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A SURVEY OF FUNGAL PATHOGENS ASSOCIATED WITH EGUSI MELON (*Citrullus lanatus*) IN SOUTH WESTERN NIGERIA

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ABSTRACT

In a survey of farmers' fields across four states of South western Nigeria, major diseases of equsi melon were evaluated and symptoms described. Pathogenicity tests of foliage and fruits were conducted in the laboratory and causal pathogens identified. Powdery mildew (Erysiphe cucurbitarum) appeared as round whitish spots on the lower surface which increased in size and later appeared as whitish talcum on the upper surface. Downy mildew (Peronospora cucurbitarum) appeared on the upper surface as small and pale green to yellow angular spot which became chlorotic and shriveled. Alternaria leaf spot (Alternaria cucumerina) appeared as small circular water soaked areas which turned dark brown to black and Cercospora leaf spot (Cercospora citrullina) appeared as circular spot with white to tan centres having dark margins. Foliar spots which began as small yellowish watersoaked areas on the veins that later turned circular, dried up, broke and became shattered were symptoms observed for the Anthracnose disease (Colletotrichum lagenarium). Fruit anthracnose started as small, circular, black, sunken canker with raised margin. Leaf blight, stem blight and fruit rot (Didymella bryoaniae) disease symptoms were observed on leaves, stems and fruits respectively. Leaf symptoms began as light brown, irregular spots surrounded by yellow borders that developed from the tip of the leaves and gradually progressed backwards. Brown lesions were observed on the stem which split open and turned dark. Lesions observed on fruits appeared as small, almost circular water- soaked areas, which enlarged, resulting in dark, firm and leathery depressed areas. Symptoms of vine wilt were observed as elongated brown lesions which developed on stems near the crown which extended as long, narrow, brown streaks causing stem to completely wilt. Wet rot of flower and fruit (Choanephora cucurbitarum) appeared as characteristic fungus growth resembling numerous pins stuck in a pin-cushion on the infected surface. The study will help to bridge the gap of information on the characteristic symptoms and causal pathogens of melon diseases in South Western Nigeria

Keywords: "Egusi" melon, South western, Field diseases, Symptoms

INTRODUCTION

Melon (*Citrullus lanatus*) is an important crop plant and vegetable in Nigeria, cultivated for its edible nutrient rich seeds popularly called "egusi" (Denton and Olufolaji 2000). Melon belongs to the family Cucurbitaceae (Oyolu 1977). The 'egusi' melon plant is a creeping, hairy, herbaceous annual crop (Cobley 1979; Tindal 1983; Burkill 1985). Melon (egusi) seeds are nutrient-rich, thus they have been found to have value in various uses, including condiment in enriching the taste and appearance of local stew (Denton and Olufolaji 2000). The oil extracted from the seed varies in quality and quantity depending on the cultivars (Adewusi *et al.*, 2000), and it is widely used for cooking, frying and in the food industries, for manufacturing margarine, salad oil and canned fish (Ajibola *et al.*, 1990), soap

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making, medicine and illuminant (Burkill 1985). It contributes to food production, land erosion control and product with economic value in domestic markets. Equsi melon contains 34-38% protein when undefatted and 69-78% when deffated. It also contains 11% starch, 2.50% soluble sugars and 12% crude fibre and ash (Burkill 1985; Nwokolo and Sim 1987; Fayemi 1999). 'Egusi' melon is often cultivated as cash crop with other arable crops in the rainy and dry seasons (Adewusi et al., 2000). Its production varies among the states. Melon is usually planted before the food crops in traditional mixed cropping system. It is sown with starch staples such as yam (Dioscorea rotundata Poir), maize (Zea mays L.), sorghum (*Sorghum bicolor* (L.) Moench) and cassava (*Manihot esculenta* Crantz), in the rainy and dry seasons in Southern and Eastern parts of Nigeria (Asoegwu 1987; Adewusi et al., 2000). Development of appropriate crop protection measures depend on the knowledge of the diseases and causal pathogens. The traditional method of intercropping melon with other crops has helped peasant farmers to evolve crop protection techniques that are readily adaptable. However, a major constraint to the adoption of these methodologies beyond the peasant farmers' condition is the difficulty in appropriate identification of crop diseases. Similarly causal agents of diseases and their peculiar characteristics are major points of reference when control strategies are being developed.

For egusi melon, there is paucity of information on the characteristic symptoms, its development and causal pathogens in South western, Nigeria. This requires to be elucidated in order to give the farmer added advantage in respect of resource utilization and specificity of efforts and results in the

bid to manage challenges posed by melon diseases on the farm.

MATERIALS AND METHODS Field surveys

During the late (August to November) and early (April and July) planting seasons, a survey for diseases was carried out in four local government areas (LGA) of four states viz Oyo (Ido, Egbeda, Ona-ara, Lagelu), Ogun (Odeda, Yewa-North, Yewa- South, Ado-Odo), Ondo (Owo, Idanre, Akoko, Irele) and Oshun (Isokan, Irewole, Ayedade, Iwo). Three villages were selected per LGA and the choice of the villages in each state was the high melon production areas. One farm per village was evaluated in this study. At onset of the visits, fifty plants were tagged per farm and subsequent observations were made from the tagged plants. Farmers' fields were monitored once a week till crop maturity. Development of the symptoms was closely observed from initiation to full expression. Furthermore, from each diseased leaf, stem, fruits and flower, samples were collected for identification of casual organisms. They were stored seperately in polythene bags, labelled and kept for further use.

Isolation of pathogens

Symptomatic leaves were rinsed in tap water and small pieces (2mm) were cut from the junction between the healthy and infected portions (four pieces per plant). These were surfaced sterilized in 0.5% NaOCI for 5 minutes, rinsed twice in sterile distilled water and placed onto Potato dextrose Agar (PDA Oxoid Ltd, Basingstoke, Hampshire, UK, 3.9%) ammended with Streptomycin sulfate (0.39% Sigma, St Louis, MO). The cultures were incubated at room temperature (28 \pm 2°C) for up to 8 days. Fungal colonies which emerged were subcultured until pure. Macroscopic and microscopic cultural characteristics were used to identify the fungi with reference to Barnett and Hunter (1998), Lucas *et al.* (1992) and Kenaga *et al.* (1971).

Pathogenicity tests

Surfaced sterilised (10% NaOCI for 5 minutes) equsi melon seeds were sown in plastic pots (21cm diameter) half filled with steam sterilized (160°C for 2h) soil in a screen house with natural lighting. Pots were watered daily and seedlings which emerged were thinned to five plants per pot. Pots were arranged in the screen house in a randomized complete block design with four replicates per treatment and control. Three to four day old melon seedlings were used to assess pathogenicity of the following fungi Cercospora citrullina, Aspergillus niger, flavus, Fusarium Α. oxysporium, Rhizopus stolonifer, Colletotrichum lagenarium and Didymella bryonaie

Effect of inoculum on the leaves of egusi melon

Test fungi were investigated for pathogenicity on leaves by spraying the leaf surface to run off with the spore suspension 6 x 10⁴–3 x 10⁶ spores/ml (0.1% (vol./vol.) to which tween 20 was added. Spore suspension of *Didymella bryonaie* were obtained by flooding 8-10day old cultures with 5 to 10ml of sterile distilled water with lactic acid (pH 4.0) was used to increase spore discharge from pycnidia to reduce clumping of spore. Spore suspension was filtered through 4 layers of cheese cloth to remove pycnidia (Williams 2003) and adjusted to 10⁴ – 10⁶ spore/ml. Four uninoculated plants served as controls. Plants were kept in the screen house and disease assessment was a visual observation of presence or absence of symptoms for 20

days after inoculation.

Effect of inoculum on the stem of egusi melon

The fungal isolates associated with the stem diseases were inoculated via stem inoculum and dipping method. Two stem surfaces of one week-old plants were punctured with sterile needle. The punctured surfaces were then sprayed with the inoculum. Uninoculated stem served as control.

Effect of inoculum on the fruits of egusi melon

Inoculation of fruits with pathogens isolated from diseased fruits was carried out on 60day-old intact fruits. The fruits were wounded by means of a scalpel and sprayinoculated to run off with the spore suspension of the pathogens. Control fruits were sprayed with sterile distilled water.

Disease assessment was a visual observation of presence or absence of symptoms after inoculation. Fungi were reisolated from the diseased tissue and compared with original isolates.

RESULTS

Pathogenicity tests

All plants inoculated with corresponding organisms showed disease symptoms similar to those observed at sampling times in the fields. Based on cultural characteristics and fungal morphology, the isolates were identified as *Colletotricium lagenarium* for anthracnose disease and Didymella bryoaniae for blight disease. For powdery mildew Erysiphe cucurbitarum symptom, was consistently isolated from diseased leaf tissues and was found to be the causal agent. Peronospora cubensis caused the downy mildew symptom of equsi melon. The pathogen frequently found associated with Alternaria

leaf spot symptom was Alternaria cucumerina and was confirmed the causal agent of the Alternaria symptom. Fusarium solani caused the vine wilt disease of the plant while Cercospora leaf spot disease was caused by Cercospora citrullina.

Isolation of fungal species

A total of 11 fungal species namely; *Colletotrichum lagenarium, Didymella bryoaniae, Peronospora cubensis, Erysiphe cucurbitarum, Cercospora citrullina, Alternaria cucumerina, Aspergillus niger, A. flavus, Fusarium oxysporum, F. solani* and *Rhizopus stolonifer* were isolated from the different diseased samples collected from the melon fields. The organisms were observed in all fields surveyed.

Symptomatology

Anthracnose disease (Colletotrichum lagenarium)

Foliar spots began as small yellowish and water- soaked areas on the veins that later

turned more or less circular (Plate 1a). Diseased tissues dried up and became dark brown while necrotic areas broke and shattered at the centre of the lesion (Plate 1b). Plants were infected at any stage of growth.

On the stem, the disease started as elongated, narrow and watersoaked lesions. Lesions sometimes became slightly sunken and yellowish in colour (Plate 2a). Lesions also penetrated the stem to cause lip canker in most cases (Plate 2b).

Symptoms observed on the fruits consisted of small, circular, black, sunken canker with raised margin. Spots were about 2.5-3cm in diameter and 0.8cm deep but later coalesced to form larger spots. Fruit rot was observed at parts where lesion was in contact with the soil (Plate 3a). Fruit pedicels were also affected, leading to the darkening, shrivelling and death of very young fruits (Plate 3b). White mycelia were sometimes found on the pedicel and fruits.



Plate 1: Anthracnose on melon leaves (arrowed); a) early stage of infection (b) late stage of infection

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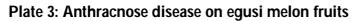


Plate 2: Stem Anthracnose showing a) slightly sunken brownish lesion on stem (arrowed) and b) lip canker on stem of melon (arrowed)



а

b



- (a) Mature fruit showing fruit rot at point in contact with the soil(b) Darkened and shrivelled infected young fruits

Leaf blight, stem blight and fruit rot (Didymella bryoniae)

Symptoms were observed on leaves, stems and fruits. On plants, small green spots were observed on the underside of cotyledons at the start of infection. Spots later became greyish green and round to irregular in shapes. Spots expanded rapidly and killed the entire cotyledon. Blighting of the cotyledon, which extended to the hypocotyls, resulted in the killing of the plant. Symptoms were also observed on older leaves close to the soil line. Leaf symptoms began as light brown, irregular spots surrounded by yellow borders that developed from the tip of the leaves and gradually progressed backwards (Plate 4a). At advanced stage, lesions became larger. Black fruiting bodies (pycnidia) covered the lesions on leaves. Leaves turned yellow, then black and eventually died (Plate 4b).

Symptom observed on stem appeared around the stem node of plants during the

fruiting period (6th – 7th week) after seedling emergence. The areas appeared oily-green with elongated light brown clearing. Affected areas later cracked and exuded sap that dried to form coloured gum deposit. Brown lesions were also observed on the stem (Plate 5). This split opened and turned brown with age. The vine cankers which produced brown exudates were also found near the soil lines. Tiny brown to black speck-like fruiting structures (pycnidia) were often seen on the infected stem or nodes. As infection progressed, lesions enlarged and vines beyond the infected nodes turned yellow, wilted and died. Lesions observed on fruits appeared as small, almost circular water- soaked areas, which enlarged, resulting in dark, firm and leathery depressed areas. As the season proaressed, cracked areas were noticed from the depressed points on the affected areas. From the cracked areas, gummy exudates emanated. The spots later became sunken and brownish black in colour (Plate 6).



Plate 4: a) Early stage and b) advanced stage of leaf blight caused by *Didymella* bryoaniae

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Plate 5: Brown stem blight (arrowed) caused by Didymella bryoaniae



Plate 6: Dark brown fruit rot with brownish gummy exudate (arrowed) caused by *Didymella bryoaniae*

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Powdery mildew (*Erysiphe cucurbitarum*) The disease appeared as round whitish spots on the lower surfaces of the oldest leaves. The spots increased in number and size, coalesced and appeared on the upper surface as whitish, talcum-like powdery growth (Plate 7). Small, black, globose, speck-sized cleistothecia eventually covered most of the lesion surfaces. At advanced stage of infection, severely affected leaves lost their normal dark green colour, turned brown and shrivelled. Symptom was not observed on fruits but fruits growing on infected vines ripened prematurely and

appeared sunburned.

Downy mildew (*Peronospora cucurbitarum*) At the early stage of infection, symptoms appeared on the upper surface of the oldest leaves near the crown. Symptoms appeared as 2-4, small and pale-green to yellow angular spots (Plate 8). The underside of the leaves opposite the yellow spots became covered with layers of greyish mycelial growth. The leaf veins confined the spots and at advanced stage, severely infected leaves became chlorotic, turned light brown and shrivelled.



Plate 7: Egusi melon leaf showing whitish talcum-like symptom of powdery mildew (arrowed)

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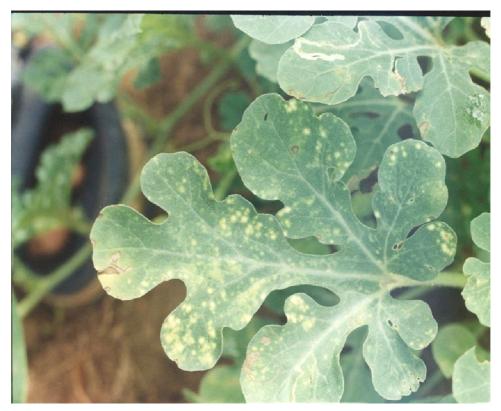


Plate 8: Leaf of egusi melon showing chlorotic lesions (arrowed) caused by downy mildew fungus

Alternaria leaf spot (Alternaria cucumerina)

Spots appeared first on older leaves near the crown of the plant. Symptoms initially appeared as small, circular and watersoaked areas. The spots increased in number, enlarged to 0.8cm in diameter and turned dark brown to black. Spots were distinct, with definite margins and concentric rings within the lesions (Plate 9a and b). On the lower surface of leaves, rings rarely occurred and the margins were indistinct. At advanced stage, severely affected leaves were entirely covered with the lesions (on which black conidia appeared). Eventually leaves shrivelled, died and dropped off.

(Alternaria Vine wilt (Fusarium oxysporum)

Both young and matured plants were susceptible to the pathogen. Infection of older plants resulted in yellowing and loss of lustre on one or more branches near the crown at the early stage. Elongated brown lesions developed on stems near the crown and extended as long, narrow, brown streaks. Salmon pink, spore masses, which appeared as pink mould, were also observed on the lesions. The affected vines and entire plants eventually wilted and died (Plate 10 a & b). Roots of severely affected plants were often decayed and appeared shredded. Sometimes the lesions form on hypocotyls thereby causing the tissue to rot.



(a)

(b)

Plate 9: Alternaria leaf spot of egusi melon showing

- a) Dark brown concentric rings of early stage of leaf spot development
- b) Coalesced spots at advanced stage of disease development.



Plate 10: Wilting of runners of egusi-melon (a) early stage and (b) advanced stage on mature plants

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Cercospora leaf spot (*Cercospora citrullina*) Few small spots (0.32 to 0.64 cm diameter) appeared first on older leaves as oily green spots. The spots later became numerous

and necrotic. Spots were small, circular to irregularly circular, with white to tan centres and dark margins (Plate 11).



Plate 11: Symptom of Cercospora leaf spot (arrowed)

Wet rot of flower and fruit (*Choanephora cucurbitarum*).

The disease affected the blossoms and fruit. The fruiting bodies consisted of white stalks (sporangiophores) with white to brown heads (sporangia), having characteristic metallic lustre that later changed to purplish black. Flowers were infected soon after they opened (Plate 12a). A characteristic fungal growth resembling numerous pins stuck in a pin-cushion developed on the infected

surface (Plate 12b). The affected tissue beneath the mass of fungus became translucent, water-soaked and rotten.

The fungus was usually confined to the end of the fruit, but during wetness, the entire fruit decayed (Plate 13a & b). The fruiting bodies did not appear on the rotted parts of fruits. Fruits also rotted backwards from the blossom end. Affected fruits dropped off prematurely.



Plate 12: Flower rot of egusi melon caused by *Choanephora cucurbitarum* showing (a) Dead flowers on a vine (b) fungal growth on infected (dead) flowers



Plate 13: Fruit rot of egusi melon caused by *Choanephora cucurbitarum* showing:

a) Rotted immature fruitsb) Rotted mature fruits

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DISCUSSION

Colletotrichum lagenarium is the causal pathogen of leaf, stem and fruit anthracnose of egusi melon. The fungus was reported by Prakash *et al.* (1974), Timechenko (1977), Pegerine *et al.* (1984), and Wei *et al.* (1991) as causal agent of anthracnose diseases on infected leaves of melon. Lesions were observed as dark brown which were more or less circular. Ferrin (2008) reported that lesions were brown to black with irregular margins often restricted by leaf veins. The differences in disease symptom presentation may be due to the differences in cultivars assessed.

Didymella bryonaie is the causal pathogen of leaf blight, stem blight and fruit rot of egusi melon. It is a disease found on all parts of plants except the root. It has been found to cause extensive damage to all above ground parts of green house grown cucumbers (Miller et al. 2009). It is reported to be the most destructive disease of cucurbits (Choi *et al.*, 2010). Robert and Kucharek (2004) also confirmed *D. Bryonaie* to be the causal organism of blight disease on squash but was found to be a minor disease on water melon in Dar-es-salam (Peregrine *et al.*, 1984).

Erysiphe cucurbitarum was confirmed the causal organism of powdery mildew in this study. This pathogen was among the pathogens listed by Hill and Waller (1999) as causal agents of watermelon diseases in the United States of America. It is reported to be a common disease of cucurbits in the field and green houses in most areas of the world and can be a major production problem. Sphaerotheca spp. and Erysiphe spp. are the two most commonly reported fungal pathogens of powdery mildew (McGrath 1997).

Pathogenicity test confirmed Peronospora *cucurbitarum* as the causal organism of downy mildew which affects only the leaves of the crop. Colucci and Holmes (2010) reported that although the disease infects only the foliage, a reduction in photosynthetic activity early in plant development results in stunted plant, yield reduction and premature defoliation which eventually results in fruit sunscald. Downy mildew has a wide host (Colucci and Holmes 2010) and this could have facilitated the wide distribution of diseases. Other infected plants could readily provide alternative host status for the pathogen until periods congenial for melon production.

Alternaria cucumerina caused the Alternaria leaf spot disease however the disease was limited to the foliage of equsi melon. Wei et al. (1991) and Buzi et al. (2002) stated that leaf spot disease caused by A. cucumerina is one of the major diseases observed on water melon in China and Italy respectively. It causes damage by defoliating the vines and reducing fruit yield, size and quality and with only partial defoliation, the fruit may sunscald and ripen prematurely on cucumber, squashes, watermelon and pumpkin (Babadoost 1989).

Fusarium oxysporum was found associated with vine wilt of egusi melon. This result confirms the reports of Wei *et al.* (1991) and Park *et al.* (1996) who reported *Fusarium oxysporum* as the causal organism of wilt of water melon. Fusarium disease has been reported on several crops such as cotton, to-mato, lettuce, sweet potatoes and beans. Musoni *et al.* (2010) reported that Fusarium wilt caused by *Fusarium oxysporum* f. sp. *phase-oli* caused growers to abandon the most popular climbing bean cultivar in Rwanda. Thus, the wide host range of fusarium spp.

makes the pathogen a major threat to melon production in South western Nigeria where climatic conditions favourable to disease development subsists.

Results of this study agrees with the reports of Peregrine *et al.* (1984) and Rego (1994) that *Cercospora citrullina* causes leaf spot diseases on various cucurbitaceae. In West Africa, cercospora leaf spot has been reported to be one of the most widely occurring leaf spot disease of cassava and found occurring in several zones (Wydra and Msikita 1998). The disease causes severe defoliation in the most susceptible cultivars but with slight yield losses.

Choanephora cucurbitarum is the causal organism of wet rot of flower and fruit in equsi melon. Symptoms of the disease observed in this study were similar to that on common garden petunia. Flowers with water soaked lesions wilted and rotted and on these whitish mycelia and monosporous sporangiola were produced (Jun and Hiromichi 2000). Choanephora cucurbitarum has been reported to cause devastating inflorescence disease of Amaranthus sp. During severe infection and favourable conditions, the inflorescence head may be completely cut off such that the plant does not produce any seed or the yield may be reduced to half or less (Adebanjo 1990).

Choanephora cucurbitareum was reported to cause the wet rot of okra (Siddiqui *et al.*, 2009). The understanding of the unique symptoms and causal pathogens of egusi melon diseases in South-Western Nigeria will facilitate easy adoption of management techniques by farmers in a bid to reduce the adverse effect of diseases on melon production.

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