U/Gishu	Moiben	Chepkoilel	2196.41	UM03	0a
U/Gishu	Moiben	Kuinet	2070.62	UM04	0a
Nandi	Chesumei	Kaptel	1978.32	NC04	25.79b
Nandi	Emgwen	Emgwen	1984.78	NE01	30.04bc
Nandi	Chesumei	Chemundu	1946.51	NC03	31.73bcd
Nandi	Aldai	Samitui	1803.25	NA01	31.79bcd
U/Gishu	Kapseret	Kapseret	2146.65	UK02	32.03bcde
U/Gishu	Kapseret	Tuiyo	2134.54	UK03	32.64bcde
Nandi	Chesumei	Kosirai	2044.48	NC01	33.11bcde
Nandi	Mosop	Kilagan	1977.03	NM01	33.7bcde
U/Gishu	Kapseret	Kapkagaron	1894.69	UK01	33.77bcde
Nandi	Aldai	Kaptumo	1925.78	NA03	34.72bcdef
Nandi	Mosop	Kaprus	1924.65	NM02	35.39bcdef
U/Gishu	Kapseret	Mlango	2038.67	UK04	35.75bcdef
Nandi	Mosop	Kapmazia	1838.55	NM06	37.57cdefg
Nandi	Aldai	Kobujoi	1824.14	NA02	38.21cdefg
Nandi	Mosop	Salient	1878.89	NM05	38.55cdefg
U/Gishu	Moiben	Moiben	2154.08	UM01	40.7cdefgh
Nandi	Chesumei	Chemuswo	2023.62	NC02	41.23defgh
Nandi	N/Hills	Nandi Hills	1998.24	NH01	41.53defgh
Nandi	Mosop	Cheptil	1951.84	NM03	41.62defgh
Nandi	Mosop	Kabisaga	1946.82	NM08	42.65efgh
Nandi	Mosop	Kapelem	1864.65	NM09	45.08fgh
Nandi	Mosop	Kabiyet	1898.72	NM04	46.7gh
Nandi	Mosop	Kapkong'ony	1872.03	NM07	50.51h
				Mean	21.37
				CV	31.2
				LSD	10.844

Values with the same letter(s) along the same column are not significantly different (P≤0.05). Legend; N/Hills-Nandi Hills; U/Gishu-Uasin Gishu

Alternaria leaf spot occurred in all the seven sub-counties surveyed. There was no significant difference (P≤0.05) among Emgwen, Nandi Hills and Moiben sub-counties but they differed significantly from Chesumei, Aldai and Kapseret. Moiben sub-county differed significantly (P≤0.05) from all the sub-counties (Table 2). Alternaria leaf spot prevalence was 100% in Mosop sub-county, 66.67% in Chesumei, 66.67% in Kapseret, 60% in Aldai but the lowest occurrence was in Nandi Hills 20%.

Table 2: Incidence of Alternaria Leaf Spot of Spinach at Various Sub-Counties of Nandi and Uasin Gishu Counties

Sub-county	Mean incidence (%)
Emgwen	6.01a
Nandi Hills	8.31a
Moiben	10.18ab
Aldai	20.94bc
Chesumei	21.98c
Kapseret	22.37c
Mosop	41.31d
Mean	21.37
LSD (5%)	21.37
CV	15.8

Values with the same letter(s) along the same column are not significantly different (P≤0.05)

The mean disease incidence was found higher in the altitudes ≤1900 metres above sea level (m.a.s.l). There was no significant difference (P≤0.05) on disease incidence at 1901-2000 and >2000 m.a.s.l but differed significantly from the disease incidence at ≤1900 m.a.s.l (Fig. 1).

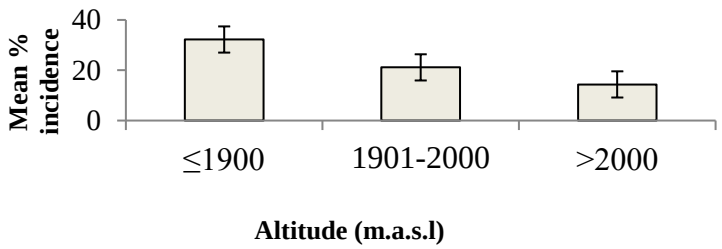

Figure 1: Incidence of Alternaria Leaf Spot at Various Altitudes in Nandi and Uasin Gishu Counties.

Cultural and Morphological characteristics of *Alternaria alternata*

Alternaria alternata showed significant variability in cultural and morphological characteristics on potato dextrose media (PDA) (Table 3). The predominant aerial mycelia growth was moderately compact which was observed in 60.87%, 26.09% had fairly compact and 13.04% had moderately luxuriant (Table 4). The isolates had grayish white cottony compact aerial mycelium (Plate 2a), rough margins and dark reverse with dark black substrate (Plate 2b). Isolates had grayish white compact aerial mycelium with black centre (Plate 2c) and wavy margins with dark green substrate (Plate 2d). Further, other isolates had dense raised grayish white aerial mycelium with small dark concentric rings and circular margins. The conidia varied in shape from mostly muriform to ellipsoidal having smooth walls and 1-3 longitudinal and 2 to 10 transverse septations with cylindrical curved beaks (Plate 2e). The conidial length ranged between 24.76 µm for the shortest to 75.14 µm for the longest and the width ranged from 6.82 µm to14.78 µm (Table 3). The conidia were multi-celled born in chains of up to 10 or more on conidiophores varying

colours from light olivaceous to dark brown as shown in (Plate 2g). The conidiophores were unbranched, arising singly or in clusters, long or short (Plate 2f and h). Conidiophores were olivaceous to olivaceous brown with majority being straight and a few curved. Further, the conidiophores were slightly swollen at the apex having terminal scars.

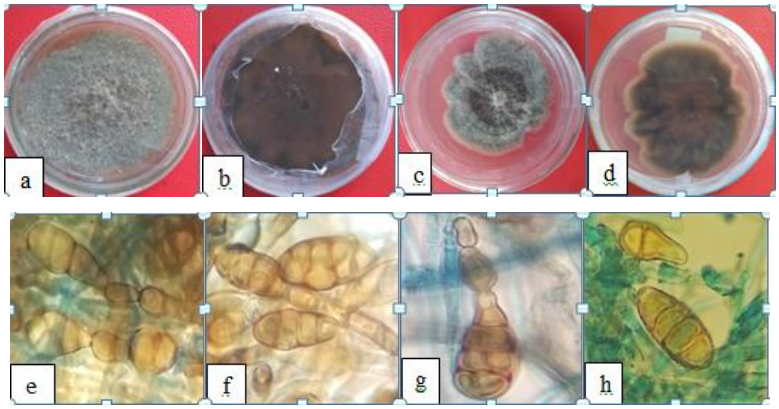

Plate 2: The Pure Cultures of *Alternaria alternata*, Front and Back (a and b) and (c and d) Respectively Showing Colour and Texture of Mycelia. Micrographs of *A. alternata*; (e) Long Conidiophores; (f) Multi-Celled Conidia; (g) Long Smooth Walled Multi-Cell Conidia with both Transverse and Longitudinal Septations, and (h) Short Single Smooth Walled Conidiophores with Cylindrical Curved Beaks.

Table 3: Cultural and Morphological Characteristics of *Alternaria alternata* Isolates
[r= -0.0833, P<0.05=0.7055, 'L' and 'W' represent the conidial size in Length and Width respectively as measured in micrometers (µm)]

Isolate	Aerial mycelium growth	Mycelial texture	Mycelial colour	Substrate colour	Growth rate mm/day	Mean spores per ml	L (µm)	W(µm)
NM01	Moderately compact	cottony	Gray white	Black	3.735	5.6×10^4	64.66	9.64
NM02	Moderately compact	cottony	Gray white	Black	3.635	6.16×10^4	65.46	9.68
NM03	Moderately compact	aerial	Black gray	Dark green	3.85	4.68×10^4	68.24	9.18
NM04	Moderately luxuriant	feathery	Black	Black	3.685	6.64×10^4	42.04	7.82
NM05	Moderately compact	cottony	Black gray	Dark black	3.615	5.96×10^4	62.42	9.21
NM06	Fairly compact	feathery	Green with black center	Dark green	3.65	4.95×10^4	71.52	13.95
NM07	Fairly compact	feathery	Gray green	Gray	3.735	6.2×10^4	69.62	14.62
NM08	Moderately compact	velvet	Gray green	Dark green	3.535	7.2×10^4	58.80	11.16
NM09	Moderately compact	feathery	Black gray	black	4.03	5.4×10^4	69.76	10.41
NC01	Moderately compact	velvet	Black gray	Gray	3.93	7.4×10^4	70.32	11.82
NC02	Fairly compact	cottony	Gray white	Black	3.7	5.16×10^4	60.34	12.61
NC03	Moderately compact	cottony	Gray white	Gray	3.05	6.76×10^4	38.65	7.02
NC04	Moderately luxuriant	velvet	Black gray	Black	3.485	4.84×10^4	40.46	9.46
NA01	Moderately compact	cottony	Gray white	Black	3.565	5.72×10^4	36.82	14.78
NA02	Fairly compact	aerial	Black gray	Dark green	4.015	5.38×10^4	72.48	12.34
NA03	Moderately compact	feathery	Olivaceous green	Dark green	4.05	6.56×10^4	73.02	10.78
UK01	Moderately luxuriant	feathery	black gray	Dark black	3.685	4.8×10^4	44.81	10.34
UK02	Moderately compact	feathery	Gray with black center	Black	2.98	5.12×10^4	28.12	6.82
UK03	Moderately compact	cottony	Gray green	Gray	3.7	6.4×10^4	24.76	8.43
UK04	Fairly compact	cottony	Gray white	Black	4.05	5.0×10^4	75.14	13.98
NH01	Moderately compact	velvet	Gray green	Gray	3.6	5.28×10^4	49.61	10.88
NE01	Moderately compact	aerial	Black gray	Gray	4	4.96×10^4	68.90	12.86
UM01	Fairly compact	aerial	Olivaceous green	Dark green	3.5 3.686086957	5.68×10^4	36.78	7.06

On mycelial colour; 26.09% of the isolates had gray white, 34.78% had black gray, 3.35% had gray with black centre, 3.35% had black, 3.35% had green with black centre, 8.70% had olivaceous green and 17.39% had gray green (Table 4). Majority (39.0%) of the isolates studied, coloured the substrate black, 26.09% had coloured the substrate dark green, 8.70% dark black and 26.09% had gray (Table 4). The predominant mycelial texture was cottony and feathery which was observed in 30.43% of the isolates, while 17.39% had velvet and aerial growth each.

Table 4: Cultural and Morphological Characteristics of *A. alternata* Isolates

Character		Percentage of isolates showing the features
Aerial mycelium growth		
	Moderately compact	60.89
	Fairly compact	26.09
	Moderately luxuriant	13.04
Mycelial texture		
	Cottony	30.43
	Aerial	17.39
	Feathery	30.43
	Velvet	17.39
Mycelial colour		
	Gray white	26.09
	Black gray	34.78
	Black	3.35
	Gray green	17.39
	Olivaceous green	8.70
	Gray with black centre	3.35
	Green with black centre	3.35
Substrate colour		
	Black	39
	Dark green	26.09
	Dark black	8.7
	Gray	26.09
Growth rate		
	Fast growth	21.74
	Moderate growth	65.22
	Slow growth	13.04
Sporulation		
	High sporulation	8.7
	Moderate sporulation	43.48
	Low sporulation	21.74

The isolates were grouped according to the growth rate into fast growth (≥4.0 mm/day), moderate growth (≥ 3.5-3.99 mm/day) and slow growth rate (< 3.5 mm/day). The growth rate ranged between 2.98 mm/day to 4.05 mm/day with a mean growth rate of 3.69 mm/day. The distributions of isolates into fast, moderate and slow growths were 21.74%, 65.22% and 13.04% respectively (Table 4). There was significant difference (P≥0.05) in growth rate among the isolates.

Sporulation

Alternaria alternata isolates showed significant difference (P≤0.05) in sporulation on PDA. Based on sporulation the isolates were grouped into 4 categories. Two (8.70%) isolates had very high sporulation (>7.0×10^4); 26.09% of the

isolates had high sporulation (6.0-7.0×10^4), 43.48% of the isolates had moderate sporulation (5.0-6.0×10^4) and 21.74% of the isolates having low sporulation, (<5.0×10^4. Some isolates from the same sub-county showed variation (P≤0.05) with respect to sporulation (Table 5). For example, isolates NM03 and NM06, NC01 and NC04, and UK01 and UK03 were isolates from the same sub-counties but showed significant variation in sporulation. When the relationship between sporulation and growth rate was compared it was established that there was no correlation between the growth rate and sporulation. The correlation coefficient was negative and low.

Table 5: Sporulation of *A. alternata* Isolates on PDA

A. alternata Isolate	Spores per ml
NM03	46800a
UK01	48000ab
NC04	48400bc
NM06	49500cd
NE01	49600cd
UK04	50000de
UK02	51200ef
NC02	51600fg
NH01	52800gh
NA02	53800h
NM09	54000h
NM01	56000i
UM01	56800i
NA01	57200i
NM05	59600j
NM02	61600k
NM07	62000k
UK03	64000l
NA03	65600m
NM04	66400mn
NC03	67600n
NM08	72000o
NC01	74000p
Mean	57326
CV	1.6
LSD	1476.6

Values with the same letter(s) along the same column are not significantly different (P≤0.05). r= -0.0833, (P<0.05) =0.7055

DISCUSSION

Disease Incidence and Field Symptoms of Alternaria Leaf Spot

Different symptoms of Alternaria leaf spot on spinach were noted during the survey and these included dark black coloured spot margin circular spots starting on the lower leaves upwards. The upper surface of the leaves showed small circular dark spots encircling a necrotic region. The leaves showed loss of vigour, chlorosis and yellowing, drying of some leaves and in severe infection resulted to death of the whole plant. These leaf spot symptoms were similar to the ones reported on spinach by Marraiki *et al.* (2012), Anuj *et al.* (2014), Czajka *et al.* (2015) and Aslam *et al.* (2019). The leaf spot prevalence varied among the sub-counties with Mosop recording the highest incidence (100%), followed by Chesumei and Kapseret. This report is the first to establish an incidence of Alternaria leaf spot in Uasin Gishu and Nandi which showed a mean of 21.37% which was lower than what Czajka *et al.* (2015) reported in Poland (50%) in spinach fields. Aslam *et al.* (2019) from similar study done in Pakistan reported an approximate disease incidence of 45%. The variations in the incidence of Alternaria leaf spot in the farms could have been due to variations in spinach varieties grown, the cropping system practiced, the stage of plant growth during the survey, the handling of the crop residue after harvest and control measures employed by farmers to control the leaf spot disease as reported elsewhere by Marcin *et al.* (2012), Czajka *et al.* (2015), Singh *et al.* (2015) and Aslam *et al.* (2019).

Cultural and Morphological Characteristics of *Alternaria alternata*

Single spore isolates of *Alternaria alternata* from Uasin Gishu and Nandi counties demonstrated variation in cultural and morphological characteristics. In the present study aerial mycelium growth, mycelial texture, mycelial colour, substrate colour, growth rate, conidia size, shape and sporulation were used to group the 23 isolates of *Alternaria alternata* on potato dextrose medium (PDA) incubated at 25ºC for 12 days which collaborated Marraiki *et al.* (2012) who had isolated *Alternaria alternata* from the spinach leaves in Saudi Arabia and reported pure cultures on PDA at 25±1ºC had grayish white colony which later became black. The conidia had 3-8 transverse septations and 1-2 longitudinal septation. Conidia were solitary or in short chains, mostly ovoid and evenly walled. The hyphae were septate, branched and brownish with olive brown conidiophore. These findings were in tandem with findings of Czjaka *et al.* (2015) who reported that pure cultures of *Alternaria alternata* had dark gray to black colonies, the septa ranged from 30.95 µm and 43.69 µm long, 11.00 and 12.81 µm in the widest area and 3.00 µm to 3.82 µm in the narrowest area. The present findings noted the septa ranging in length from 24.76 µm to 75.14 µm and the width from 6.82 µm to 14.62 µm. The same results were similar with reports of Aslam *et al.* (2019) from *Alternaria alternata* isolates from spinach in Pakistan. Pervaize *et al.* (2018) also reported similar findings on *Alternaria alternata* isolated from alfa alfa.

Bhaat *et al.* (2000) isolated *Alternaria alternata* from spinach in Anusandham Samithan, India and reported that the isolates on PDA at 25±1ºC had circular and olivaceous black colonies; branched flexuous conidiophores emerging from cicatrize; conidia in long chains, golden brown, obyriform with cylindrical beaks, smooth with 6-8 transverse and several longitudinal septa. Their results are in concurrence with our present results. Further, Sanjeev *et al.* (2017) reported growth rate of 5.6 mm/day in *A. alternata* isolated from brinjals which slightly deviates from the present study (3.69 mm/day), the mycelial growth was dull white with fluffy growth at the centre with excellent sporulation. Similar results had earlier been reported by Singh *et al.* (2001). Chethana *et al.* (2018) studied 6 isolates of *Alternaria alternata* causing purple blotch

disease of onion and reported a significant variation in mycelial colour of the isolates, growth rate, sporulation, conidial length and width. The mycelial colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Isolates varied in sporulation ranging from 0.5×10^5 conidia/ml for low sporulating isolate to 4.5×10^5 conidia/ml for highly sporulating isolate which were similar though with slight variations with results of the current study. The conidial shape was obclavate with colour being golden brown or light brown. The conidial length ranged between 26.89 µm for the shortest to 76.15 µm for the longest and the width ranging from 9.50 to 23.82 µm. Their findings were in tandem to the findings from the present study.

Alternaria alternata isolated from pigeon pea yielded abundant branched, brownish septate mycelia. Conidiophores were simple, septate, olive-brown and varying in length with terminal conidia being either in short chains or solitary. Fully developed conidia measures from 10-30 by 5-12 µm with low coned beak or beakless. Conidia had 3-7 transverse septa, 1-5 longitudinal septa and in chains of 5-15 conidia (Mamta *et al.*, 2013). Their results are in tandem with the findings from the current study and those of Raja and Reddy (2007) and Meena *et al.* (2014). Devappa and Thejakumar (2016) reported a variation in mycelial colour, substrate colour and mycelial texture of *Alternaria alternata* on PDA medium incubated at 30ºC for 10 days. They reported a growth rate of 8.5 mm/day which differed to the present mean of 3.69 mm/day. Dipak *et al.* (2013), while studying *A. alternata* (Fr.) Keissler from *Gerbera jamesonii*, reported similar findings. However, they recorded a varying growth rate from the present study (10.08 mm/day to 3.69 mm/day). Further, they reported a maximum sporulation (0.61×10^4 spores mm^{-2}) in PDA maintained at 27±1ºC for 10 days. The sporulation was recorded within a period of 48 hours, thereby explaining the difference observed in the current. Guo *et al.* (2011) and Waghunde and Patil (2010) noted that the maximum sporulation was obtained at 25ºC which is within the temperature at which the present study was done. Barry and Themis (2001) studied 70 isolates of *A. alternata* and noted that there was a great variation in mycelial colour ranging from lettuce green to olive green colonies with white margins, growth rate of about 7 mm/day which deviates slightly from our findings. The conidia were formed in chains of 6 to 14 in length having ovate shape with apical extensions. Their findings were in agreement with the present study and those of Hashem *et al.* (2014).

The slight variations observed in growth rate and sporulation could be due to a number of factors such as; difference in incubation conditions, composition and availability of nutrients in the medium as reported by Dipak *et al.* (2013) and Devappa and Thejakumar (2016). Dipak *et al.* (2013), Varma *et al.* (2007) and Singh *et al.* (2007) noted variability due to different geographical locations of isolates within Alternaria species. Cultural variability in Alternaria species exists and the characteristics significantly serve the identification of races of the isolates being tested (Rotem, 1994).

CONCLUSIONS

The results from the study verified *Alternaria alternata* as the pathogen causing Alternaria leaf spot on spinach, which can be is a threat for spinach crops for future cultivation.

The *Alternaria alternata* isolates from the spinach showed significant difference in cultural and morphological characteristics pertaining to myeclial growth, mycelial colour, substrate colour, conidial size and growth rate.

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