Prevalence, Incidence and Severity of Anthracnose in *Carica Papaya* Fruits in Baringo and Elgeyo-Marakwet Counties

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Abstract

Papaya is the fourth most important fruit crop in Kenya after oranges, mangoes and bananas. It has also become an important export crop in the arid and semi-arid lands. Anthracnose caused by Colletotrichum gloeosporioides in pawpaw (Carica papaya) infects pawpaw fruits both in the field and also under postharvest conditions resulting in huge economic losses. Therefore, information on the epidemiology of the disease and the extent of damage provides vital information for the management of the disease. Field surveys were conducted in major pawpaw-growing areas of Baringo and Elgeiyo-Marakwet Counties from March 2016 for 15 months during the fruiting stage, in order to determine the incidences, prevalence and severity of anthracnose disease. A total of 32 farms in 8 areas (4 farms per area), 20 in Baringo and 12 in Elgeyo-Marakwet, that had pawpaw at the fruiting stages were sampled in the two counties using the line transect method. In each farm a 9 m side of a 'W' shaped transect was used to assess the incidence of anthracnose on papaya. Significant differences (p =0.05) in disease prevalence in Carica papaya were reported in Baringo (95 %) and Elgeyo-Marakwet (83 %). The percent disease incidence of anthracnose infections on fruits was 9.23 ± 1.22 % in Baringo County and 4.5 ± 1.1 % in Elgeyo Marakwet County. The severity of anthracnose disease was higher in Baringo than Elgeyo Marakwet and was found to positively correlate with the incidence. In regions where lower incidences were reported, there was low severity of the anthracnose disease. In a severity scale of 1-5, the severity was predominant in the scale of 1 and 2. There is need to characterize the isolates of C. gloeosporioides infecting papaya in the two counties. Additionallya detailed study on influence of various ecological factors on the distribution and epidemiology of C. gloeosporioides are necessary in order to inform integrated disease management strategies of anthracnose of papaya in this region.

Keywords: *Colletotrichum Gloeosporoides*, Epidemiology, Pawpaw, Transect

INTRODUCTION

There is consistent lack of research in papaya anthracnose in Kenya, despite the obvious signs that the fruit has a number of diseases. This has resulted in reduced ability to control and manage this disease.

Papaya (*Carica papaya* L.) (Caricaceae) is one of the most popular fruits grown widely under tropical and sub-tropical climates (da Silva *et al.,* 2007). Its production varies widely across the world. The world production of the crop has been estimated in the range of approximately 7.2 million metric tonnes (FAOSTAT, 2016; Evans and Ballen, 2017). *C. papaya* is among the most consumed fruit in many parts of Africa by humans (Oboh *et al*., 2015; Oyedemi *et al*., 2016), while other parts of the plant are fed to domestic animals (Imran *et al*., 2009).

In Kenya, the area under *C. papaya* ranges between 300,000–500,000 hectares producing about 60,000 metric tonnes - 150,000 metric t/year of papaya fruits (FAOSTAT, 2016). It is grown in almost all areas in Kenya but the major areas are the counties of the former provinces of Eastern, Rift Valley, Nyanza and Western regions.

Pawpaw anthracnose is reported to be the most important disease affecting pawpaw worldwide (Pomper *et al*., 2010). Anthracnose caused by *Colletotrichum gloeosporoides* is a fungal disease that attacks all parts of the pawpaw plant at any stage of growth (Torres-Calzada *et al*., 2012), exhibiting symptoms such as small, brownish spots with light-coloured centers on the leaves and twigs (Ryan *et al*., 1994).

Tiny cracks on the leaf spots indicate fruiting structures of the fungus. On panicles, the symptoms first appear as small black or dark brown spots that may enlarge or coalesce to kill the flowers before fruits are produced. Blighted flowers are dry and their colour varies from brown to black (Rivera – Pastran, 2016). Petioles, twigs and stems, are also susceptible and the typical black expanding lesions found on leaves can be found on them. Two types of symptoms are found on fruits. The commonest is a dark-brown lesion that is slightly sunken with raised rims (Nwofia and Ojimelukwe, 2012). The disease is a problem during cool, wet springs (McGinnis, 2005). Variegated varieties are more susceptible (Hernandez-Albiter *et al*, 2007).

Incidence of pawpaw anthracnose has been reported to range between 32 % in South Africa and 64.6 % in Costa Rica and can reach 100 % when fruits are produced under wet or very humid conditions. Similarly, post-harvest anthracnose incidence on pawpaw can also reach as high as 100 % on fruits produced in wet or high humid conditions (Kamdoum *et al*., 2018). The fungus attacks primarily the fruit of papaya, mature fruits being more susceptible (Janisiewiez and Jeffers, 1997). Petioles and leaves may be infected, but this is thought to be important only as a source of the fungus for further fruit infection. The fungus may produce a pink mass of spores in the middle of the older spots. The pathogen grows into the fruit, resulting in softening of the fruit and an off flavour, becoming dirty brown, soft and finally rots (Pomper *et al*., 2010). Research on postharvest diseases of perishable fruits, particularly papaya anthracnose in Kenya, is limited. In view of the above, the study was conducted with the objective of determining the prevalence, incidence and severity of anthracnose in *C. papaya* fruits.

METHODOLOGY

Study area for anthracnose prevalence, incidence and severity in *C. papaya*

This study was conducted in Elgeyo-Marakwet and Baringo Counties. Both Baringo and Elgeyo-Marakwet Counties are in the Rift Valley of Kenya. Baringo County is bordered by Elgeyo-Marakwet County to the West and lies between Latitudes 0°13" South and 1°40" north and Longitudes 35°36" and 36°30" East. Elgeyo-Marakwet County extends from latitude 0°20′ to 1°30′ North and longitude 35°0′ to 35°45′ East and borders Baringo County to the East. The regions receive bimodal annual rainfall ranging between 800 - 1100 mm. The long rainy season occurs from March to June with the peak period in April and May while short rainy season occurs between August to November. The average temperature is 23 °C with a minimum of 15ºC and maximum is 30°C.

Sample size

Samples of papaya that showed symptoms of anthracnose disease were collected from Marigat, Koriema, Kapkelelwa, Mochongoi Lower and Barwessa areas in Baringo County and Kimwarer, Cheptebo and Tambach Lower areas in Elgeyo-Marakwet County. The sample size for the number of farms was computed using Yamane (1967) formula: thus $n = \frac{N}{1 + Ne^2}$.

Where n = required responses

N = Population

e^2 = error limit (0.1 for samples between 100 to 1000)

Number of households of papaya will be determined as:

$$\text{n for respondents} = \frac{12239}{1 + 12239 * 0.1^2} = 99.189 \approx 99$$

Field survey of anthracnose prevalence, incidence and severity in *C. papaya*

Survey of papaya anthracnose disease was carried out in farmer's fields and market centres along accessible routes in the two counties. In the market, samples of papaya fruits were examined for the presence of the disease and information on the source of the fruits was sought from the traders. The survey adopted exploratory survey research design to evaluate the occurrence, prevalence, incidence and severity of anthracnose in *C. papaya* (Kumar, 2019). The presence or absence of the disease in a County or Sub-County represented occurrence or non-occurrence of papaya anthracnose

In all the selected farms from the two counties disease assessment was conducted. A line 'W' transects of 9m long on each side were applied randomly and the number of pawpaw plants counted, while noting plants with fruits and those showing anthracnose symptoms. Papaya fruits from each transect within the farms were visually inspected for the presence of symptoms of papaya anthracnose disease. Disease incidence (%) was determined as percent anthracnose infected plants or fruits per field or market.

Disease prevalence, incidence and severity were determined as:

$$\% \text{ prevalence} = \frac{\text{Number of farms with anthracnose disease affecting fruits}}{\text{Total number of farms surveyed}} \times 100$$

$$\% \text{ incidence} = \frac{\text{Number of fruits showing symptoms of anthracnose}}{\text{Total number of fruits surveyed}} \times 100$$

Disease severity (%) on papaya fruits was rated on 1 to 5 scale, where 1 = 0 % of fruit area affected, 2 = 1-25%, 3 = 26-50%, 4 = 51-75 %, and 5 = 76-100 % fruit area affected as described by Bautista-Banos *et al*., (2002). During the survey papaya varieties were also noted.

Papaya plant parts in the field were inspected for the presence of symptoms of papaya anthracnose disease. Diseased samples of papaya plant parts and fruits were collected, stored in a keep-cool box and transported to the Laboratory, University of Eldoret, for isolation of the causal pathogen.

Isolation and identification of *C. gloeosporoides*

A total of 320 fruit samples that were randomly picked from the *C. papaya* plant parts were taken to the laboratory for further confirmation of the occurrence of *C. gloeosporoides*. In the laboratory, the parts were washed in running tap water and 1cm^2 area from the symptomatic part was cut and disinfected by immersing in 1 % NaOCI solution for 1 minute, then rinsed thrice with sterile distilled water and dried. The diseased samples were placed onto PDA media and incubated at room temperature 25 ± 2^{0}C for two days.

The emerging fungi were sub cultured by cutting from the hyphal tip to obtain pure cultures. From 10-14 days old cultures, cultural and microscopic (x40) observation to identify *C. gloeosporoides* was done. Microscopically, the anthracnose pathogen in each papaya fruit was recorded and classified under different *Colletotrichum* species.

Data analysis

One-Way Analysis of Variance (ANOVA) was carried out with the statistical software SAS v. 9.0. Percent incidence of anthracnose of papaya assembled from the field was subjected to arcsine transformation. Least Significant Difference (LSD) at 5% probability level was used to separate the means. Disease severity ratings were square root transformed before statistical analysis.

RESULTS

Occurrence and severity of anthracnose on papaya

The symptoms of infection were initially observed on the leaves as small angular, brown to black spots that coalesced to form large extensive lesions on the leaf, and occurred all around the edges of the leaves. On the fruits of pawpaw, the symptoms were characterized by occurrence of tear strains of linear necrotic regions on the skin associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface. Later, these lesions become enlarged, rounded, sunken brown to black in colour. The centers of these lesions were often covered with pink, gelatinous masses of spores especially during moist, warm weather. The mean disease incidences of anthracnose from the two counties showed a higher disease incidence of 9.23 ± 1.22% in Baringo County and 4.5 ± 1.1% in Elgeyo-Marakwet County (Figure 1).

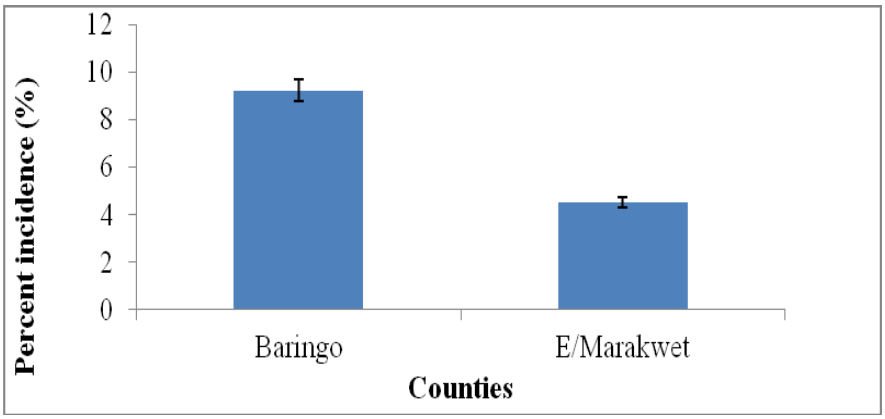

Figure 1: The mean incidence of papaya anthracnose in Baringo and Elgeyo Marakwet Counties

The highest mean anthracnose disease incidence among the locations studied showed that Mochongoi location of Baringo County had the highest incidence and was least in Tambach location of Elgeyo-Marakwet County being 11.2 % and 3.4 %, respectively (Figure 2). However, the incidence of anthracnose differed significantly (p= 0.0004) among the locations where sampling was done.

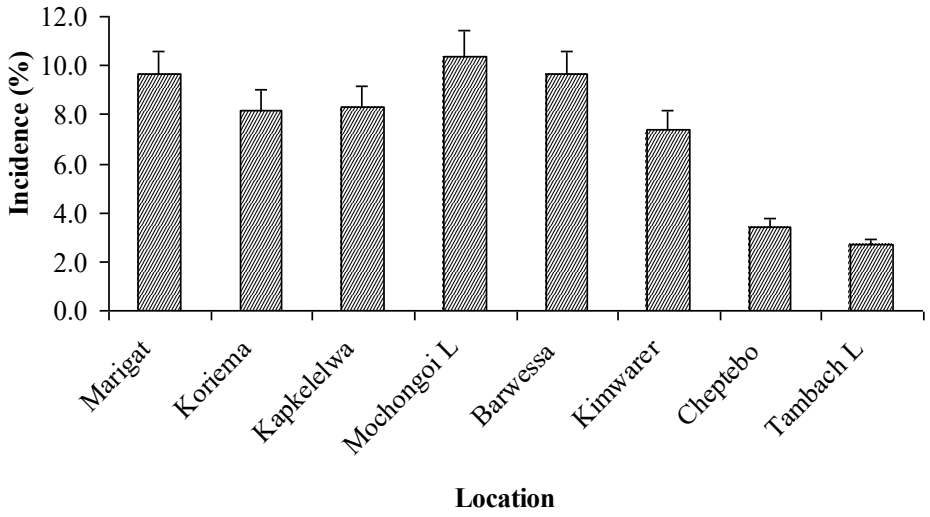

Figure 2: Incidence of anthracnose of *C. papaya* fruits in locations of Baringo and Elgeyo-Marakwet Counties.

Symptoms of papaya trees based on leaf defoliation and fruit yellowing were assessed on altitude ranges of 1051 to 1400 m above sea level. The field survey showed high leaf defoliation incidence of 23 % recorded in an altitude range of 1251-1400 m asl, and lower a leaf defoliation of 8 % recorded in altitude range of 1351–1400 m asl (Table 1). High fruit yellowing of 14 % was recorded in an altitude range of 1101–1150 m asl; with the lowest fruit yellowing of 3 % recorded in altitude range of 1351–1400m asl. The incidences on the field leaf defoliation and fruit yellowing were not significant (P= 0.05) and appeared to decrease with rise in elevation. Overall, the disease incidence was higher in infecting the papaya leaves rather than on the fruits.

Table 1: Altitude range (m asl) and symptoms of papaya anthracnose in Baringo and Elgeyo-Marakwet Counties

Altitude range (m above sea level)	Incidence of symptoms (%)	
	Leaf defoliation	Fruit yellowing
1051 - 1100	16	12
1101 - 1150	21	14
1151 - 1200	19	11
1201 - 1250	22	13
1251 - 1300	23	10
1301 - 1350	11	05
1351 - 1400	08	03

The prevalence of anthracnose in *C. papaya* in the farms was 95% in Baringo and up to 83 % in Elgeyo-Marakwet County (Table 2), which was found to differ significantly with county of occurrence and altitude (χ^2 = 45.2312, P = 0.00). When the disease occurrence was considered against the elevation (m above sea level) the disease was not found occurring on the altitude above 1280 m asl in Baringo County, moreover in altitude of 1062 m asl, the disease incidence decreased. In Elgeyo-Marakwet County, altitude ranging between 1300 to 1400 m asl did not show the presence of the disease.

Table 2: Prevalence of anthracnose of *C. papaya* in Baringo and Elgeyo-Maraket Counties

County	Area (Location)	Village	Altitude	Prevalence
Baringo	Marigat	Kaptich	1062	++-
		R7	1060	++-
		R1	1032	+++
		L3	1057	+-+
	Koriema	Koriema	1100	+++
		Kimalel	1150	+++
		Patkawanin	1151	+++
		Kibingor	1130	+++
	Kapkelelwa	Kapkelelwa	1280	---
		Kisok	1275	+--
		Oinobmoi	1230	+++
		Kurumbsoo	1012	+++
	Mochongoi L	Sandai	1010	+++
		Loboi	1008	+++
		Molok	1012	+++
		Kaptombes	1011	+++
	Barwessa	Barwessa	1000	+++
		Muchukwo	1009	+++
		Likwon	1002	+++
		Chesongo	1005	+++
Elgeyo Marakwet	Kimwarer	Kimwarer	1260	+++
		Seko	1256	+++
		Kapokpok	1250	+++
		Emsea	1230	+++
	Cheptebo	Cheptebo	1200	+++
		Chekobei	1205	+++
		Chepsigot	1230	+++
		Biretwo	1300	---
	Tambach L	Nyawa	1400	---
		Kapshokwei	1380	++-
		Kewapsos	1350	++-
		Sangeta	1310	+++

Legend
+++ showed occurrence of the disease among farmers during all the three surveys
++- showed occurrence of the disease during the first two surveys and absence in the last survey
+-- showed occurrence in only one survey during the study
--- showed absence of the disease during the three surveys

Generally, the severity of anthracnose disease was more in Baringo than Elgeyo-Marakwet County and was found to differ according to the incidence (Table 3). In regions where lower incidences were reported (<8%), there was low severity of the anthracnose disease basically at level 2 (1-25%) followed by level 1 (0%). However, when incidences of anthracnose disease were higher, the severity of the disease increased mainly to levels 4 (51-75%) and 5 (>75%).

Table 3: Severity of anthracnose disease in *C. Papaya* in the field

			Disease severity					Mean
County	Location	Village	1	2	3	4	5	
Baringo	Marigat	Kaptich	1.9	2.6	0.8	0.9	0.8	1.40
		R7	0.4	1.1	1.5	1.2	6.8	2.20
		R1	1.1	2.9	2.1	3.2	3.2	2.50
		L3	1.7	3.6	1.4	1.1	0.2	1.60
	Koriema	Koriema	0.8	0.5	0.2	3.1	3.1	1.54
		Kimalel	0.7	0.7	1.5	3.4	4.0	2.06
		Patkawanin	0.4	0.4	0.8	4.5	2.4	1.70
		Kibingo	0.3	0.3	0.4	2.2	3.1	1.26
	Kapkelelwa	Kapkelelwa	0.7	0.7	0.9	3.3	4.7	2.06
		Kisok	1.1	1.1	1.4	1.7	2.1	1.48
		Oinobmoi	0.8	0.8	1.0	3.5	2.4	1.70
		Kurumbsoo	1.9	2.6	1.1	1.1	0.3	1.40
	Mochongoi L	Sandai	2.2	3.8	0.9	0.6	1.0	1.70
		Loboi	1.2	1.2	1.5	2.5	3.9	2.06
		Molok	0.2	0.2	0.2	5.4	5.4	2.30
		Kaptombes	0.4	0.4	0.5	4.3	5.8	2.30
	Barwessa	Barwessa	1.4	1.4	1.7	3.2	0.9	1.72
		Muchukwo	1.2	1.2	1.5	3.7	3.6	2.24
		Likwon	1.5	1.5	1.9	4.5	0.3	1.94
		Chesongo	2.5	3.2	1.0	1.2	1.1	1.80
Elgeyo Marakwet	Kimwarer	Kimwarer	0.7	3.3	1.2	1.1	2.3	1.72
		Seko	0.9	2.9	1.3	1.1	1.3	1.50
		Kapokpok	0.8	2.1	0.5	1.2	1.5	1.22
		Emsea	1.3	3.4	1.1	0.9	0.7	1.48
	Cheptebo	Cheptebo	1.5	2.4	0.6	0.2	0.3	1.00
		Chekobei	1.3	2.3	0.7	0.3	0.0	0.92
		Chepsigot	0.8	1.2	0.7	0.7	0.6	0.80
		Biretwo	0	0.0	0.0	0	0.0	0.00
	Tambach L	Nyawa	0	0.0	0.0	0	0.0	0.00
		Kapshokwei	0.7	1.2	0.5	0.5	0.4	0.66
		Kewapsos	1.1	1.3	0.5	0.4	0.2	0.70
		Sangeta	1.1	1.3	0.6	0.5	0.4	0.78

DISCUSSION

Prevalence, incidence and severity of anthracnose in *Carica papaya* fruits

Although it was possible to identify the infected fruits and leaves of the pawpaw plant visually, identification was only possible when disease had progressed significantly within the plant. It was positively determined that the pawpaw fruits were infected with anthracnose. The symptoms were characterized by tear strain symptoms exemplified by the occurrence of linear necrotic regions on the fruit and leaves associated with superficial cracking of the fruit epidermis causing an alligator skin consistent with those of other studies (Saini *et al*., 2016; Snowdon, 2017; Aktaruzzaman *et al*., 2018). The anthracnose spots symptoms on green fruits were dark brown to black with a pale margin and ventricular in shape. These increased in size and became sunken and coalesced to form large spots similar to Saini *et al*., (2016) report. The authors also noted that as the disease increased these lesions become enlarged, rounded, sunken and brown to black in colour. The symptoms of infection on the leaves initially occurred as small angular, brown to black spots that coalesced to form large extensive lesions on the leaf, common around the edges of the leaves.

The disease has been reported to appear on the ripening portions of the fruit, occasionally the green portions of the fruit may become infected (Dickman and Alvarez, 1983). The centers of these lesions were often covered with pink, gelatinous masses of spores especially during moist, warm weather. Symptoms first appear as brown superficial discolorations of the skin and then develop into circular, slightly sunken area, when under humid condition an encrustation of salmon pink spores are observed (Srivastava and Tandon, 1971), which is in tandem with the findings of this study.

The findings on the prevalence of anthracnose in Baringo and Elgeyo-Marakwet Counties concur with those found in other studies in Europe (Torres-Calzada *et al*., 2012), Bangladesh (Hamim *et al*., 2014a and b) and Ethiopia (Mekonnen *et al*., 2015). In the two counties higher altitudes showed decline in the incidence of the disease. These studies concur with other reports that show that high altitudes areas frustrate the survival of *C. gloeosporoides* (Oniha and Egwari, 2015).

The study also determined the overall incidence of anthracnose disease based on observation of 2682 fruits. The study indicated that the incidence of anthracnose was 9.23 ± 1.22% in Baringo County and 4.5 ± 1.1% in Elgeyo Marakwet County, therefore a higher incidence of anthracnose in the fruits sampled from Baringo than Elgeyo-Marakwet County which may be attributed to the differences in weather conditions as anthracnose occur in more hot and humid conditions that supports spore germination (da Silva *et al*., 2007). The incidence is often lower in areas that experience lower temperature. Baringo County is known to experience hot and humid conditions than Elgeyo-Marakwet County and therefore one of the probable causes of the observed higher incidence of anthracnose disease.

The altitude was also found to affect the incidence of the anthracnose disease but this may be related directly to the variations in the temperature and other altitude related effects as established by McConnel *et al*., (2018). The occurrence, prevalence and incidence of anthracnose were found to differ significantly (P= 0.05) with altitude. The differences in the prevalence of anthracnose disease were associated with the locality as well as altitude. This showed that the lower the altitude range, the higher the

symptoms development of *C. papaya*, and the higher the altitude range, the lower the cases of anthracnose symptoms as in Biretwo and Nyawa in Elgeyo-Marakwet County. Leaf defoliation and fruit yellowing followed a similar pattern (P=0.05) with the change in altitude range which, is in agreement with other known studies done elsewhere (Oniha and Egwari, 2015; Tasiwal *et al.,* 2009).

The severity of anthracnose disease in *C. papaya* showed a trend similar with the disease incidence. Generally, the severity of the anthracnose disease was more in Baringo than Elgeyo Marakwet probably attributed to the weather conditions and altitude. Furthermore, the severity of the disease tended to correlate with incidence as also observed by Hossain *et al*., (2010). In areas where incidences of the disease were low, there were less severe cases of the disease most probably due to the exposure of the region to virulent forms of *C. gloeosporoides* as was explained by Hamim *et al*., (2014b). Likewise, when incidences of anthracnose disease were higher, the severity of the disease increased mainly to levels 4 and 5 suggesting that the pathogen may have undergone resistance. It is also probable that at higher incidences, the severity was higher due to the multiplicity of the pathogens infecting the plants. This may be associated with the economics of the pathogen control where after prolonged exposure and attempts at controlling the pathogens, the local community members appear to accept the problem (Ready *et al*., 2017). There is also a possibility that higher incidences cause more exposure of the fruits to pathogens and with the help of environmental conditions become more severe.

There is need to characterize the isolates of *C. gloeosporoides* infecting papaya in the two counties. In addition, there is need for a detailed study on influence of various ecological factors on distribution and epidemiology of *C. gloeosporoides* to form an integrated disease management strategy in this region.

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