

OCCURRENCE OF BLACK SPOT DISEASE OF BRASSICAS CAUSED BY *ALTERNARIA* SPECIES IN KENYA

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ABSTRACT

Brassicas are important vegetable crops in Kenya for domestic economy. They are cultivated by over 90% of the smallholder farmers each with approximately 1 to 2.5 ha of land. Black spot disease of brassicas caused by *Alternaria* spp. is one of the major constraints to increased yield in Kenya. There is very little information available on *Alternaria* spp. affecting brassicas in Kenya. The objective of the present study was to establish the pathogenic *Alternaria* spp. affecting brassicas in Kenya and to determine the distribution and incidence of the disease in farmer's fields. This was done by carrying out a field survey in 13 selected districts in Kenya with varying agro-ecological zones. The correlation of environmental variables with incidence and Brassica cultivars was analyzed by Analysis of Variance using SAS Computer package release 6.12 and means separation by least significance difference and student Newman-Keuls test. Out of 89 farms surveyed, 46 (51.7%) had black spot. The farms with cabbage were 52 with 28 (53.8%) having black spot, while farms with kale were 37 with 18 (48.6%) having the disease. The disease incidence on cabbage and kale farms was 30.3% and 8.7%, respectively. *Alternaria* species identified from black spot infected cabbage and kale were *A. brassicicola* and *A. japonica* with the former predominant (64.4%) of cabbage and the later predominant (66.7%) of kale. Both pathogens appeared in 17.8% and 11.1% of infected cabbage and kale farms, respectively. The present findings are useful in the development of Brassica cultivars resistant to Kenyan isolates of *Alternaria* species. This is the first report implicating *A. brassicicola* and *A. japonica* as causal agents of black spot disease of brassicas in Kenya.

Keywords: *Alternaria* species; black spot; cabbage; incidence; kale; Kenya

INTRODUCTION

Brassica (*Brassica oleracea L.*) are important vegetable crops consumed locally and are recognized for the health and nutritional benefits and cash income. Brassicas grown in Kenya include kale, cabbage, broccoli, cauliflower, and Brussels sprouts. Health benefits include reduction of some forms of cancers. Nutritionally, they are high in beta-carotene, vitamin C and fibre. They are important vegetables normally cultivated by over 90% of the smallholder farmers in Kenya each with approximately 1-2.5 ha of land. In 2005, cabbage and other brassicas in Kenya were planted in 35,000 ha with yield of 550,000 metric tonnes at 15,714 kg/ha (CABI, 2007). Low productivity of brassicas in Kenya is due to a number of factors such as the use of disease susceptible cultivars and low quality seeds (Spence *et al.*, 2005). Brassica production in Kenya is mainly concentrated at higher altitudes with reliable rainfall.

Alternaria black spot is one of the most common and destructive diseases of brassicas worldwide. A complex of three *Alternaria* species namely *A. brassicicola*, *A. brassicae* and *A. japonica* is responsible for important yield losses. Significant reductions in yield quantity and quality (Verma and Saharan, 1994) are the result of several factors including reduced photosynthetic potential, accelerated senescence, premature pod shatter and shriveled seed (Shresta *et al.*, 2000). Brassica hosts can be affected in all stages of growth and typical symptoms include black necrotic lesions surrounded by chlorotic areas on seedlings, leaves, stems and siliquae (Jasalovitch *et al.*, 1995; Mac Kinon *et al.*, 1999). *Alternaria* black spot disease is one of the main causes of low yields in brassicas in Kenya (Spence *et al.*, 2005). *Alternaria* species can be serious at higher tropical elevation (Tewari, 1991). There is little information available on *Alternaria* black spot disease affecting brassicas in Kenya. This is a major drawback in planning for strategic management of the disease.

As a management strategy, use of resistant cultivars would probably be the most effective

and economical control option. Unfortunately, such resistance is not usually expressed in commercially available varieties of Brassica (Tewari, 1991). The objective of the current study was to undertake a field survey in selected districts in Kenya to determine the occurrence and incidence of black spot disease on farmers' fields, and to identify *Alternaria* species causing the disease. The information would be useful in breeding programmes aimed at developing disease resistant/tolerant Brassica cultivars.

MATERIALS AND METHODS

Field survey and collection of samples. The *Alternaria* black spot survey was carried out in 13 districts selected in Kenya. The districts were: Uasin Gishu, Keiyo, Marakwet and Molo from Rift Valley province, Mt. Elgon from Western province, Kisii Central and Siaya from Nyanza province, Kiambu West, Nyeri Central and South Nyandarua from Central province, Meru Central from Eastern province, and Taita Taveta from Coast province. Leaves with black spot symptoms were collected from the farmer's fields and kept in a cool box containing ice (4°C), and transported to the laboratory. Among the brassicas sampled with diseased symptoms included cabbage cultivars Gloria F1, Pructor F1, Victoria F1, Amigo F1, Copenhagen market, and Red cabbage; broccoli; cauliflower; and kale cultivars Collards, thousand head and a local cultivar. The samples from the field were stored in the laboratory in a refrigerator set at 4°C until the fungi were isolated.

Disease occurrence was taken as the presence or absence of black spot disease in a district. Disease incidence (%) was recorded as the average per cent black spot due to *Alternaria* species in a 10-metre row of cabbage or kale plants in four replications (4 rows). The farms with cabbage or kale that were surveyed in a particular district or agro-ecological zone (AEZ) were on average five kilometres apart. The data gathered in each farm included,

cabbage variety, kale variety, cropping system, stage of plant growth, altitude in metres above sea level (masl), longitude, latitude, rainfall and temperature. Global positioning system was used to obtain information on altitude, longitude and latitude. Data on rainfall and temperature were obtained from the nearest meteorological station. Data on *Alternaria* black spot incidence were analysed by Analysis of Variance (ANOVA) procedure and General Linear Models (GLM) procedure using the SAS computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by t-tests using least significant difference (LSD) and Student-Newman-Keuls (SNK) test.

Fungal isolation. Small pieces (0.5-1.0 cm²) from the edge of necrotic/discoloured portions of leaves were cut using a sterilized scalpel and surface sterilized in 2.5 % sodium hypochlorite for 1-5 minutes. The tissues were then washed in three series of sterile distilled water and blotted dried with sterile paper towel and a small piece sliced off each end so that residual sterilant was not carried over. Five pieces were placed on each plate containing potato dextrose agar (PDA) and replicated twice. The plates

were incubated at 25°C in a 12-hour light/dark cycle for 48 hours (Brayford, 1991). After 24- 48 hours of growth, isolates were re-isolated from the ends of the cut pieces using sterile fine pointed needle onto fresh PDA medium. These were incubated at 25°C in a 12-hour light/dark cycle for 7 days.

Identification of *Alternaria* species using conidial morphology. Using a sterile needle, small mycelia of seven-day old fungal cultures were placed in sterile glass slides, stained with cotton blue in lactophenol, covered with a glass cover slip and observed under light microscope fitted with an Olympus camera (Model SC 35). The morphology of conidia of the fungal isolates was used to identify the various species of *Alternaria* (Ellis, 1988).

RESULTS

Occurrence and incidence of *Alternaria* black spot disease of brassicas. *Alternaria* black spot occurred in all districts surveyed, and it appeared in both cabbage and kale cultivars. Out of a total of 89 farms surveyed, 46 farms (51.7%) had black spot disease (Tables 1 and 2).

Table 1. Incidence of Alternaria black spot disease on cabbage cultivars grown at various altitudes, agro-ecological zones, annual average rainfall and annual mean temperatures.

Province	District	Division	Altitude (masl) ¹	AEZ ²	Mean Rainfall (mm) range	Mean Temp. (°C) ³ range	Cabbage cultivar	<i>Alternaria</i> spp.	Mean incidence (%)
Central	Kiambu West	Kikuyu	2221	LH2	1100-1300	15.2-17.6	Broccoli	<i>A. japonica</i>	10.0
Central	Kiambu West	Lari	2361	UH1	1200-1600	13.5-15.2	Pructor F1	<i>A.brassicicola</i>	52.5
Central	Kiambu West	Limuru	2217	LH2	1100-1300	15.2-17.6	Red cabbage	<i>A.brassicicola</i>	30.0
Central	Kiambu West	Limuru	2417	UH1	1200-1600	13.5-15.2	Pructor F1	<i>A.japonica</i>	33.0
Central	Kirinyaga	Central	1700	UM1	1400-1700	17.5-19.3	Pructor F1	<i>A.brassicicola</i>	22.8
Central	Kirinyaga	Gichugu	1675	UM1	1400-1700	17.5-19.3	Pructor F1	<i>A.brassicicola</i>	75.0
Central	Kirinyaga	Gichugu	1693	UM1	1400-1700	17.5-19.3	Gloria F1	<i>A.brassicicola</i>	100.0
Central	Kirinyaga	Gichugu	1725	UM1	1400-1700	17.5-19.3	Amigo F1	<i>A.brassicicola</i>	2.8
Central	Nyeri North	Kieni West	1922	LH5	650-850	15.6-16.9	Gloria F1	<i>A.brassicicola</i>	20.0
Central	South Nyandarua	South Kinangop	2532	UH1	1150-1600	10.0-14.6	Gloria F1	<i>A.brassicicola</i>	94.8
Central	South Nyandarua	South Kinangop	2544	UH1	1150-1600	10.0-14.6	Broccoli	<i>A.brassicicola</i>	77.3
Central	South Nyandarua	South Kinangop	2552	UH1	1150-1600	10.0-14.6	Gloria F1	<i>A.brassicicola</i>	100.0
Central	South Nyandarua	South Kinangop	2605	UH1	1150-1600	10.5-14.5	Gloria F1	<i>A.brassicicola</i>	30.0
Coast	Taita Taveta	Mwatate	1526	UM3	900-1100	19.5-19.9	Gloria F1	<i>A.brassicicola</i>	93.3
Coast	Taita Taveta	Mwatate	1609	UM2	1100-1400	18.4-19.5	Cauliflower	<i>A.brassicicola</i>	27.5

Coast	Taita Taveta	Wundanyi	1590	UM2	1100-1400	18.4-19.5	Red cabbage	<i>A. japonica</i>	45.0
Eastern	Meru Central	Abogeta	1837	LH1	1300-1500	15.2-18.0	Amigo F1	<i>A.brassicicola</i>	10.8
Eastern	Meru Central	Kibirichia	2277	LH1	1300-1500	15.2-18.0	Gloria F1	<i>A.japonica</i>	41.3
Eastern	Meru Central	Kibirichia	2292	UH1	1200-1600	13.5-15.2	Victoria F1	<i>A.brassicicola</i>	29.3
Nyanza	Kisii Central	Keumbu	1716	UM1	1400-2100	18.0-20.5	Pructor F1	<i>A.brassicicola</i>	47.8
Rift Valley	Keiyo	Chepkorio	2728	UH1	1200-1700	11.2-15.0	Copenhagen	<i>A.brassicicola</i>	37.5
Rift Valley	Keiyo	Chepkorio	2734	UH1	1200-1700	11.2-15.0	Gloria F1	<i>A.brassicicola</i>	100.0
Rift Valley	Marakwet	Kapcherop	2284	LH1	1250-1350	15.0-16.6	Gloria F1	<i>A.japonica</i>	80.8
Rift Valley	Marakwet	Kapcherop	2293	LH1	1250-1350	15.0-16.6	Gloria F1	<i>A.japonica</i>	76.8
Rift Valley	Molo	Elburgon	2584	UH1	1200-1800	10.5-14.5	Pructor F1	<i>A.brassicicola</i>	100.0
Rift Valley	Molo	Elburgon	2590	UH1	1200-1800	10.5-14.5	Victoria F1	<i>A.brassicicola</i>	100.0
Rift Valley	Molo	Elburgon	2593	UH1	1200-1800	10.5-14.5	Pructor F1	<i>A.brassicicola</i>	100.0
Western	Mt. Elgon	Kapsakwony	1916	LH2	1300-1700	18.8-20.6	Gloria F1	<i>A.brassicicola</i>	37.3
Mean									56.3

Key: ¹masl: metres at sea level; ²AEZ (agro-ecological zone): UH1 = sheep-dairy zone, LH1 = tea-dairy zone, LH2 = wheat-maize-pyrethrum zone, LH5 = lower highland ranching, UM1 = coffee-tea zone, UM2 = main coffee zone, UM3 = marginal coffee zone (Jaetzold and Schmidt, 1983); ³temp.: temperature

Table 2. Incidence of Alternaria black spot disease on kale cultivars grown at various altitudes, agro-ecological zones, annual average rainfall and annual mean temperatures.

Province	District	Division	Altitude (masl) ¹	AEZ ²	Mean Rainfall	Mean Temp.	Kale cultivars	<i>Alternaria</i> species	Mean incidence (%)
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					(mm) range	(°C) ³ range			
Central	Kiambu West	Lari	2387	UH1	1200-1600	13.5-15.2	Lks	<i>A.japonica</i>	30.3
Central	Kiambu West	Lari	2398	UH1	1200-1600	13.5-15.2	Lks	<i>A.brassicicola</i>	23.0
Central	South Nyandarua	Njabini	2676	UH1	1150-1600	10.0-14.6	Lks	<i>A.japonica</i>	9.3
Central	South Nyandarua	Njabini	2682	UH1	1150-1600	10.0-14.6	Lks	<i>A.brassicicola</i>	25.0
Central	South Nyandarua	Njabini	2704	UH1	1150-1600	10.0-14.6	Lks	<i>A.japonica</i>	11.5
Central	South Nyandarua	South Kinangop	2666	UH1	1150-1600	10.0-14.6	Lks	<i>A.japonica</i>	19.5
Eastern	Meru Central	Kibirichia	2277	LH1	1300-1500	15.2-18.0	Thk	<i>A.japonica</i>	18.8
Nyanza	Kisii Central	Central	1805	UM1	1400-2100	18.0-20.5	Lksu	<i>A.japonica</i>	20.0
Nyanza	Siaya	Karemo	1282	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	16.3
Nyanza	Siaya	Ugunja	1238	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	19.8
Nyanza	Siaya	Ugunja	1241	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	18.3
Nyanza	Siaya	Ugunja	1244	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	19.3
Nyanza	Siaya	Ukwala	1207	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	14.8
Nyanza	Siaya	Wagai	1374	LM1	1500-1900	20.5-21.7	Lksu	<i>A.japonica</i>	22.3
Nyanza	Siaya	Ugunja	1313	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	19.5
Nyanza	Siaya	Wagai	1312	LM2	1450-1600	21.4-22.3	Lksu	<i>A.japonica</i>	12.0
Rift Valley	Uasin Gishu	Soy	2143	LH3	900-1100	15.1-17.9	Kc	<i>A.brassicicola</i>	10.0
Western	Mt. Elgon	Kapsakwo ny	1961	LH2	1300-1700	16.4-18.8	Lksu	<i>A.brassicicola</i>	22.0

Mean										17.9
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Key: ¹masl: metres at sea level; ²AEZ (agro-ecological zone): UH1 = sheep-dairy zone, LH1 = tea-dairy zone, LH2 = wheat-maize-pyrethrum zone, LH3 = wheat-barley zone, UM1 = coffee-tea zone, LM1 = main sugarcane zone, LM2 = marginal sugarcane zone (Jaetzold and Schmidt, 1983); ³temp.: temperature; ⁴Kale cultivars: Lksu = local kales propagated by suckers; Lks = local kales propagated by seed from informal seed sector; Kc = kales (collards) from formal seed sector; Thk = thousand head kale

The farms with cabbage were 52 with 28 (53.8%) having black spot, while farms with kale were 37 with 18 (48.6%) having the disease. The disease mean incidence on cabbage and kale farms (all farms) were 30.3% and 8.7%, respectively. In the farms with the disease, cabbage cultivars had higher susceptibility to the disease with a mean incidence of 56.3% and a range of 2.8% to 100%; while kale cultivars had lower susceptibility to the disease with a mean of 17.9% and a range of 9.3% to 30.3%.

Disease incidence of *Alternaria* black spot on farmers' fields was affected by agro-ecological zones, altitude, annual average rainfall and annual mean temperatures on cabbages and kales (Tables 1 and 2). The disease was found from the highland to lowland zones, at higher and lower altitudes, at lower and moderate temperatures, and in areas with lower and higher rainfall. The disease incidence was significantly higher ($P \leq 0.05$) in the highlands (upper and lower highland zones) (43.3%) than in the midlands (upper and lower midland zones) (33.0%). The disease incidence was also significantly higher ($P \leq 0.05$) at higher altitudes (2001 to 3000 masl) (49.1%) than at lower altitudes (1000 to 2000 masl) (29.1%).

Conidial morphological of *Alternaria* species. Based on conidia morphology, two *Alternaria* species were implicated to cause black spot of brassicas in the areas surveyed in Kenya, namely *A. brassicicola* and *A. japonica*. *Alternaria brassicicola* is a small-spored, long-chain species. Conidia are in long multiple-branched chains; the initial few conidia are strikingly larger than subsequently formed conidia which tend to be progressively smaller as the chain lengthens. Conidia are

acropleurogenous, straight, ovoid to nearly cylindrical, beakless but pseudorostrate due to the presence of secondary conidiophores, with 1-11 transverse and up to 7 longitudinal septa, although many conidia lack longitudinal septa. Septa and the conidial wall are deep chestnut-brown in edge view and medium yellowish-brown to reddish-brown in face view. Conidia are moderately constricted at the septa, and are 10-130 μm long and 6-20 μm wide.

Alternaria japonica conidia are solitary or in short chains of 2-4. Conidia in culture are short to long ovoid, beakless, strongly constricted at the transverse septa, smooth-walled, mid-brown and only darkening slightly when mature, they tend to be somewhat variable in size and septation, some having 2-3 transverse septa and 1-2 longitudinal septa, and a size range of 35-45 x 20-24 μm , while others have 5-7 transverse septa with 1-2 longitudinal septa, and a size range of 55-70 x 18-22 μm .

Occurrence of *Alternaria* species on brassicas. The main symptom of *Alternaria* black spot disease in the farmer's fields was necrotic centres surrounded by chlorotic areas on true leaves, petioles and stems of cabbage and kale. Older plants were more susceptible than younger ones, hence causing accelerated senescence. *Alternaria* species identified from black spot infected cabbage and kale were *A. brassicicola* and *A. japonica* with the former predominant (64.4%) of cabbage and the latter predominant (66.7%) of kale (Tables 1 and 2). Both pathogens appeared in 17.8% and 11.1% of infected cabbage and kale farms, respectively. The two species occurred either singly or as mixtures. The farmer's fields with *Alternaria* species were 46 out of a total of 89 farms sampled during the survey. *A.*

brassicicola was significantly ($P \leq 0.05$) more prevalent (52.6 % incidence) than *A. japonica* (26.9% incidence) in the field (combined cabbage and kale fields). The cabbage cultivars that had *Alternaria* black spot symptoms caused by one of the two species were Gloria F1, Pructor F1, Victoria F1, Amigo F1, Copenhagen market, Red cabbage' broccoli, and cauliflower. The kale cultivars that had *Alternaria* black spot symptoms caused by one of the two species were collards, thousand head and local kales.

DISCUSSION

The present study identified *A. brassicicola* and *A. japonica* as causal agents of *Alternaria* black spot in Kenya. The disease is worldwide in distribution (CABI, 2007). Although the disease has been previously found to exist in Kenya, no attempts have been made to identify the various *Alternaria* species causing it (Spence *et al.*, 2005). *Alternaria brassicicola* has a wide distribution. It is prevalent in areas where cultivation of crucifers is extensive. *Alternaria brassicicola* has been reported in 20 African countries, including Tanzania and Uganda in East Africa (CABI, 2007). It had not been reported in Kenya. *Alternaria japonica* is not as widespread as *A. brassicicola*. It has been reported in three African countries, Egypt, South Africa and Zimbabwe (CABI, 2007). It had not been reported in Kenya. The conidial morphology of *A. brassicicola* and *A. japonica* isolates from Kenya are similar to observations made earlier (Neergard, 1945; Wiltshire, 1947; Changsri and Weber, 1963; Ellis, 1971; Rotem, 1994; Simmons, 1992; Yu, 1992; Corlett and MacLatchy, 1996).

Alternaria black spot incidence in the farmer's fields ranged from 0.0 to 100%. This has implications on seed yield and quality. The disease causes economic losses in several different ways (Humpherson-Jones, 1992; Strandberg, 1992; Rotem, 1994; Verma and Saharan, 1994; Shresta *et al.*, 2000). The symptoms of *Alternaria* black spot on cabbage and kale observed in the present study were similar to observations made by earlier workers (Poapst *et al.*, 1979; Humpherson-Jones, 1992;

Paul and Rawlinson, 1992; Yu, 1992; Howard *et al.*, 1994; Verma and Saharan, 1994; Chung and Huang, 1993). Seed infection causes reduced germination and seedling vigour, in addition to pre- and post-emergence damping-off, and affects the sale and use of infected/infested seed. Lesions on leaves, stems and siliques reduce the photosynthetic area and accelerate senescence in the plant. The causal agents are responsible for major seed yield losses in the oleraceous brassicas and they are the most important component of its economic impact. The unsightly cosmetic blemishing or rotting of the head or wrapper leaves in vegetable brassicas as a consequence of disease causes downgrading and crop losses in both fresh and stored produce. *Alternaria brassicicola* often occurs in conjunction with *A. brassicae*, *A. japonica* and some other pathogens of the Brassicaceae. This confounds precise estimates of losses caused individually by this pathogen in the field.

Alternaria brassicicola is the most widespread and predominant pathogen causing significant losses especially in cabbage cultivars grown in Kenya. In vegetable brassica, *A. brassicicola* is the dominating *Alternaria* species (Humpherson-Jones, 1985) and causes serious disease problems in vegetable production (Corlett and MacLatchy, 1996). *A. japonica* is widespread in the Northern hemisphere. Both pathogens were found to be prevalent in the upper agro-ecological zones, at higher elevation and at moderate to high rainfall conditions. The pathogen is greatly influenced by weather with the highest disease incidence reported in mild, wet seasons and in areas with relatively high rainfall (Humpherson-Jones, 1989). *Alternaria brassicicola* and *A. japonica* cause black spot of cultivated and wild crucifers (Smith *et al.*, 1988). Although kale cultivars showed tolerance to local *Alternaria* isolates, informal seed systems pose a threat to the spread of seed-borne pathogen. This particular source of kale seed to smallholder farmers has been identified as a major source of the spread of *Alternaria* spp. (Spence *et al.*, 2005). Intensive cultivation of susceptible cultivars of these brassicas may also enhance high infection of seeds.

The management of *Alternaria* black spot should be given a priority because of the seedborne nature of the two identified species of *Alternaria*, the fact that the pathogens can survive in infected plant debris, has a wide host range, and the plant parts that they can attack, for example, pods, inflorescence, leaves, seeds, stems and whole plant (CABI, 2007). Various options of disease control are available for black spot of brassicas, but the most sustainable one is through breeding for resistance/tolerance.

The present findings are useful in the development of brassica cultivars resistant to Kenyan isolates of *Alternaria* species.

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