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Report of *Sclerotinia sclerotiorum* as the causal organism of the leaf spot and stem blight disease of African yam bean *(Sphenostylis stenocarpa).*

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ABSTRACT

Objectives: There has been a dearth of information on the fungal field diseases, which affect the production of African yam bean *(Sphenostylis stenocarpa)* in Nigeria. This study was therefore carried out to assess the fungal field pathogens including *Sclerotinia sclerotiorum* associated with the crop .

Methodology and Results: Seed varieties used in the study were obtained from the Institute of Agricultural Research and Training, Ibadan and International Institute of Tropical Agriculture, Ibadan. Ten seed varieties were planted using a Completely Randomised Block Design in five replicates. Disease assessment was carried out weekly between April and September 2013. The leaves, pods and stems were assessed for disease symptoms and taken to the laboratory for pathogen isolation. The healthy and diseased specimens were cut into small pieces of 3mm diameter and disinfected in 5% Sodium Hypochlorite (NaOCl). Pathogenicity of the organism was done using foliar spray method on a 7 days old AYB plant. (Dinghra and Sinclair, 1985). There was the presence of white cottony mycelia on leaves close to the base of the plant on field as well as round black sclerotia on the leaves and plates in the laboratory. Using the appropriate morphological guides as illustrated in Barneth and Hunter (2010), the organism was identified as *Sclerotinia sclerotiorum*.

Conclusions and Application of Findings: The pathogenicity test confirmed the organism as the causal factor of leaf spots and stem blight of AYB. The organism has previously been reported on some members of the *Fabaceae* family; however, this probably is its first report of pathogenic invasion of African yam bean. The soil used for planting could be considered a possible medium of disease transmission since *S.sclerotiorum* is known as a soil organism. This study has shown that AYB is susceptible to *S. sclerotiorum*

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa)* is a highly nutritious legume consumed as both seed and tuber in many parts of Africa especially sub-Saharan Africa. The crop is identified with various common names. Some of the indigenous lingual synonyms for AYB in Africa according to Kay (1987) are: “Diegemtenguere” (Mali), “Girigiri” (Hausa, West Africa), “Norouko” and/or “Roya” (Sudan),

"Okpududu" (Igbo, Nigeria) and "Sese" (Yoruba, Nigeria). The crop is used extensively in various dietary preparations; in most West African communities, the seed grains are boiled and eaten with other staples such as yam, plantain, cassava, corn/maize, etc. A popular snack is produced from the grains through roasting particularly in Enugu/Nsukka area of Nigeria. The seed is a highly priced commodity especially in West Africa where it is often preferred over other grain legumes (Obizoba and Nnam, 1992; Okpara and Omaliko, 1995). West Africans prefer the seeds to the tubers while the tubers are important as food in East and Central Africa, especially among the Bandudus, the Shabas and the tribe at Kinshasha in Zaire (Potter, 1992; Nwokolo, 1996). The seed and tuber of AYB contain different food fractions and minerals that are comparable to other food legumes. The tubers are very high in protein content more than twice that in Sweet potatoes (*Ipomea batatas*) or Irish (*Solanum tuberosum*) potatoes and very much higher than those in yam and cassava (Amoatey *et al.*, 2000). In addition, AYB as a profuse nodulator improves soil nutrient through atmospheric nitrogen fixation.

Plate 1a: Creamy seeds of AYB

Plate 1b: Brown seeds of AYB

Plate2: Pods and tuber of AYB.

African yam bean (*Sphenostylis stenocarpa)* is a crop that continues to attract significant attention due to its potential for improving the problem of protein deficiency and malnutrition in sub-Saharan Africa. Its ability to survive in various ecological climes confers on the crop the advantage of being naturally resistant to many pathogenic organisms. Ameh and Okezie *(2005)* identified wilting, powdery mildew and root gall as diseases of AYB but further availability of information on the diseases of the crop are scanty. *Sclerotinia sclerotiorum* is generally known to be the causal organism of the white mould disease. It has a wider host range than its counterpart *S.minor*. (Franklin, 2001). Its host range includes broccoli, cabbage, cauliflower, carrot, celery, bean, lettuce, parsnip, tomato, pepper, eggplant, broad bean, beet, melon. (Franklin, 2001). It is therefore important to conduct an investigation into the pathogenicity of this organism on AYB. This will contribute to increasing the volume of information needed for the improvement of the crop.

MATERIALS AND METHODS

Research location: Planting was carried out at the Teaching and Research farm of the Federal University of Agriculture, Abeokuta. Ogun State Nigeria.

Land preparation and planting: The land was ploughed and harrowed. Seeds were planted in five plots of ten lines each in a Randomised Complete Blocked Design (RCBD).The dimension of each plot was 2m x 1m with a row of 1m. Seed varieties (creamy white and brown) obtained from Oja oba and Bodija markets in Oyo State, Oba Adesida market in Akure and Oja Oba and Iyere markets in Owo Ondo State were used in the study. Standard agronomic practices of weeding, thinning and staking, were conducted.

Assessment and isolation of pathogens. : Disease assessment was carried out within a period of six months (April and September 2013). Plants were tagged for proper monitoring, and assessment of infected plant tissues commenced 3 weeks after planting which was carried out weekly till pods were mature for harvesting. The infected parts were promptly collected, labelled and stored in polyethene bags. The infected tissues were cut alongside healthy tissues with a sterile scalpel. In the laboratory, diseased leaf and stem parts were surface sterilized in 1% Sodium Hypochlorite (NaOCl) for 10 min and rinsed in three changes of sterile distilled water. Five segments of diseased parts were placed on each petri dish containing Potato Dextrose Agar (PDA) culture medium. The plates were incubated for 3 to 4 days at room temperature 28±2°C and observed daily from the 2nd day. Fungal colonies obtained after incubation were sub cultured on PDA until pure cultures were obtained. The identification of the fungi was carried out using appropriate morphological criteria as illustrated in Barnett and Hunter (2010).

Pathogenicity test: Pathogenicity test was carried out in the screen house of the Department of Biological Sciences, Federal University of Agriculture, Abeokuta. Ogun State. Soil used was sterilized at 160°C for 2 hours. Five kilograms of the sterilized sandy loamy soil was filled into a perforated plastic bucket (10 cm diameter) and arranged in a completely randomized design of three treatments per organism. Five seeds were sown per bucket. The plants were thinned to two plants per bucket two weeks after germination of the seeds. Spores were counted using a haemocytometer. Inoculum suspension was prepared using a modified method of (Mew 1987, Mew *et al.* 1981). A 7 days old culture was washed with 5ml of 4.0 %lactic acid and scrapped aseptically into distilled water to which 0. 5 % surfactant (tween 80) was added. The seedlings were thereafter sprayed to run off (foliar spray method) (Dinghra and Sinclair, 1985). Polyethene bags were used to cover the plants for 24 hours to create humidity. For controls, plants were sprayed with distilled water.

RESULTS AND DISCUSSION

Symptom emergence of the leaf spot disease was first noticed two months after planting and progressed into maturity and harvest of the crops. The infected leaves showed very small black spots all over the leaves, which increased gradually at 0.2±0.1 mm. These spots coalesced to become ringed necrotic spots which were conspicuous by the mid of the growing season, however not all symptoms tagged matured into the conspicuous leaf spots.

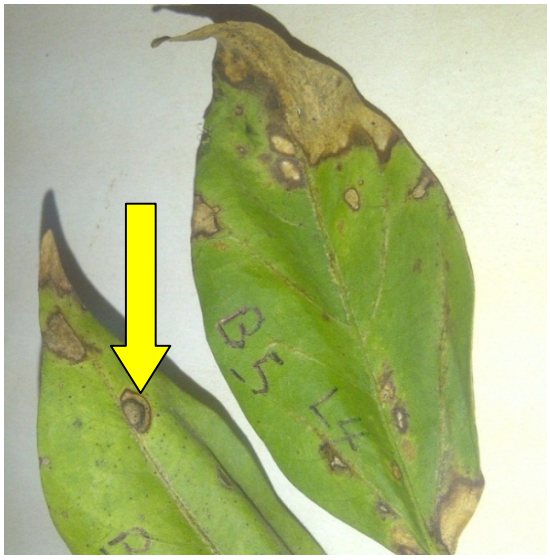

Plate1a: leaf showing symptoms leaf spot caused by *Sclerotinia sclerotiorum*

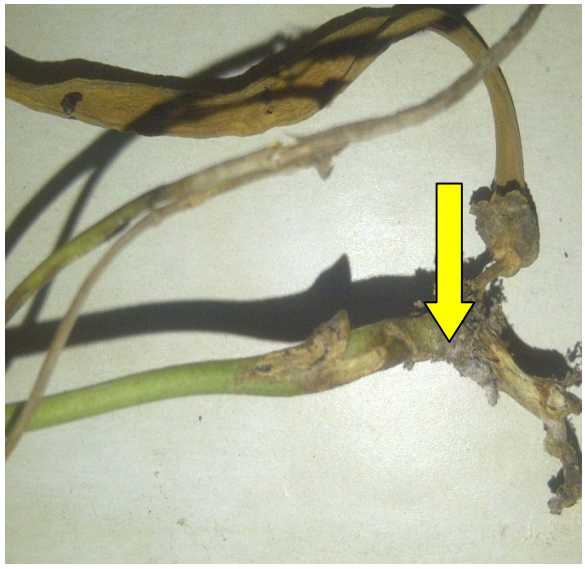

Plate 1b: Stems of AYB showing signs of *Sclerotinia sclerotiorum*

The spots were bordered by black margins and light brown necrotic lesions within. The stems had presentations of lesions, which were water- soaked and had, white cottony mycelia on their surface especially close to the soil line. The pods of the crop were also affected; however, spot lesions were ringed and water-soaked. This agrees with reported characteristics of plants affected by white mould disease (Franklin, 2001). Signs of the causal organism were seen as small black sclerotia and mass of white cottony mycelia on leaves and stems close to the soil. The symptoms culminated in the general wilting of the plant leaves. *Sclerotinia sclerotiorum* is widely known as a soil borne organism however, its invasion of the leaves and pods of AYB could be explained by the contamination of the growth medium (soil) with sclerotia of the organism. Previous studies showed *S. sclerotiorum* as capable of pathogenic invasion of crops through sclerotia infected soil and airborne spores (Franklin, 2001). The presence of few pathogenic organisms associated with the crop on field could be responsible for the crops' ability to survive in varied ecological environments (Adewale and Dumet, 2011). This presents an ecological advantage over many other crops in the fabacea family like cowpea and peas (Adewale and Dumet, 2011).

CONCLUSION

We recommend careful observation of soil for the signs of *Sclerotinia sclerotiorum* before planting. Farmers are as well encouraged to plant properly treated seeds free of disease innocula. This probably is the first report of *Sclerotinia sclerotiorum* causing leaf spot and stem blight disease on AYB. Additional investigation is needed at the molecular level to consolidate the information given in this work.

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