INCIDENCE OF LEAF BLIGHT DISEASE OF EGUSI MELON IN SOUTH-WEST NIGERIA

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ABSTRACT

Egusi melon (Citrullus lanatus (Thumb) Mansf.) is an important vegetable crop grown for edible seeds and oil in West Africa. Leaf Blight Disease (LBD) is one of the major constraints to its production, with potential to cause economic damage. The objective of this study was to investigate the incidence and distribution of leaf blight on Egusi melon in Southwestern Nigeria. A survey of LBD of Egusi melon was conducted in 2015 and 2016, in five southwestern States of Nigeria (Ogun, Oyo, Osun, Ekiti and Ondo States). Twenty plants each, were randomly sampled from 150 farms comprising 30 farms each, from each State. The distribution of different Egusi melon varieties planted was recorded. “Bara” cv. was the most cultivated variety (51.6%); followed by “Bojuri” (30.4%) and “Serewe” (18%). Leaf blight was observed in most farms in the five States, from 73% in Osun and Oyo states to 83% in Ondo State. Disease incidence and severity varied with locations and cultivars, and ranged from 0.0-87.5±18% and 1.0±0-4.5±0.8 in Osun State to 20.0±19 - 95.0±4.5% and 2.3±1.5 - 5.0±0 in Ondo State. Out of the twelve fungal pathogens from ten genera isolated from infected plants, only Colletotrichum truncatum, C. gloeosporioides and Lasiodiplodia theobromae caused Leaf blight on Egusi melon.

Key Words: Colletotrichum gloeosporioides, Colletotrichum truncatum, Lasiodiplodia theobromae

RÉSUMÉ

Le melon Egusi (Citrullus lanatus (Thumb) Mansf.) Est une importante culture légumière cultivée pour les graines et l’huile comestibles en Afrique de l’Ouest. La maladie de brûlure foliaire (LBD) est l’une des principales contraintes à sa production, avec le potentiel de causer des dommages économiques. L’objectif de cette étude était d’étudier l’incidence et la distribution de la brûlure foliaire du melon Egusi dans le Sud-ouest du Nigéria. Une enquête sur la LBD du melon Egusi a été faite en 2015 et 2016, dans cinq États du sud-ouest du Nigéria (États d’Ogun, Oyo, Osun, Ekiti et Ondo). Vingt plantes dans chaque État ont été échantillonnées au hasard dans 150 fermes comprenant 30 fermes chacune, de chaque État. La distribution des différentes variétés de melons Egusi plantées a été enregistrée. «Bara» cv. était la variété la plus cultivée (51,6%); suivi de «Bojuri» (30,4%) et «Serewe» (18%). La brûlure foliaire a été observée dans la plupart des exploitations agricoles des cinq États,
INTRODUCTION

Egusi melon (Citrullus lanatus (Thumb) Mansf.) is a vegetable crop commonly cultivated in West Africa for edible nutrient rich seeds, popularly called “Egusi” in Nigeria (Denton and Olufolaji, 2000; van der Vossen et al., 2004). It belongs to the family Cucurbitaceae (Schippers, 2000). Four genera of this family, namely Cucumis, Citrullus, Cucumeropsis and Lagenaria are referred to as melon. Example of the genus Cucumis is C. melo L. (true melon), that of Citrullus are C. lanatus Thumb. Matsum and Nakai (Watermelon) and Citrullus lanatus (Thumb) Mansf. (Egusi melon). The common Cucumeropsis species is C. mannii Naud (white-seeded Egusi melon); while the species of Lagenaria (gourd melons) that is Egusi melon is Lagenaria abyssinica (Ajuru and Okoli, 2013).

The scientific name for Egusi melon has been variously given as Citrullus lunatus (Thumb.), Citrullus vulgaris Shrad or Colocynthis citrullus L. (Philip, 1977; Ogunremi, 1978). However, Lowe and Soladoye (1990) indicated that Egusi melon was formerly known as Colocynthis citrullus (Linn) O. Kitzer, but is now confirmed to be Citrullus lanatus (Thumb) Mansf. The crop is native to the West African region. It is a trailing, hairy and herbaceous crop (Agba et al., 2009), usually intercropped with other crops such as yams, sorghum and cassava, in peasant farms (Adewusi et al., 2000; Agba, 2004). Nigeria is the highest producer of melon in Africa, producing about 569, 398 tonnes over an area of 1,085,998 ha, with an average yield of 5,243 kg ha⁻¹, followed by Democratic Republic of Congo (62,487 tonnes) and Cameroun (52, 940 tonnes) (FAO, 2016).

The common Egusi melon cultivars in Nigeria are “Bara”, “Serewe” (Citrullus lanatus (Thumb) Mansf) and “Bojuri” (Lagenaria abyssinica). In the South-western and Northern Nigeria, “Bara” is the most common, characterised by prominent thick, black or white colour seed edges (Oyolu, 1977; Fayemi, 1999). This cultivar has the widest distribution (Denton and Adeniran, 1990; Denton and Olufolaji, 2000). Unlike “Bara”, “Serewe” lack pronounced seed edge (van der Vossen et al., 2004) and the characteristic thin seed coat; and lack of seed edge has made the “Serewe” seed to have higher shelling percentage, thus providing more edible endosperm than “Bara” (Denton and Olufolaji, 2000). “Bojuri” plant, which is broad-leaf unlike others, has bigger and longer seed than the other two, and without pronounced seed edge but usually with terminal white or brown patches.

In spite of the vast nutritional and industrial significance of Egusi melon seeds, its production and yield are very low in Nigeria, owing to many factors which include excessive rainfall, low soil pH and increased incidence of pathogens such as, fungi, bacteria, nematodes and viruses (van der Vossen et al., 2004). Incidence and management of a variety of diseases have been reported on other members of the family Cucurbitaceae; but little information is available on Egusi melon. Most of the time, diseases affecting this crop are described with reference to other related crops such as watermelon (C. lanatus var. lanatus), which have received more research attention.
Fungal diseases, namely powdery mildew, downy mildew, *Alternaria* leaf spot, *Cercospora* leaf spot, Anthracnose disease (*Colletotrichum lagenarium*), leaf blight and fruit rot (*Didymella bryoniae*) and fruit and flower wet rot (*Choanephora cucurbitarum*) have been reported on Egusi melon in Nigeria (Kehinde, 2008). Among these, Anthracnose and leaf blight were reported to be the predominant foliar fungal diseases of the crop in Southwestern Nigeria (Kehinde, 2011). However, there are limited reports and lack of updates on the important Egusi melon diseases, especially on the distribution and current status of blight in Nigeria. This study investigated the incidence and causal organism of leaf blight disease of Egusi melon in South Western Nigeria.

**MATERIALS AND METHODS**

**Incidence and severity of leaf blight.** A field survey was conducted in Ogun, Oyo, Osun, Ekiti and Ondo states of Nigeria, during Egusi melon growing season, in the South-west Nigeria (between April and July) of 2015 and 2016. Information on the Egusi melon producing areas obtained from the Agricultural Development Projects (ADPs) of the States, was used in the choice of survey locations. Twenty blight infected symptomatic plants were randomly sampled per farm (Kehinde, 2011) with two farms per location, three locations per local government areas (LGAs) and five LGAs per State; making 150 surveyed farms from the five States. Survey was conducted by randomly walking through the farm diagonally to collect leaf samples. Geo-references of each point surveyed were determined using Global Positioning System equipment (eTrex Garmin, Taiwan).

Disease incidence on the fields was assessed using the formula:

\[
\text{Incidence (\%) = \frac{n}{N} \times 100}
\]

Where:

- \( n \) = number of plants showing symptoms on the field;
- \( N \) = Total number of plant on the field.

Disease severity was determined as:

\[
\text{Disease severity = Proportion of total damaged tissue \times 100} \\
\text{Total surface area of the plant parts}
\]

This was converted to a disease rating scale of 1-5 (Kehinde, 2011):
- 1 = No symptom;
- 2 = <10% damaged portion (mild symptom);
- 3 = (11 – 30% damaged portion (moderate symptom);
- 4 = 31 – 50% damaged (severe symptom);
- 5 = >50% damaged portion (very severe symptom).

The frequency of occurrence of isolated fungi was calculated following Ebele (2011) as follows:

\[
\text{Frequency of occurrence (\%) =} \\
\frac{\text{Number of times a fungus was encountered}}{\text{Total number of fungi isolated}} \times 100
\]

**Isolation of fungi associated with Leaf blight disease.** A diagnostic test was carried out at the Pathology Laboratory of the Nigeria Agricultural Quarantine Service, Moor Plantation Ibadan, Nigeria. Leaf samples with blight symptom were washed with sterile distilled water, and 5 mm² of the infected parts were cut with sterile scalpel blade, at the boundary of the healthy and infected tissues. The excised leaves were sterilised for two minutes, in 10% Sodium hypochlorite (Klement et al., 1990). They were then rinsed five times with sterile distilled water and blotted to dry between sterile paper towels, before plating on potato dextrose agar (PDA). These pieces were aseptically transferred with scalpel into Petri dishes containing 15 mL of PDA.
The inoculated plates were incubated at 28 ± 2 °C for 3 to 7 days, and examined for fungal growth using compound microscope (Navite Olympus, China). Fungal isolates from each of the samples were sub-cultured on PDA to obtain pure cultures for identification. Cultural and morphological characteristics of the fungal isolates were recorded. Wet mounts of the isolates were prepared on a microscope slide and stained with lactophenol cotton blue. The mounts were observed under a compound microscope at X40 magnification. Detailed structural features of each isolate were recorded and features of the organisms were compared with those described in a Standard Manual for Fungi (Barnett and Hunter, 2003).

Pathogenicity test. Pathogenicity test was carried out in a screenhouse, using 14 day-old Egusi melon seedlings. A fungal suspension of 2.1x10⁶ spores mL⁻¹ was prepared from the 7 days old culture plates of the isolated fungi. All the fungi isolated from survey sampled were used. The test was conducted in a 3 by 12 factorial (3 cultivars: “Serewe”, “Bara”, “Bojuri” and twelve isolates) experiment in a completely randomised design, replicated three times. Two seeds were planted per pot, filled with 5 kg sterilised soil, to raise seedlings on which fungal isolates were inoculated to confirm causal organisms of Egusi melon diseases.

A spore suspension of 2.1x10⁶ spores mL⁻¹ was sprayed on the leaf surfaces to run off using a 250 mL hand-operated sprayer, and covered with a transparent polythene sheet for 48 hours to maintain high humidity for disease initiation. Control plants were sprayed with only sterile water. Appearance of blight and other disease symptoms were observed for five weeks. Daily watering of plants was carried out. Re-isolation of the pathogen was done from the plant showing disease symptoms within two to five weeks post-inoculation and isolates were identified using standard procedures (Barnett and Hunter, 2003).

Data were analysed by ANOVA, using the Statistical Analysis Software (SAS, 2008). Means with significant differences were separated by Student Newman Keuls (SNK) Test at 5% level of significance.

RESULTS AND DISCUSSION

Three Egusi melon cvs, were found in the five States, of which “Bara” was the most cultivated (51.6 %) in all the LGAs, followed by “Bojuri” (30.4 %). “Serewe” (18 %) was least cultivated in both years. Leaf blight symptoms were observed in all the LGAs in the five States. Out of 30 farms per State, blight was observed on 22 farms (73 %) in Osun and Oyo, 23 (77 %) in Ogun and Ekiti and 25 farms (83 %) in Ondo State. This confirms the presence of leaf blight on the crop in Nigeria, as previously reported (Chiejina, 2006; Kehinde, 2008). Blight incidence and severity were generally high across the five States, although this varied with farm locations and cultivars (Table 1). In Ogun State, the highest incidence and severity (90.0 % and 5.0) were observed on “Bojuri” cv. in Odeda LGA, with lowest (25.0±7% and 4.0) on “Bara” from Ado-odo Ota.

Ise-orun LGA had the highest leaf blight incidence and severity (70.0% and 3.8±0.4) on “Bara” in Ekiti State; while “Serewe” at Ekiti West had the least (6.7±0.5 % and 2.0). Ondo State had the highest incidence of leaf blight among the five States, with 95.0±4.5% incidence and 4.7±0.5 severity on “Bojuri” at Akure North and least (20.0 % and 2.3±1.5) on “Bara” in Akoko North-east. Also, “Bara” cv. had the highest (87.5±18% and 4.5±0.8) in Ayedire LGA of Osun State; while blight was not observed (0.0 and 1.0%) on “Bara” and “Serewe” at Ife South. The highest incidence and severity observed in Oyo State (77.0±14 and 3.8±1.3%) was on “Bara” at Ibarapa East; whereas Atisbo had the least (35.0±20 and 2.8±0.5%) on the same cultivar.

The observed blight incidences were generally not too distant from the range of 82 - 100% earlier reported by Kehinde (2008). Higher leaf blight incidence and severity observed on “Bara” in three (Ekiti, Osun and
### TABLE 1. Incidence (%) and severity of leaf blight on Egusi melon cultivars in five South Western States in Nigeria

| State     | LGA          | Incidence Bara cv. | Incidence Serewe cv. | Incidence Bojuri cv. | Severity Bara cv. | Severity Serewe cv. | Severity Bojuri cv. |
|-----------|--------------|---------------------|----------------------|----------------------|-------------------|---------------------|---------------------|
| Ogun      | Odeda        | 60.0±24a            | 40.0±0c              | 90.0±0a              | 3.8±1.5a          | 1.5±0b              | 5.0±0a              |
|           | Odogbolo     | 56.0±11a            | 50±11b               | -                    | 4.0±0.7a          | 3.5±0a              | -                   |
|           | Ijebu N.E.   | 55.0±6a             | 70.0±0a              | 40.0±0b              | 3.3±0.5a          | 5.0±0a              | 2.0±0c              |
|           | Ado-odo ota  | 25.0±7b             | 30.0±0c              | 32.5±25b             | 4.0±0a            | 4.0±0a              | 2.5±1.0c            |
|           | Yewa N.      | 30.0±8ab            | 30.0±0c              | 32.5±5b              | 3.3±1.0a          | 3.0±0a              | 3.8±0.5b            |
| Ekiti     | Emure        | 40.0±0ab            | 40.0±0a              | 63.3±6a              | 2.6±0.9b          | 2.0±0b              | 3.3±0.6a            |
|           | Ise-oru      | 70.0±0a             | -                    | 62.0±11a             | 3.8±0.4a          | -                   | 3.2±0.5a            |
|           | Ekiti S.W.   | 40.0±0ab            | -                    | 71.6±25a             | 2.0±0b            | -                   | 4.0±1.3a            |
|           | Ekiti W.     | 23.8±20c            | 6.7±0.5b             | 32.0±18b             | 2.3±1.0b          | 2.0±0b              | 2.8±0.8a            |
|           | Ijero        | 47.5±17ab           | 40.0±0a              | 57.5±27a             | 3.8±0.5a          | 3.5±0a              | 4.0±0.8a            |
| Ondo      | Owo          | 92.0±7a             | 70.0±0a              | 86.7±5.8a            | 5.0±0.5a          | 3.0±0b              | 4.3±0.6ab           |
|           | Odigbo       | 90.0±0a             | 80.0±0a              | 83.3±15.3a           | 4.0±0ab           | 4.0±0a              | 4.3±0.6ab           |
|           | Akure N.     | -                   | -                    | 95.0±4.5a            | -                 | -                   | 4.7±0.5a            |
|           | Akoko N.E.   | 20.0±19b            | 20.0±0b              | 32.5±22b             | 2.3±1.5b          | 3.0±0b              | 2.3±1.5c            |
|           | Akoko N.W.   | 27.5±17b            | 30.0±0b              | 25.0±18b             | 3.0±1.2ab         | 3.0±0.4b            | 2.7±1.2bc           |
| Osun      | Ayedire      | 87.5±18a            | 70.0±4b              | -                    | 4.5±0.8a          | 4.0±0a              | -                   |
|           | Iwo          | 75.0±17a            | 80.0±0a              | -                    | 4.2±0.8a          | 4.0±0a              | -                   |
|           | Ola Oluwa    | 75.0±10a            | -                    | -                    | 4.1±0.7a          | -                   | -                   |
|           | Ife S.       | 0.0±0c              | 0.0±0cd              | 34.0±20a             | 1.0±0b            | 1.0±0b              | 3.2±0.8a            |
|           | Ori-ade      | 24.0±11b            | 35.0±0c              | 26.0±9a              | 3.4±0.6a          | 3.5±0a              | 3.6±0.5a            |
| Oyo       | Ibarapa E.   | 77.0±14a            | 58.3±4b              | 60.0±0ab             | 3.8±1.3a          | 2.0±0.6b            | 2.0±0b              |
|           | Ibadan S.W.  | 50.0±19b            | 56.7±12b             | 70.0±0a              | 3.6±0.6a          | 4.0±0a              | 4.0±0a              |
|           | Ido          | 35.0±16b            | 70.0±0a              | -                    | 2.8±1.0a          | 4.0±0a              | -                   |
|           | Atisbo       | 35.0±20b            | 40.0±0c              | 35.0±16c             | 2.8±0.5a          | 3.0±0.7a            | 2.8±0b              |
|           | Iseyin       | 60.0±0ab            | 60.0±0b              | 50.0±12b             | 3.7±0.5a          | 4.0±0a              | 3.8±0.4a            |

- = variety not found, LGA = Local government area. Values are mean data of 2015 and 2016 surveys. Value with similar letter along the column for each State are not significantly different by Student Newman Keuls Test (P = 0.05)
Oyo) of the five States is conceivable due to wider distribution of the cultivar in Southwestern Nigeria, arising from the cultivar’s better preference by the people as well as its high susceptibility to the disease. Leaf blight has been reported to have produced the highest incidence and severity compared to other folia diseases of Bara and Serewe cvs. in South-west Nigeria (Kehinde, 2011). High rainfall and humidity of South-west Nigeria, during the Egusi melon cropping season might have also contributed to the high incidence and distribution of the disease.

Fungal isolates from the samples produced some disease symptoms on Egusi melon plants (Plate 1) from the pathogenicity test. Twelve fungal pathogens from ten genera isolated and identified from the infected samples (Fig. 1)
Leaf blight of Egusi in Nigeria had some similarity with previous reports (Bankole and Joda, 2004; Kehinde, 2011). Though there is limited information on the economically important pathogenic fungi of Egusi melon, some of these isolated fungi have been reported earlier from the crop in South-eastern Nigeria (Chiejina, 2006). Meanwhile, the available reports focussed more on stored Egusi melon seeds than field plants. For instance, a study on stored seeds of “Serewe” variety in Nigeria showed incidence of Aspergillus niger, A. flavus, A. fumigatus, A. granulosis, F. solani, Botrydiplodia theobromae and Penicillium citrinum (Bankole and Joda, 2004).

Kehinde (2013) reported isolation of Erysiphe cucurbitarum (Powdery mildew), Peronospora cucurbitarum, (Downy mildew) Alternaria cucumerina, (Alternaria leaf spot) Cercospora citrullina (Cercospora leaf spot), Colletotrichum lagenarium (Anthracnose), Didymella bryanii (leaf and vine blight and fruit rot) and C. cucurbitarum (Wet rot of flower) from Egusi melon plants in Nigeria.

The most frequently occurring field fungal pathogens associated with blight from this study (Fig. 1) were Choanephora cucurbitarum (29.8 %), followed by L. theobromae (26.0%) and Curvularia lunata (9.3%). Some of these recently identified Egusi melon pathogens (C. cucurbitarum, Colletotrichum spp., Fusarium spp. and L. theobromae) have been earlier reported in Egusi melon (Bankole and Joda, 2004; Kehinde, 2008; 2011; 2013); while other...
identified Egusi melon fungi not observed in this study might not be associated with blight disease.

The observed uneven occurrence and distribution of the Egusi melon associated pathogens in the study areas might be due to variation in environmental factors such as temperature, relative humidity, soil pH, soil fertility and preferred Egusi melon cultivars. Such differences in disease distribution have been described by Baimey et al. (2009) as a natural occurrence, resulting from differences in the host plant, soil, climate, cropping patterns and cropping history. Though in the same agro-ecology, there are some variations in the climate, soil type, cropping system and cultivar preference among the five Southwestern States (Eludoyin et al., 2017).

These effects of variations in climate on host susceptibility and plant infection mechanisms have been reported by Hellin et al. (2014). The common practice of mixed cropping among the Egusi melon farmers in South-western Nigeria (Kehinde, 2011) could vary the establishment and distribution of blight disease, especially where Egusi melon is mixed with blight susceptible crops. In States like Ondo and Ekiti with preference to Bojuri cv. which is a broad-leaf, disease development will differ from that of Oyo State where Bara cv. is prevalent.

Pathogenicity test confirmed Colletotrichum gloeosporioides, Lasiodiplodia theobromae, (Syn. Botryodiplodia theobromae) and Colletotrichum truncatum as causal organisms of leaf blight on Egusi melon (Plate 1). This is contrary to Kehinde’s (2013) report of Didymella bryoniae as the causal pathogen of leaf blight and fruit rot of Egusi melon. We could not observe gummy stem blight symptoms or exudation of gummy material and water soaked leaf lesions of the Egusi melon plants from the study area, which are characteristic of diseases by Didymella bryoniae in cucurbits (Sudisha et al., 2006; Keinath, 2011). The different observation might be attributed to varying environmental factors such as relative humidity, temperature, soil moisture and soil pH, capable of affecting fungi pathogenicity (Topit and Sovali, 2010).

The three identified leaf blight causal pathogens have been reported to have caused various diseases in many crops (Nasimento et al., 2010; Suparman et al., 2018). Lasiodiplodia theobromae was reported to have a very wide host range, causing diseases such as blight, dieback, root rot and post-harvest rot in different plants in the tropical and subtropical regions (Urbez-Torres et al., 2008; Kumar and Singh, 2009). It was reported as one of the major field pathogens in South East Nigeria (Bankole, 1998) that caused leaf blight on orchid (Catasetum fimbriatum) in Brazil (Lopes et al., 2009) and root rot and leaf blight symptoms on rice in Nigeria (Claudius-Cole, 2018). It is also a tropical and subtropical cosmopolitan soil borne fungus (Burgess et al., 2006), with a wide host range which includes banana, mangoes, cocoa (Twumasi et al., 2014), cassava, yams (Ogundana, 1983) and coffee and coconut (Rosado et al. 2016).

Many species of Colletotrichum similarly cause blights of aerial plant parts and post-harvest rots (Saxena et al., 2016). Though known mainly for causing anthracnose, many Colletotrichum species also incite other diseases such as dieback, leaf spot, flower rot, fruit rot, stem end rot and root rot (Bruce et al., 2013). Colletotrichum truncatum causes anthracnose/pod blight of soybean (Jagtap et al., 2014); while Leaf blight of musli (Chlorophytum borivilianum) is caused by C. capsici (Sattar et al., 2005) and C. dematium (Banginwar et al., 2012). Leaf blight of Azadirachta indica by C. dematium has also been reported (Bhanumathi and Rai, 2007).

Leaf blight of Egusi melon is capable of causing economic damage on the crop in Nigeria and can reduce the photosynthetic ability of melon plants, making the plants more vulnerable to the effects of other diseases. The weather conditions of the survey area are conducive for fungal disease development. For instance, C. gloeosporioides requires high
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humidity of 85 – 90% and optimal temperature of 20 - 28 °C for survival on cassava (Bruggen et al., 1990). Such rain distribution and temperature range, which are obtainable in the southwestern Nigeria, might have enhanced the blight development by the fungi.

CONCLUSION

This study confirms the incidence of leaf blight on three Egusi melon varieties in the Southwestern Nigeria, and provides updates on the distribution of the disease. Blight is prevalent on “Bara” Egusi melon cultivar which is mostly preferred in the surveyed area and its incidence is highest on farms in Ondo State, but the least in Osun State. Twelve fungi are associated with blight on the crop; while Colletotrichum truncatum, C. gloeosporioides and Lasiodiplodia theobromae are the causal organisms of leaf blight disease of Egusi melon.

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