INCIDENCE OF FUNGAL FOLIAR BLIGHTS ON DIFFERENT MANGO CULTIVARS IN SOKOTO METROPOLIS

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Abstract

A field survey was carried out to investigate the incidence of fungal leaf blights disease of mango (Mangifera indica) in Sokoto metropolis using random sampling methods. Survey was carried out in six mango growing areas; Arkilla, Dambuwa, Gagi, Illela, More and the permanent site of the Usmanu Danfodiyo University, Sokoto areas. A total of 125 healthy and diseased plant leaves were collected by detached technique. The diseased leaves were taken to the laboratory for culture, isolation, and identification of pathogens. Disease incidence was determined using disease index and severity scale of 0-4 rating. Results from isolation revealed Colletotrichum gloesporioides and other pathogenic fungi. The incidence of leaf blight was greater in UDUS area with percentage of occurrence of (56.75%) while it was lower in Arkilla area (31.65%). In relation to the disease incidence in different mango varieties, the incidence was higher with Mai bindiga Mango variety (78%) whereas, improved variety was the least infected (25.8%). Therefore, the statistical analysis conducted showed that at 5 % disease severity from the study was not significant with mango location but it was with Mango variety. Anthracnos caused by C. gloesporioides was severe due to cultural practices in the areas. Enlightening farmers on proper cultural practises aimed at boosting mango yield and reducing pathogen invasion is recommended.

Keywords: Incidence, Fungi, Foliar, Blights, Mango, Cultivars.

Introduction

Mango (*Mangifera indica*) locally known as Mangoro (Hausa), in Nigeria. The guinea and Sudan zones of Nigeria are credited with producing greater percentage of the fruit in the country. Earlier studies indicated that Agricultural Research Institute in Zaria was the centre where improved mangoes varieties were obtained (Avav and Uza, 2002). Mangoes are grown in eighty-five countries worldwide. Sixty-three of such countries provided more than 1000 metric tons in 1999. Developing countries account for about 98% of the total world production (Yusuf and Salau, 2007). Despite lack of encouragement to large scale production of tropical fruits, Nigeria occupies 8th position in world ranking of mango producing countries as at 2002. This suggests the potential of tropical fruits in the country. Sokoto state occupies 10th position among the main mango producing states in the country which included Jigawa, Plateau,

Yobe, Kebbi, Niger, Kaduna, Kano, Bauchi, Adamawa, Taraba, and FCT (Yusuf & Salau, 2007; FAOSTAT, 2007). Mango tree vegetation in Sokoto metropolis is under threat by unidentified pathogens. Diseases has rendered its production non attractive to both farmers and home gardeners in the region. So far many works were made by researchers on mango fruits collected from different parts of Sokoto metropolis, but little or no attention is paid to its foliar diseases. Most of the research carried out on pest and diseases associated with mango in Nigeria were mainly concentrated/limited to humid regions; the southern part of the country with little or no work done in the northern parts. Anthracnose is presently the most important field and post harvest disease of mango widely distributed in all mango-growing regions of the world (Okigbo & Osuinde, 2003; Stovold, 2004; Nelson, 2008; Uliwa, 2008; Awa *et al.*, 2012).

Sampling Technique A randomized sampling method were used in survey and isolation leaf pathogens. A systemic field survey of mango tree diseases were carried out at the Usmanu Danfodiyo University permanent site, Arkilla, Dambuwa, Gagi, More and Illela all within Sokoto metropolis. To determine the frequency of occurrence of diseased mango trees at the study area, at each site, 20 mango trees were randomly selected and inspected. The frequency of occurrence was taken as the number of mango trees affected by the disease expressed as percentage of the total number of mango trees at a location. A visual assessment technique was used with which many plantations were evaluated in a relatively short time as put forward by Okigbo and Osuinde (2003). Both healthy and diseased leaves were collected randomly without favour for the disease assessment.

Collection of Diseased Mango Samples Diseased samples were collected into closed paper envelops and not plastic bags to avoid creating a micro climate condition for the specimen (Zainab & Shinkafi, 2016). Diseased leaves collected were sorted into envelops according to different varieties as identified by the local farmers. The envelopes where identified (i.e. numbered serially 1-25) and the varieties were also stated on each envelop. A minimum of four mango varieties were obtained from each location with the exception of More (along Tashan illela /bakin gada) where five varieties were collected. In each envelop, five leaves where randomly plucked and identified (i.e. numbered serially 1-5 and scored/rated based on appearance of spots/lesions on leaf) using a masking tape. A total of 125 leaves where collected from the six locations and were taken to Usmanu Danfodiyo University of Sokoto Herberium for verification of samples varieties then later to Mycology laboratory of same institution for isolation and identification of the fungal pathogen(s).

Assessment of Incidence and Severity of *Mangifera indica* in the Study Area

A systemic field survey of foliar disease of mango was carried out in the six selected mango-growing areas (Usmanu Danfodiyo University permanent site, Arkilla, Dambuwa, Gagi, illela and More) to determine foliar fungal disease frequency of occurrence and severity. Following Awa *et al.* (2012) and Abubakar (2009) methods with slight modification, twenty (20) mango trees in each sampling location were randomly selected. On each tree, five on-tree mango leaves were plucked, examined, and scored. Leaf spots were assessed by modifying the standards that has been in used for the assessment of leaf anthracnose of mango proposed by Awa *et al. (*2012). Scale 0 to 4 was used instead of 1 to 5 where scale 0 represents no leaf lesions/spots 1 represents 1 to 3 lesions, 2 represents 4 to 6 lesions, 3 represents 7 to 15 lesions and 4 where more than 70 per cent of leaf surface is covered with lesions. Disease incidence (percentage of diseased leaves), and disease severity (percentage of area affected on the leaf on average) were then obtained by the following formula:

1. No disease symptoms on leaves (rated zero).
2. 1-25% of leaf area covered with spots (scored as 1).
3. 26-50% of leaf area covered with spots (scored as 2).
4. 51-75% of leaf area covered with spots (scored as 3).
5. 76% or more (scored as 4).

$$\textbf{Disease Incidence (D I \%)} = \frac{\mathbf{x}}{\mathbf{N}} \times \mathbf{100}$$

OR

$$\text{D. I (\%)} = \frac{\text{No. of infected trees}}{\text{Total number (healthy and infected) trees assessed}} \times 100$$

$$\text{Disease Severity (D.S \%)} = \sum \frac{a + b}{N.Z} \times 100$$

(Awa *et al.*, 2012).

OR

$$\text{D. S (\%)} = \frac{\text{Sum of all diseased leaves ratings/scores}}{\text{Total number of leaves examined x maximum rating/score}} \times 100$$

Where;

Σ (a+b) = Sum of infected/diseased leaves and their corresponding score scale.

N = Total number of sampled trees

Z = Highest/maximum score scale/rating

X = Number of infected/diseased trees (Awa *et al.*, 2012).

Preparation of Culture Media for the Growth of Fungi using PDA

Potato Dextrose Agar (PDA) was prepared from Irish potato tubers as a carbon source. 200 g of peeled fresh Irish potato tubers was cut into pieces of about 1 cm^3 and weighed into a 1000 ml beaker. 500 ml of distilled water was poured on to the diced potatoes and boiled gently on a hot plate until soft. The supernatant was filtered using a doubled layered muslin clothe into a litre conical flask. 10g of D-glucose and 15 g of plain agar were added after which the filtrate was then made up to one litre with distilled water. The conical flask was corked using cotton wool and foil paper. The mixture was homogenized on a hot plate and then sterilized in an auto cleave at 121 ^{0}C for 15 mins. The medium was allowed to cool to about 45 ^{0}C before pouring aseptically into 9 cm diameter Petri dishes. (Stevens, 1981).

Isolation of Fungal Pathogens

At the laboratory, all the eight varieties of mango obtained from the field were sorted separately. Isolation was carried out according to variety and location obtained (Zainab & Shinkafi, 2016). Leaves samples collected from Dambuwa and Gagi were not isolated because the varieties obtained from these locations were well represented in all the major mango growing regions surveyed in this research. Leaves with highest score scale from each variety were used for inoculation. Two centimetres of the infected mango leaf tissue were excised with a sterilized razor blade at the point of progression of disease symptom; then surface sterilized by dipping into 70 per cent ethanol solution for 2 minutes. The tissues were then rinsed in three changes of sterilized distilled water and were dropped on sterile paper towels, allowed to dry before plating them onto PDA (potato dextrose agar) in Petri dishes. A total of 17 petri dishes of PDA media were plated for mango leaf tissue (i.e. the five varieties obtained from More, four varieties each

from UDUS permanent site, Arkilla and Illela respectively). These were incubated for 6 days at room temperature (25-28 ^{0}C) under light to enhance fungal growth following Okigbo and Osuinde (2003).

Purification

After some days, the plates were inspected to observe the growth of pathogens on the media. Some young mycelium emerging from the tissues were sub cultured on fresh PDA media with the help of a sterile loop to obtain pure isolates of the pathogens. They are checked and transferred on to new PDA media weekly and kept at room temperature (21-25 ^{o}C) to maintain pure cultures at all times for correct identification of fungal pathogens. Isolated colonies were sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were dentified from each variety by visual examinations and viewing under electronic microscopes (Zainab & Shinkafi, 2016).

Identification of Foliar Fungal Pathogens

After three consecutive times of purifying the plates following Saeed (2012), they were inspected again to for growth and purity. Some of the pure cultures obtained, on Potato dextrose agar, had colonies that were whitish to dark grey with thick to sparse lawns of aerial mycelium when viewed from the top of Petri dishes similar to Awa *et al.* (2012) description and were greenish to orange or dark brown centre bordered by creamy surrounding when viewed from the reverse side of the Petri dish. When viewed under the microscope, conidia were observed to be hyaline; single celled and cylindrical with obtuse ends. The fungus was, identified to be *Colletotrichum gloeosporioides*. A total of 48 pure cultures were obtained (out of which *c. gloesporiodes* had the highest occurrence of 12 plates). Some plates exhibited similarities in morphological growth and were therefore divided into groups. Eleven different groups with similar growth morphologies of isolates within each group were identified and three distinct and morphologically different from any isolate were also identified as *Aspergillus fumigatus, Pestalotiopsis mangiferae* and *Phomopsis mangiferae.* A maximum of three pure isolates that have similar morphology were carefully selected from each group of eleven isolates and assumed that they were all the same. They were grouped according to the growth shown (colony characteristics). Purification was done for the various groups to ensure pure isolates were obtained at all the times. Temporary slides from each group of mango leaf isolates were made after carefully selecting the purest and observed under light/electronic microscope. Some unidentified spores were observed to be similar to *Pseudofusicoccum spp.* and were identified as such. A total of eleven pathogens (*Alternaria longipes, Aspergillus fumigatus, Aspergillus niger, Colletotrichum gloesporioides, Fusarium oxysporum, Fusarium mangifera Pestalotiopsis mangiferae, Phoma mangiferae, Phomopsis mangiferae, Pseudofusicoccum spp.*, and *Rhizopus oryzae*) were identified and were then described and classified based on conidia and colony morphology as described by Barnett and Hunter (1998), Ellis and Hermanis (2003), Palvic *et al*. (2008), Guo-yin *et al. (*2013), Nyongesa *et al.* (2015), Britz *et al.* (2002), Iqbal *et al.* (2008) and Gagkaeva (2008). Stock cultures of all the isolates were maintained on PDA slopes in McCartney bottles at 4°C in the dark.

Pathogenicity Tests

Preparation of Spore Suspension

The procedures that were adopted are in accordance with Abubakar (2009), Okigbo and Osuinde (2003) and Awa *et al.* (2012). The preserved pure cultures of the isolated pathogen were grown on PDA in the laboratory until they sporulate. Ten milliliters (10 ml) of distilled water were added to each Petri dish for all the eleven pathogens identified from this experiment using sterile syringe. The mycelia mat from the culture in each Petri dish was harvested using a sterile scalpel. The mycelia were shaken vigorously or blended in an electric blender (pending on power supply) for five minutes (5 mins). 200 ml of distilled water were added in to eleven (500 ml) conical flasks for each of the pathogen and filtered separately using a sterile double layered muslin clothe for each suspension.

Leaf Inoculation (Detached Technique)

Detached leaf technique was used for leaf inoculation. New leaves apparently healthy and free from leaf spots were collected, washed, and surface sterilized. The leaves were then sprayed with the spore solution of the respective fungal isolates using hand fitted with sterile gloves and placed into paper envelopes lined on the inside with moist paper tissue, covered with moist paper towels and incubated in a humid chamber for 2 weeks at 28 ^{o}C (Awa *et al.*, 2012) .

Re-isolation of fungal foliar pathogens

The causative organisms in previously diseased parts were re-isolated on potato dextrose agar as described earlier in isolation of pathogen (section 3.6). The characteristics of the re-isolated fungi were compared with their original isolates to confirm Koch's postulates. (Abubakar, 2009; Awa *et al.*, 2012).

Results

More area from the field survey carried out had the largest number of Mango tree population with this research estimating the figure at 1,800 (figure 1). There are climatic and socio economic factors explaining this reality. The trees are planted on the northern bank of River Rima, which supplies moisture for the most part of the year. On the southern bank of the river lies the city of Sokoto, a sprawling urban centre providing a huge demand for the mango fruits. As further depicted in figure 1, Usmanu Danfodiyo University, Sokoto (UDUS) is the environment home to the second largest concentration of mango trees, with this research estimating a total of 580 mango trees in the area, followed by Illela area, where an estimated total of 280 mango trees thrive. The remaining areas of Arkilla, Dambuwa and Gagi were home to an estimated total of 110, 65 and 38 trees respectively**.**

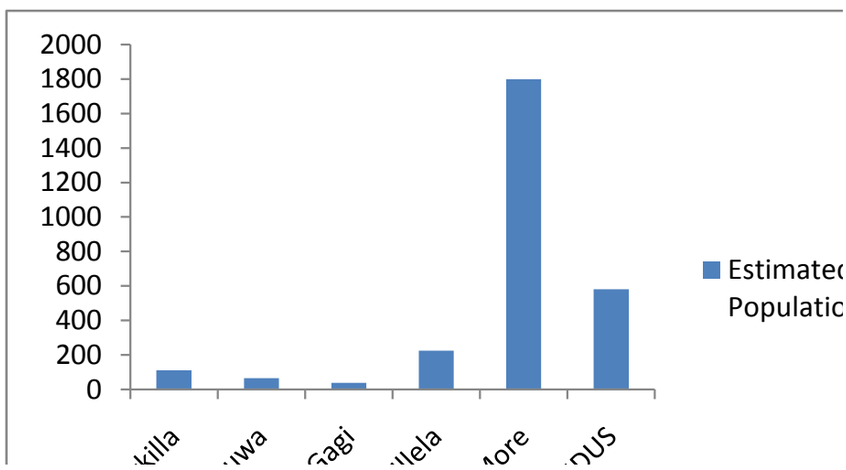

Figure 1: Estimated mango plant population in sokoto metropolis.

The graph in figure 2 show some interesting results. More area had the largest number of infected mango trees, with an estimated number of 200, followed by UDUS for which an estimated total of 72 infected trees were obtained.

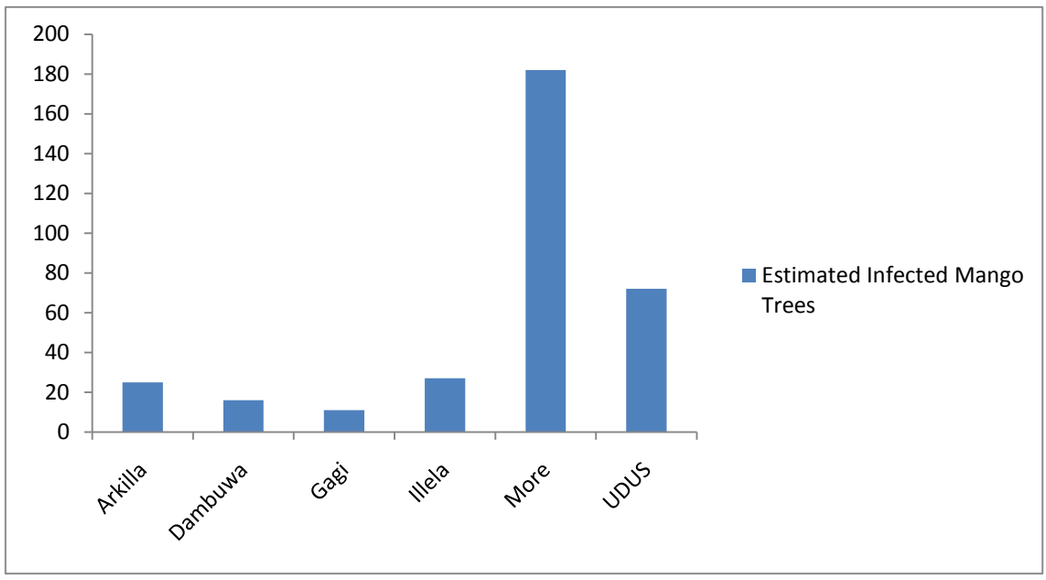

Figure 2: Estimated Number of Infected Trees (August 2015).

Although tree population varied from one area to another, Figure 3 shows that, all the five sampling locations except More area accounted for 16% of the sample each. Because of its overwhelming mango tree population. More accounted for 20% of the sample.

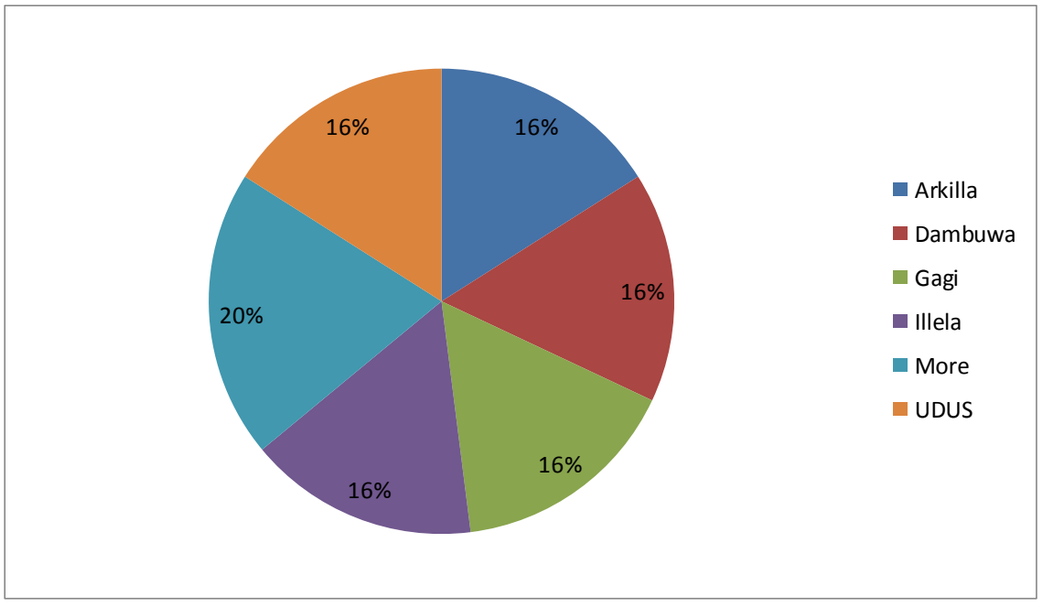

Figure 3: Distribution of Mango sample by Location.

Figure 4 indicated the results of distribution of Mango Variety by Location. From the results obtained it is clear that Dankamaru mango variety had the highest representation in the sample, due to the predominance of this variety in Sokoto and its environs. A further illustration of the relative dominance of Dankamaru as a variety of mango tree in Sokoto (figure 4) indicated that, two cultivars (Dandogo, and Mai Bindiga) are found only in one location each. Babban Turmi is found only in Illela and More, while improved variety in Illela and Gagi. Dankamaru is found in all locations except Dambuwa. Binta siga is found in all locations except in Gagi while Peter is found in four places, except in Illela and UDUS.

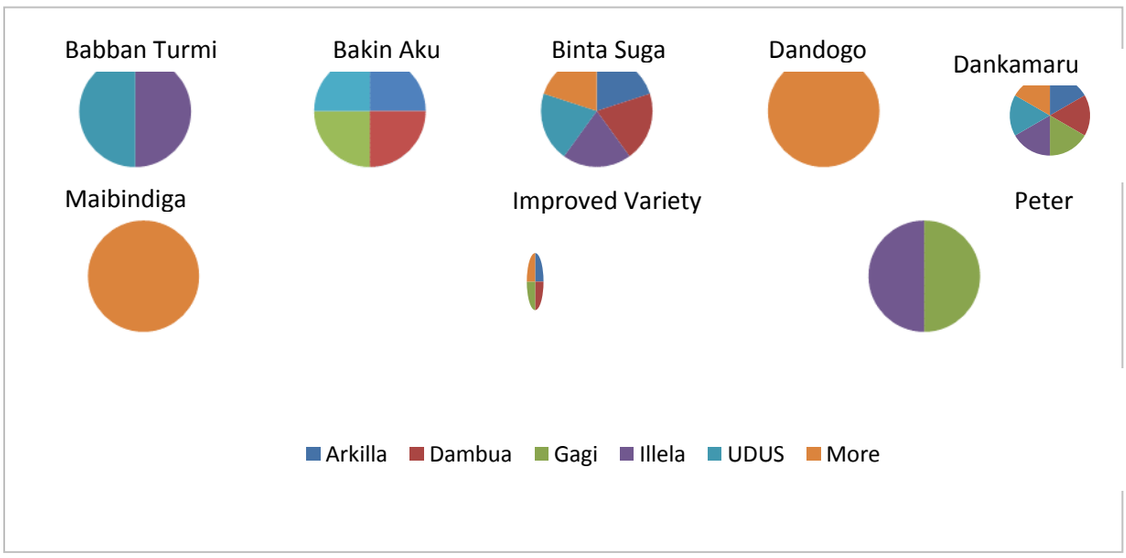

Figure 4: Distribution of Mango Variety by Location.

The bar graph shown in Figure 5 indicated the incidence of mango leaf infection. From the graph, it is evident that judging by the two measures of leaf infection (i.e. leaf score and disease severity) UDUS had the highest incidence of Mango disease severity with (56.75%), followed by Dambuwa with (45.50%), More area (44.48%), lllela (37.00%), Gagi (34.15%) and Arkilla (31.65%) in that order.

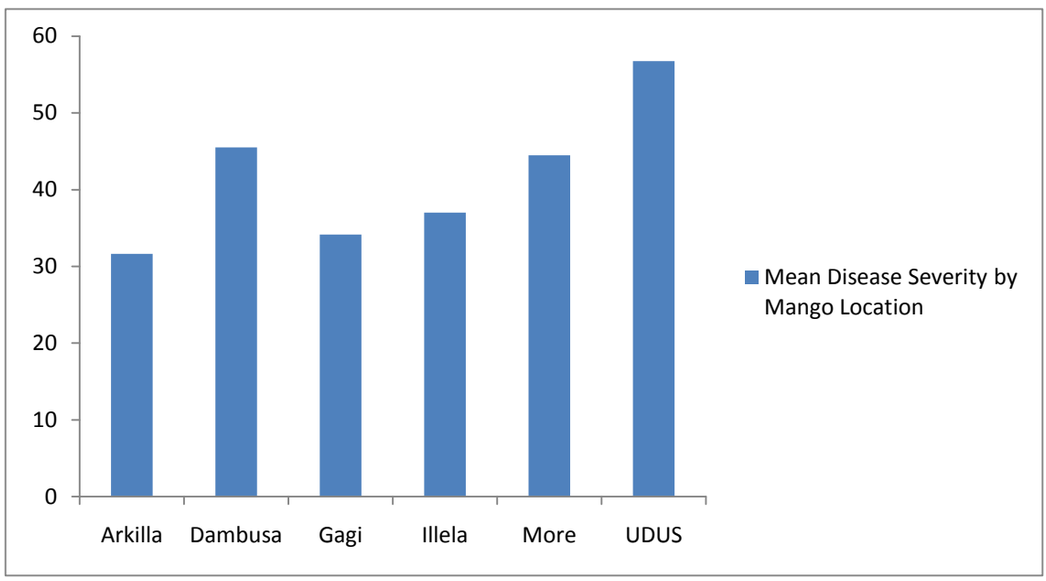

Figure 5: Mean Disease Severity by Mango Location.

Figure 6. Show the mean disease severity in relation to mango variety. From the results obtained it can be concluded that the mango variety with the highest disease severity is Mai Bindiga (78%) followed by Dandogo (53%), and Peter (44.85%). Others are Binta siga (37.4%), Dankamaru (34.93%), Babban Turmi (29.1%) and Improved variety (25.8%).

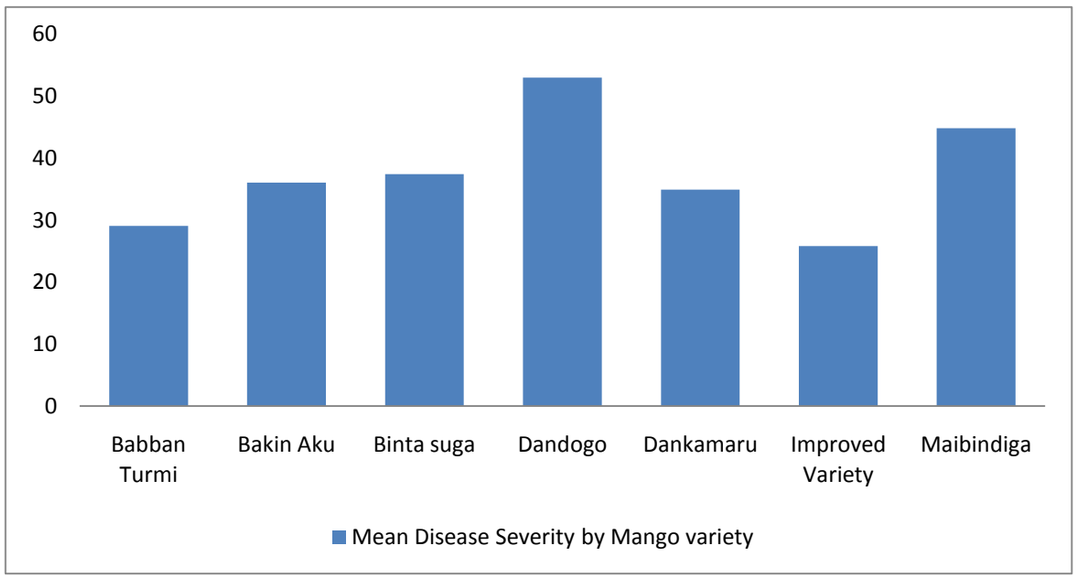

Figure 6: Mean Disease Severity by Mango Variety.

The frequency distribution of disease severity of the overall samples was indicated in Figure 7. As the figure suggested, the leaves with the modal incidence of infection are those with infection rate ranging from 26-50% of their surface area. A striking feature of the graph in Figure 7 is that the shape ofthe disease distribution from the sample for this study appears nearly like a normal distribution, centring on the estimated mean leaf score of 2.05.

The results of testing for statistical significance in infection across mango trees in different locations revealed that from the results, F- statistic of 2.15 was obtained with a significance of 6.42%. Thus the F-statistic for this result is not significant, suggesting that average disease severity for mango leaves does not significantly vary with the location. When the test was applied to see if disease severity varied with mango variety, a different conclusion was reached. F-statistic of 2.86 was obtained, with a significance level of 0.61 per cent. Thus, the F-statistic in this case is significant and it can be concluded that average mango leaf disease severity shows significant variation across mango varies. To check which of the mango varieties were responsible for this significant variation, pair wise comparison of means (Duncan's test) were conducted. The bold face results indicate the mango varieties exhibiting significant variations in mean disease severity.

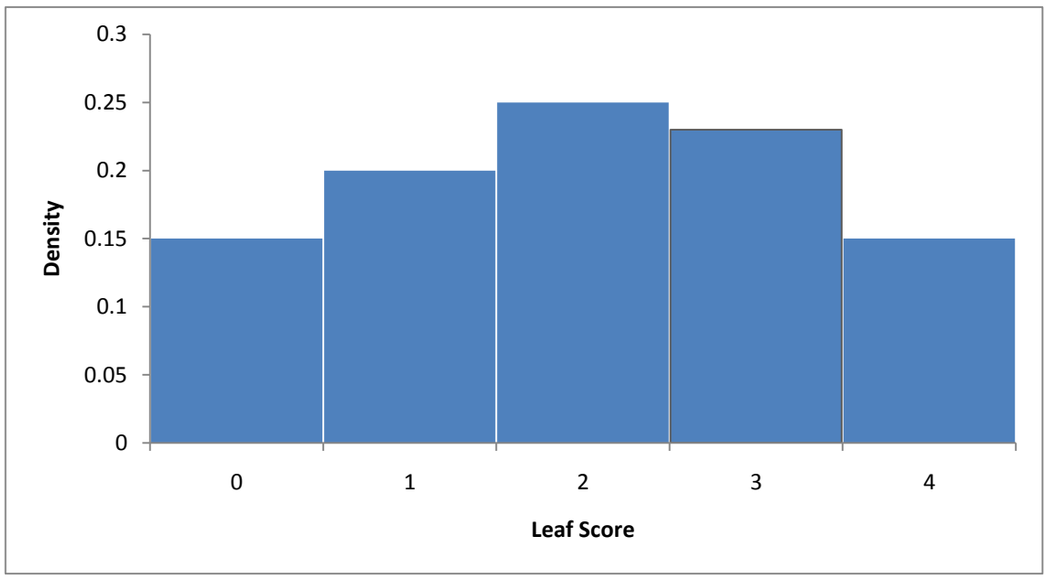

Figure 7: Frequency Distribution of Disease Severity overall sample.

Discussion

Most of the pathogens isolated from the study are known to infest mango fruits and other vegetables (Saeed, 2012). The results from this study however revealed that same pathogens could also infest not only mango fruits but also its leaves.

According to Stovold (2004), wet conditions favour most diseases especially anthracnose disease. This was similarly stated by Agrios (2005), that many pathogens, especially foliar (leaf) pathogens, need a film of water on the plant to begin growth, penetrate the host, and establish infection. This is why wet or humid weather is so important in the development of many fungal and bacterial diseases. The regular water supply from the River Rima to the mango trees at More seems to explain the susceptibility of the trees in that location to these pathogens.

C. gloesporioides were isolated from all the areas surveyed. Therefore, this could explain why Okigbo and Osuinde (2003), Stovold (2004), Uliwa (2008), Nelson (2008), and Awa *et al.* (2012), unanimously agreed that anthracnose is presently the most important field and post harvest disease of mango widely distributed in all mango-growing regions of the world.

Phoma mangiferae is the second largest reoccurring pathogen (isolated from Illela, More and UDUS). Both More and UDUS are lowland regions which are prone to flooding during the rainy season while Illela is among the characteristically dry elevated upland regions in the Sokoto. Saeed (2012) reported that *Phoma* sp. causes phoma blight in mango and can only be observed on matured or old leaves because it is a weak pathogen that invades stress plants. It causes spot that are fully developed and are characterized by dark margin and dull necrotic centre. Okigbo and Osuinde (2003), similarly identified and isolated some leaf spot pathogens from south-eastern part of Nigeria among which are *Macrophoma mangiferae* and *Pestalotiopsis mangiferae*. Diedhiou *et al.* (2007) also identified *Phoma mangiferae, Alternaria* sp. and *Aspergillus niger*, among other pathogens as major causes brown and black spots on mango leaves. Agrios (2005), further reported that Phoma spp. cause numerous diseases of vegetables and other annual plants. They are often present with other weak pathogens. Therefore, the mimickery of lowland environmental condition to that of the south-eatern part of country explained the reasons behind the isolation of these similar pathogens in this northern part of Nigeria.

Fusarium mangiferae was isolated only from UDUS (along Kofar Mata). Being a lowland, the region is usually humid and most mango trees are partially submerged during the wet season and dry most time of the year. This fluctuations could disrupt some physiological activities in the plants. Iqbal *et al*. (2008) reported that *Fusarium mangiferae* is one among many fungi responsible for mango malformation diseases resulting from various biotic and abiotic stresses. Malformation is the most important malady causing colossal losses every year.

Although there are no previous reports of *Pseudofusicoccum* sp., *Alternaria longipes* and *Rhizopus oryzae* isolations from mango, it has been isolated and identified from this study. *Alternaria longipes* was isolated from Illela, More and UDUS. *Pseudofusicoccum* sp., from More while *Rhizopus oryzae* from Illela. *Alternaria longipes* and *Rhizopus oryzae* could also be the cause of fungal leaf spot on mango in Sokoto and its environs.

Aspergillus niger was reported by earlier studies as the possible pathogen causing dusty charcoal spores of the black mold affecting mango leaves which are easily be dispersed by air from infected fruits.(Khaskheli *et al.,* 2008; Nyongesa *et al.*, 2015; Diedhiou *et al.*, 2007).

Conclusion

Anthracnose was found prevalent in all the mango-growing areas surveyed, the occurrence and severity was probably more influenced by environmental conditions and cultural practices rather than climatic factors in the areas. Fruit yield drastically reduced in areas with highest anthracnose disease occurrence and severity, an indication that anthracnose disease phenomenon has negative correlation with fruit yield. This phenomenon was largely influenced by environmental conditions and cultural practices. There are no effective controls against plant p athogenic fungi.

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