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(RESEARCH ARTICLE)

Pathogenicity of fungi associated with leaf spot disease of sweet potato (*Ipomea batatas* L. (Lam) in Makurdi, Benue State, Nigeria.

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Abstract

The screen house experiment was conducted to determine the pathogenicity of leaf spot fungi of sweet potato in Makurdi situated in the Southern Guinea Savannah of Nigeria. The experiment was a completely randomized design with twelve replicates. The variability in pathogenicity of four leaf spot fungi namely: *Aspergillus flavus* (Link), *Aspergillus tamarii* (Kita), *Macrophomina phaseolina* (Tassi), and *Fusarium verticillioides* (Sacc.) on sweet potato variety TIS-3164 was evaluated in the screen house using the spore suspension of the fungi at 15x 10^6 spores/ ml on four weeks old plants. All four isolates were pathogenic and induced necrotic symptoms on sweet potato plants. The leaf spot lesions were widest in sweet potato plants inoculated with *A. tamarii* (9 cm) followed by *F. verticollioides* (5 cm), *A. flavus* (4 cm) and *M. phaseolina* (3.20 cm). Sweet potato plants inoculated with spore suspension of the test fungi recorded significantly ($P < 0.05$) lower number of leaves with sweet potato plants inoculated with *F. verticillioides* recording the least number of leaves (30.75). At 4weeks after planting (WAP), the application of the spore suspension of *A. tamarii* produced significantly lower leaf area of 5.56cm^2. At 4 weeks after inoculation (WAI), percentage leaf defoliation was highest on sweet potato plants inoculated with *A. flavus* (44.50 %) and least when sweet potato plants were inoculated with *M. phaseolina* (34.30 %). The inoculation of the sweet potato plants with all test fungi had no significant effect on the vine length. The study demonstrated the pathogenicity of all four test fungi on sweet potato.

Keywords: Pathogenicity; Sweet potato; Leaf spot; Lesion; Symptoms.

1. Introduction

Sweet potato (*Ipomea batatas* L. (Lam) is a nutritive root crop popularly cultivated in the Southern and Northern Guinea Savannah agro ecology of Nigeria. Nigeria is ranked the third largest producer of sweet potato after China and Uganda. Nigeria cultivated 2.5% of the sweet potatoes produced in 2010 with per capita annual consumption of 22.3 Kg [1, 2]. Nigeria is the largest producer of sweet potato in West Africa with 4 million hectares cultivated and 23534 hg/ha yield in 2018 [3]. Sweet potato production in Nigeria is a source of employment which has a good profit margin and contributes to farmers' income [4]. Sweet potato production in Nigeria and Benue State in particular is a competitive and profitable enterprise supporting the rural farmers [5, 6]. Sweet potato is classified as a low glycemic crop suitable in the diet of the diabetic [7, 8]. Fresh sweet potato tuber contains about 80% non-starch carbohydrate, 8- 29% starch, 2.4% protein and 1.38 % mineral matter. One hundred milligrams of sweet potato tuber contains 26.25 mg ascorbic acid, 31.20 mg Phosphorus, 16.50 mg Calcium, 0.0078 mg carotene and 0.56 mg niacin [9].

The sweet potato tuber is consumed by boiling and eating with stew, drying and milling into flour, roasting, frying into chips, pounding and eating with soup or made into pottage or fermented to make a local drink 'kunu' [10]. It is used in the production of starch syrup, beverages and yoghurt in China and Japan [4]. Sweet potato is also used as raw material

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in the pharmaceutical and food industries for making drugs, candy, noodles, ethanol and as bio-fuel [2, 8]. The leaves are dried and fermented into silage and used as animal feed [4].

The production of sweet potato is constrained by the incidence of leaf spot diseases which reduces the photosynthetic area and consequently reduces yield [2, 11]. Several fungi have been reported to be causal agent of sweet potato leaf spot in the rain forest zone of Nigeria and elsewhere in the world [11]. There is dearth of information on the fungi inciting sweet potato leaf spot in Benue State in the Southern Guinea Savannah agro-ecological zone of Nigeria. There is the need to identify these fungi and determine their pathogenicity on sweet potato. The study was conducted to determine the pathogenicity of four leaf spot fungi isolated from sweet potato in Makurdi, Nigeria.

2. Material and methods

2.1. Isolation of Test Fungi from infected Sweet potato leaves

Infected sweet potato leaves showing characteristic leaf spot from an infected sweet potato field in the Teaching and Research Farm of the Federal University of Agriculture, Makurdi, Benue State, Nigeria (latitude 7°41' N - 7°45' N and longitude 8°35' E - 8°37' E 98 m above sea level) were collected and packaged in paper bags to the Pathology Laboratory of the Federal University of Agriculture, Makurdi, Nigeria for isolation of leaf spot fungi. Two millimetres long sections of the sweet potato leaves excised from the margins of necrotic leaf spot were sterilized for one minute in 10 % commercial Sodium hypochlorite solution after which they were rinsed in three changes of sterile distilled water (SDW) and blotted dry on sterile filter papers.

Potato Dextrose Agar (PDA) was prepared by adding 39g in 1 litre of SDW in a conical flask. The flask was autoclaved at 121°C for 15 minutes. After autoclaving, the media was allowed to cool to about 40°C and Streptomycin Sulphate was added at the rate of 0.2g/L. The media was then poured in 9cm Petri dishes and allowed to solidify. The leaf tissues were plated on PDA. The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30 ± 2°C) for 7 days. Pure culture was obtained by sub culturing unto fresh PDA plates.

Microscopic examination was done by examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol cotton blue and examined under the microscope for fungal structures. Pure cultures were identified using compound microscope and compared with reference manual [12].

The cultures were further confirmed at the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2.2. Pathogenicity of fungi associated with sweet potato leaf spot

The pathogenicity of the isolated fungi was conducted at the screen house situated at the Teaching and Research Farms of the Federal University of Agriculture, Makurdi. The experiment involved micro plots which consisted of 30 cm x 15 cm x 0.3 cm black polythene bags filled with 11 kg of heat sterilized sandy loam soil collected from the Teaching and Research Farm of the Federal University of Agriculture, Makurdi. Twenty five centimeter (25 cm) vine length of sweet potato variety TIS-3164 sourced from National Root Crops Research Institute Umudike, Nigeria with at least three nodes per vine was planted at 45° in each micro plot and maintained in the screen house at a temperature of 34°C – 39°C and relative humidity of 51 % -75 % for four weeks before inoculation.

2.3. Preparation and application of inoculum

The inocula were prepared from seven day old pure cultures of each test fungi on PDA using the method adopted from [11]. Spore suspensions were obtained by flooding the plates with 20 ml SDW and dislodging the spores with a sterile glass rod. The spore suspension were made up to 200 ml, filtered through double layer cheesecloth and centrifuged at 180 rpm for 10 minutes. The spore concentration was determined using a haemocytometer.

The four weeks old sweet potato plants were inoculated by spraying the spore suspension ($15x10^6$spores/ ml) of each isolate using a hand sprayer to run off. The control plants were un-inoculated and sprayed with sterile distilled water. All plants were covered with moistened polythene bags for 48 hours after which they were removed. The experiment was laid out in a completely randomized design with twelve replicates on an experimental plot size of 10 m x 5 m with a total of 60 micro plots spaced 15 cm apart with an alley of 0.5 m. No fertilizer was applied and micro plots were kept clean by hand pulling of weeds.

2.4. Data collection

Lesion size was obtained by measuring the average diameter of the lesion incited by each test fungi. Leaf spot lesions on sweet potato plants were inspected and recorded weekly up to six weeks after inoculation. The leaf spot severity assessment was done using the modified scale of 0-3 adopted from [13] and used as a criterion for virulence of each fungal isolate.

0 = leaves without spot (non virulence).
1 = leaves with less than 5 spots (mild virulence).
2 = leaves with 5-10 spots (Moderate virulence).
3 = leaves with more than 10 spots (Severe virulence).

Ten leaves were tagged per treatment for data collection covering the period of six weeks after inoculation. Agronomic data were recorded on number of leaves, leaf area which was the average product of the length and width of the leaves in cm^2, lesion size (diameter) and vine length at 2, 4 and 6 weeks after inoculation (WAI). Percentage leaf defoliation was determined as the number of leaflets defoliated per plot relative to the total number of leaves per plot assessed as a percentage. The fungi were re-isolated from the advancing leaf spots and the morphology compared with the original cultures.

2.5. Data analysis

Data were subjected to analysis of variance using GenStat 9 th edition. Significant means were separated using Fishers least significant difference (F-LSD) at 5% level of probability.

3. Results

Data presented in Table 1 shows the number of leaves of sweet potato plants inoculated with spore suspension of fungi in the screen house. The number of leaves by sweet potato plants inoculated with rhe spore suspension of all four test fungi was not significantly different at 2 WAI. At 4 WAI, sweet potato plants inoculated with *A. tamarii* recorded significantly ($P < 0.05$) lower number of leaves (34.92) compared with the control (42.17). The number of leaves recorded on sweet potato plants inoculated with *M. phaseolina* (40.08) and *F. verticillioides* (38.67) were not significantly different ($P > 0.05$) from the control (43.17). However at 6 WAI, all sweet potato plants inoculated with spore suspension of the test fungi recorded significantly ($P < 0.05$) lower number of leaves compared with the control. Sweet potato plants inoculated with *F. verticillioides* recorded the least number of leaves (30.75) which was significantly ($P < 0.05$) lower compared with the number of leaves recorded on plants inoculated with *M. phaseolina* (35.75) and *A. tamarii* (36.08).

Table 1 Effect of spore suspension of test fungi on the number of leaves of Sweet Potato leaves in the Screen house.

Fungi	**Number of leaves**		
	2 WAI	4 WAI	6 WAI
Macrophomina phaseolina	34.25	40.08	35.75
Aspergillus tamarii	38.25	34.92	36.08
Aspergillus flavus	33.92	37.67	33.00
Fusarium verticillioides	36.58	38.67	30.75
Control	33.25	42.17	48.00
FLSD (0.05)	NS	4. 08	4.67

NS- Not significant

Table 2 shows the leaf area of sweet potato plants inoculated with fungi in the screen house. At 2 WAI, the application of the spore suspension of *A. tamarii* produced the least leaf area of 5.56 cm^2 which was significantly ($P < 0.05$) lower compared with the leaf area produced by the plants inoculated with the spore suspension of all other fungi and the control. At 4WAI, inoculation of sweet potato plants with the spore suspension of all four test fungi produced significantly ($P <0.05$) lower leaf area compared with the control. Sweet potato plants inoculated with the spore suspension of *A. flavus* recorded leaf area of 11.53 cm^2 while those sweet potato plants inoculated with the spore suspension of *A. tamarii* recorded the least leaf area of 9.31 cm^2. At 6WAI, inoculation of sweet potato plants with the spore suspension of the test fungi produced lower leaf area with similar trend as recorded at 4WAI.

Table 2 Leaf Area of Sweet Potato Plants inoculated with fungi Spore Suspension in the Screen house.

Fungi	Leaf Area (cm^2)		
	2 WAI	4 WAI	6 WAI
Macrophomina phaseolina	6.46	10.10	16.96
Aspergillus tamarii	5.56	9.31	15.34
Aspergillus flavus	6.77	11.53	19.67
Fusarium verticillioides	6.78	11.37	18.04
Control	6.67	12.56	21.50
FLSD (0.05)	0.23	0.55	2.00

The weekly record of the lesion development of test fungi on sweet potato leaves is presented in Table 3. Leaf spots appeared on the inoculated sweet potato leaves within three days after inoculation. In the first two weeks after inoculation, the lesions appeared as multiple dark brown lesion spots with a diameter of 0.90 cm incited by *M. phaseolina* which was significantly lower than the lesion incited by *A. tamarii* (4.90 cm). At 4 WAI, the lesion development on the leaf indicated that *A. tamarii* recorded the highest lesion diameter of 9.00 cm while the least lesion diameter of 3.20 cm was recorded in sweet potato leaves inoculated with *M. phaseolina*.

Table 3 Weekly assessment of the lesion diameter of leaf spots incited by test fungi

Fungi	Lesion size (diameter in cm)			
	1 WAI	2 WAI	3 WAI	4 WAI
Macrophomina phaseolina	0.90	1.60	2.20	3.20
Aspergillus tamarii	4.90	6.50	7.20	9.00
Aspergillus flavus	1.30	2.60	3.40	4.00
Fusarium verticillioides	1.80	3.70	4.10	5.00
Control	0.00	0.00	0.00	0.00
FLSD (0.05)	1.60	2.57	1.60	1.80

Data presented in Table 4 shows the severity rating (virulence) of isolates on sweet potato plants in the screen house. All four isolates were pathogenic and induced necrotic symptoms on sweet potato leaves during the period of the study compared with the un-inoculated control which had no leaf spot. Although there were no significant differences in the severity of leaf spots incited by all test fungi, at 6WAI, leaf spot on the sweet potato plants inoculated with the spore suspension of *A. flavus* recorded the highest severity score of 2.83 (severe virulence) while the least severity score was recorded on sweet potato plants inoculated with the spore suspension of *A. tamarii* with a severity score of 2.42 (moderate virulence).

Table 4 Severity of leaf Spot on Sweet Potato inoculated with the spore suspension Test Fungi in the Screen house.

Fungi	Disease Severity		
	2 WAI	4 WAI	6 WAI
Macrophomina phaseolina	2.75	2.67	2.67
Aspergillus tamarii	2.75	2.67	2.42
Aspergillus flavus	2.92	2.75	2.83
Fusarium verticillioides	2.92	2.75	2.67
Control	0.00	0.00	0.00
FLSD (0.05)	0.30	0.38	0.44

Table 5 shows the percentage leaf defoliation of sweet potato plants inoculated with test fungi. It was observed that the leaf spot lesions coalesced to leaf necrosis and then the leaflets were defoliated. Leaf defoliation was significantly higher ($P < 0.05$) on sweet potato plants inoculated with test fungi compared with the un-inoculated control with no leaf defoliated. At 2 WAI, *Aspergillus tamarii* incited highest leaf defoliation of 18.27 % followed by *Fusarium verticillioides* 17.82 %, *Aspergillus flavus*17.61 % and *Macrophomina phaseolina* 17.46 %. At 4WAI, percentage leaf defoliation was highest on sweet potato plants inoculated with *A. flavus* (44.50 %) and least when sweet potato plants were inoculated with *M. phaseolina* (34.30 %).

Table 5 Effect of Test Fungi on the Percentage Leaf defoliation of Sweet Potato Plants

Fungi	Percentage leaf defoliation	
	2 WAI	4 WAI
Macrophomina phaseolina	17.46	34.30
Aspergillus tamarii	18.27	37.80
Aspergillus flavus	17.61	44.50
Fusarium verticillioides	17.82	37.20
Control	0.00	0.00
FLSD(0.05)	5.26	16.03

Data presented in Table 6 shows the vine length of sweet potato plants inoculated with test fungi. All four fungi had no significant effect on the vine length of sweet potato plants throughout the period of the experiment. At 2 WAI, the sweet potato plants inoculated with all four fungi grew longer than the control. Sweet potato plants inoculated with *A. tamarii* produced longer vines at 2 and 4 WAI while the sweet potato plants inoculated with *M. phaseolina* produced the longest vine at 6 WAI.

Table 6 Vine length of sweet potato plants inoculated with Test Fungi

Fungi	Vine Length (cm)		
	2 WAI	4 WAI	6 WAI
Macrophomina phaseolina	115.60	159.20	179.00
Aspergillus tamarii	120.50	161.70	176.60
Aspergillus flavus	112.00	155.10	175.50
Fusarium verticillioides	108.50	145.70	174.20
Control	92.80	150.20	170.80
FLSD (0.05)	NS	NS	NS

NS= Not significant

4. Discussion

The study showed the pathogenicity of *Aspergillus flavus, Macrophomina phaseolina, Aspergillus tamarii* and *Fusarium verticillioides* in inciting leaf spot disease on sweet potato plants. This indicates that the four test fungi are associated with leaf spot disease on sweet potato in Makurdi, located in the Southern Guinea Savannah agro - ecological zone of Nigeria. Previous reports identified *Fusarium culmorum, Cercosporella, Cochlobolus lunatus, Fusarium lateritium,* and *Fusarium solani* as leaf spot pathogens of sweet potato in Rivers and Delta States in the rainforest zone of Nigeria [11,14,15,].

The isolation and pathogenicity of *A. flavus* on sweet potato in this study is in contract with the report of Agu et al. [16] which identified *A. niger* as inciting sweet potato rot in Awka, Nigeria. *Aspergillus flavus* which was pathogenic in this study is reported to exist on decaying substrates and in the soil [17]. *Fusarium verticillioides* was also found to be pathogenic in leaf spot disease of sweet potato in this present study. This corroborates the findings of [18] which reported *Fusarium* species as being able to cause leaf spot infection under favourable temperature and humidity condition. Ilondu [11] also agreed that *Fusarium* spp could incite leaf spot disease in a variety of plants.

Macrophomina phaseolina which was one of the least virulent in this present study have been reported as a seed and soil fungus capable of inciting leaf spot and has a wide host range of about 500 plants where it causes necrotic symptoms on sweet potato, cowpea, groundnut, ginger, sunflower and castor [19, 20, 21, 22]. Furthermore [19] noted that the severity of *M. phaseolina* was directly related to the population of viable sclerotia established in the host between 24 and 48 hours of inoculation. Ndiaye et al. [22] also reported that *M. phaseolina* incited wilting and drying of cowpea leaves with high incidences at temperatures greater than 36oC.

The inoculation of test fungi in this study resulted in lesion development on the leaves of sweet potato plants and progressed to defoliate necrotic leaves over time. Similar observation was made by [23] on groundnut under natural infection and was attributed to inoculum buildup.

Aspergillus flavus in this study initiated 44.50 % leaf defoliation on sweet potato plants. Waliyar et al. [24] reported that leaf losses of between 25% and 43% could result in the disruption of the photosynthetic process, lesser pods and lower fruit quality. The report of [25] associated leaf defoliation to reduced growth and photosynthetic capacity of tomato plants. Gorbet et al. [26] reported a negative correlation between leaf lesions and leaf defoliation and yield of groundnut.

5. Conclusion

The study demonstrated the pathogenicity of all four test fungi on sweet potato leaves. Lesion diameter was highest in sweet potato plants inoculated with *A. tamarii* (9.00 cm) followed by *F. verticollioides* (5.00 cm), *A. flavus* (4.00 cm) and *M. phaseolina* (3.20 cm). The inoculation of the sweet potato plants with spore suspension of all test fungi had no significant effect on the vine length.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

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