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Incidence and Severity of Vine Rot and Wilt Disease of *Telfairia occidentalis* Caused by *Athelia rolfii* in Southern, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UYI and OGO Design the experiments, write the first draft. Authors UYI and UL methodology and data curation/visualization. Authors UYI and DAO write the final copy. Authors OGO and UL Literature search and proof reading. All authors read and approved the final manuscript.

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ABSTRACT

Telfairia occidentalis is an important vegetable crop that is intensively grown in Southern Nigeria for its utilization in home dishes and commercialization. However, its production is being limited by vine rot infection caused by *Athelia rolfsii*. Information on the epidemiology in growing regions is important for the disease management. Hence, this study was conducted to investigate the incidence and severity of vine rot disease of *Telfairia occidentalis* in commercial fields across Abak, Akwa Ibom State. A total of nine established *T. occidentalis* fields in three locations, including the

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Cross River Basin Development Authority, Abak-Irrigation Project, were visited during the peak of dry season (November – December), 2021 and raining season (June - August), 2022 respectively. The fields were scored for vine rot disease incidence and severity using a well described scale. Random samples from symptomatic plants were collected and taken to the laboratory for fungi isolation and identification. Koch postulate was carried out to confirm the causal agents on one susceptible genotype of *T. occidentalis* in the study area. Overall number of plants showing varying levels of symptoms reaches 84% and total values for mean incidences' rate (3.31 ± 0.06 and 3.19 ± 0.06) were recorded for both sampling period respectively. The result of this study revealed that the prevalent fungal pathogen that is responsible for vine rot and wilt disease of *T. occidentalis* in the study is *A. rolfsii* and presented information on the level and severity of infection that is indicative of the need to implement appropriate control measures for *A. rolfsii* disease in the study area.

Keywords: Abak; Akwa Ibom State; Athelia rolfsii, CRBDA; incidence; severity; susceptibility; Telfairia occidentalis.

1. INTRODUCTION

The vine rot disease is an emerging disease of fluted pumpkin and other important agricultural crops in some regions of the world [1]. The causal agent, *Athelia rolfsii* (Curzi) Tu & Kimbrough is a soil borne fungal necrotroph that is prevalent in most tropical and subtropical regions [2], Farr and Rossman 2017). It is a member of the order atheliales, which comprise of several members that are potential pathogens of plants and symbionts of animals [3]. The pathogen was first reported on field grown tomato but currently, over 500 different plants are host of *A. rolfsii* infection [4]. Agroecological areas that is characterized by high relative humidity and soil-water level alongside warm temperature range are preferred conditions for disease establishment and level of susceptibility by different host plant is not limited to host growth stage [5,2,6].

Generally, fungal infection is facilitated by wet field conditions but the level of infection may largely depend on the virulence of the pathogen and quantity of infectious propagules on the field [7,8]. Yet, fluted pumpkin production in Southern Nigeria is dependent largely on natural rainfall which present an unavoidable favourable situation for infection by most fungal pathogens in the season. Also, fluted pumpkin is a vine, creeping crop and these predisposes the plants to infection by soil borne pathogens. The vine rot pathogen, *A. rofsii* like most fungal pathogens has the ability to relay on plant debris and form resting stages (sclerotia) in soil for a long time [9]. This often serve as a primary source of inoculum for disease initiation. Symptoms of Vine rot disease includes the presence of whitish fan shape fluffy mycelial, accompanied by whitish-brownish sclerotia on plants [10] Kator et al., 2015). Up to 70% losses may be incurred on T. occidentalis fields due to infection by fungal pathogens in general [11].

In Akwa Ibom, *Telfairia occidentalis* is intensively grown as a commercial leafy vegetable by subsistence and commercial growers, the production and marketing of its products represent a major source of livelihood to many resource-poor farmers in this region [12,13]. The fresh and tender leaves are utilized in many local dishes, while older leaves are good sources of animal feed [14,15]. Extracts from the leaves are used as traditional remedies to ailments such as asthma and restore loss blood count [16,17]. Root and fruit infection initiated by *A. rolfsii* has been reported in North-central, Nigeria [9], but the prevalence of the pathogen as well as the disease severity has not been reported in Southern Nigerian. Hence, this study is carried out to investigate the prevalence of *A. rolfsii* in *Telfaria occidentalis* fields in Abak Akwa Ibom State and to also evaluate the rate of incidence and level of severity of vine rot disease in the study area.

2. MATERIALS AND METHODS

2.1 Field Sampling and Collection of Diseased *Telfairia occidentalis* Plants

Telfairia occidentalis established plots were sampled for diseased plants showing vine rot and wilt symptoms at the Cross-River Basin Development Authority (CRBDA), Abak Irrigation Project (4°57, 7°47) and in two other locations: Abak Usung-atai and Ibagwa (4°55, 7°48 and 4°54, 7°47) within Abak Local Government Area, Akwa-Ibom State. The sampling areas are approximately three kilometres apart and CRBDA is known to be the highest producer of *T. occidentalis* in Akwa Ibom State. Field survey was done during the peak of dry season (November – December), 2021 and rainy season (June – August) 2022 respectively, when the plants were 3 - 4 months old on field. The sampling locations has an average annual temperature range of 23.7ºC - 32.33ºC, average relative humidity of 79.57% and an average elevation of 49m above sea level (“Weather and climate”, n.d: online).

Diseased *T. occidentalis* plants showing symptoms of wilting, rot, presence of whitish mycelia around the vine and sclerotia were collected into zip lock bags and taken to the laboratory for pathogen isolation and identification.

Incidence and severity data was obtained using the formula of Cardoso et al, [18] given as

$$\frac{n}{N} \times 100.$$

Where n is the number of plants showing vine rot and wilt disease symptoms and N is the total number of plants assessed.

Vine rot and wilt disease symptoms was rated using the scale by Borisade et al, [8] with slight modifications.

2.2 Isolation and Pathogen Identification

Samples from field survey were surface sterilised by dipping in 0.75% hypochlorite solution for 1 minute, rinsed repeatedly with distilled water and air dried on sterilized surface. About 0.5 – 1 cm portion of the sterilised plants parts having mycelia mat from each sampling locations were aseptically cut using sterile surgical blades and placed in 9mm petri dishes containing PDA media for 4 – 7 days under ambient temperature. Emerging fungal mycelial mat were teased in 2 drops of lactophenol blue solution and viewed under the microscope. Fungal species showing features of *A. rolfsii* according to Prasad [19] were sub-cultured into freshly prepared Potato Dextrose Agar (PDA) media amended with 0.02% chloramphenicol to inhibit bacterial growth.

2.3 Koch Postulates

The mostly grown cultivar of *Telfairia occidentalis* (Ubong-abasin) in the study area was used to test the pathogenicity of the isolated *Athelia rolfsii* species. Inoculum from 8-day-old cultures was used to colonize sterilized millets that was prepared by autoclaving at (121 $°C$) for 10 minutes and allowed to cool down completely for 24 hours. Five grams of *A. rolfsii* colonized millets were used to inoculate five weeks old *T. occidentalis* plants at the base. The inoculated areas were covered with transparent nylon for 24 hours to enhance quick establishment of the pathogen. The plants were then observed for symptoms of infection.

2.4 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistics for Pure and Social Science (SPSS) package. Incidence and severity data were analysed using descriptive statistics and Mean were separated using Duncan Multiple Range Test (DMRT).

3. RESULTS

3.1 Symptoms, Percentage Number of Infected Plants and Frequency of *A. Rolfsii* Disease on *Telfairia occidentalis* Plants

Athelia rolfsii infection characterized by water-soaked patches, vine rot and rapid wilting of *Telfairia occidentalis* plants (Fig. 1) was observed in all the sampled plots with varying level of

Table 1. Disease rating scale used in scoring vine rot and wilt disease on *T. occidentalis* observed in the study area

Rating scale	Description
0	No symptoms of disease
1	<25 percent of the plant’s parts showing symptoms
2	25 to 50 percent of the plant’s parts showing symptoms
3	50 to 75 percent of the plant’s parts showing symptoms
4	> 75 percent of the plant’s parts showing symptoms/ dead plants

symptoms throughout the sampled seasons. Fig. 2 (a and b) show the level of symptoms and frequency of infected plants. The number of diseased plants showing symptoms >75% were higher in both seasons (33.5 and 29.2) respectively (Table 2). Also, overall number of diseased plants scored in percentage (16.8 + 20.2 + 13.5 + 33.5 = 84%) within the sampled plants population and locations were more than the non-symptomized plants (16%). Across the sampling areas, number of diseased plants recorded was higher in Location B, followed by A and C respectively. Similarly, severity of infection recorded in percentage was highest in Location B in both sampling periods (72.6 and 64.6%) respectively. (Fig. 3 and Table 3).

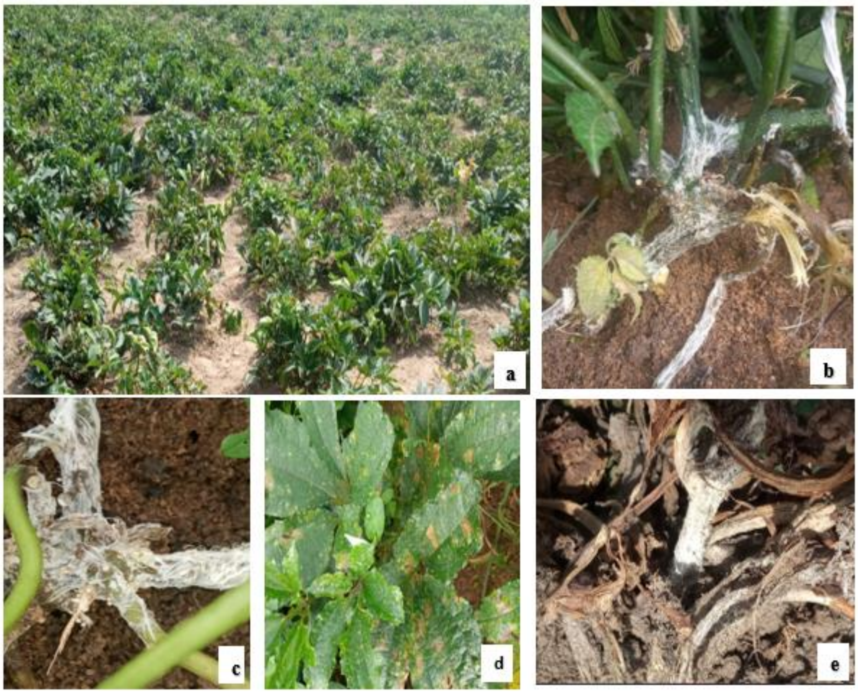

Fig. 1. a) Overview of sampling plot: b, c, d) symptoms of vine rot and patches on leaves caused *A. rolfsii* infection on *T. occidentalis*: e) wilted *T. occidentalis* plants caused by *A. rolfsii*

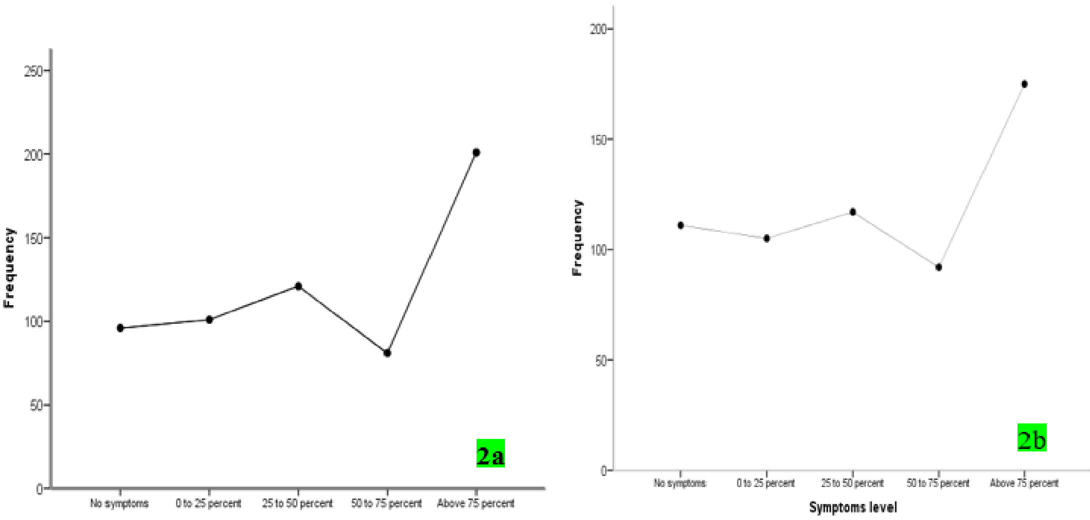

Fig. 2. a and b: Symptoms level and number of plants showing symptoms during 1st and 2nd sampling period

Table 2. Description of disease rating scale and number of diseased plants (%) per sampling period

Rating scale	Sampling periods / Level of disease incidence			
	1st Sampling (raining season)		2nd Sampling (dry season)	
	Frequency	Percentage (%)	Frequency	Percentage (%)
No symptoms	96	16.0	111	18.5
<25 percent	101	16.8	105	17.5
25 to 50 percent	121	20.2	117	19.5
50 to 75 percent	81	13.5	92	15.3
> 75 percent	201	33.5	175	29.2
Total	600	100.0	600	100.0

Table 3. Number of diseased plants and severity of infection per sampling season and location

Sampling locations	Frequency	% Number of diseased plants	% Severity during 1st sampling	% Severity during 2nd sampling
Location A	200	33.3	62.6	55.6
Location B	300	50.0	72.6	64.6
Location C	100	16.7	55	51.6

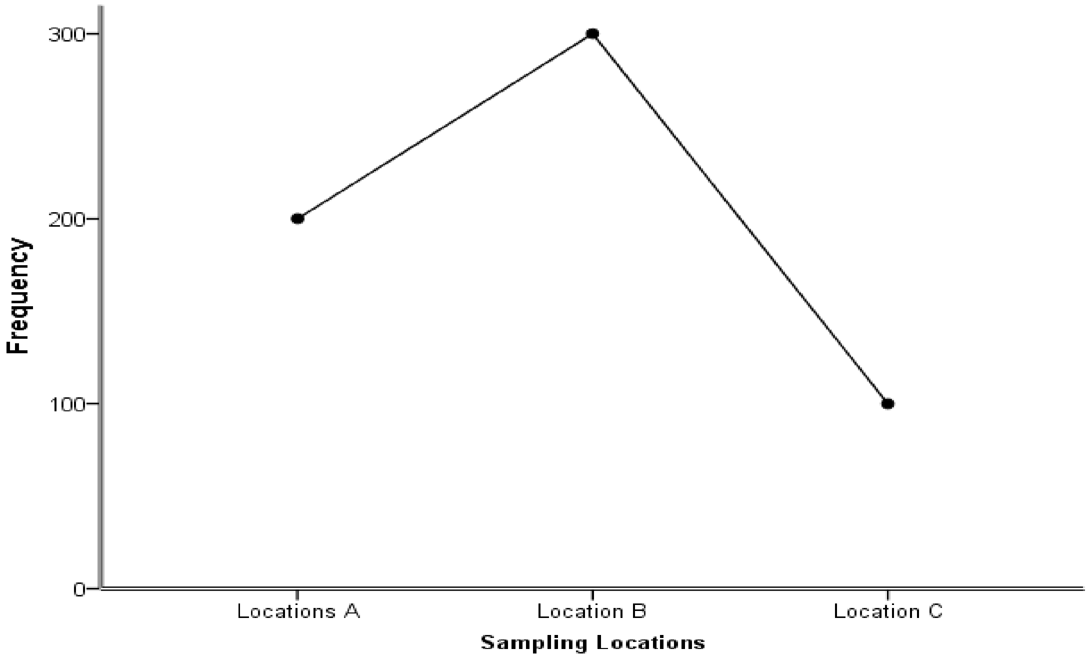

Fig. 3. Number of diseased plants across the sampling areas

3.2 Morphological Identification of Fungal Pathogen

Pure culture of the fungal pathogen isolated from diseased *Telfairia occidentalis* plants had whitish, branchy, fluffy mycelia growth on PDA media and continued to grow rapidly outside the petri dishes and on the walls of nearby objects. Old cultures > 15days formed whitish sclerotia that later turned brownish with varying sizes. Fig. 4 a, b and c show mycelial growth of 8- and 12-days old cultures and sclerotia collected on old fungal plates respectively. The following growth features align with other reported literatures; hence the pathogen was morphologically identified as *Athelia rolfsii.*

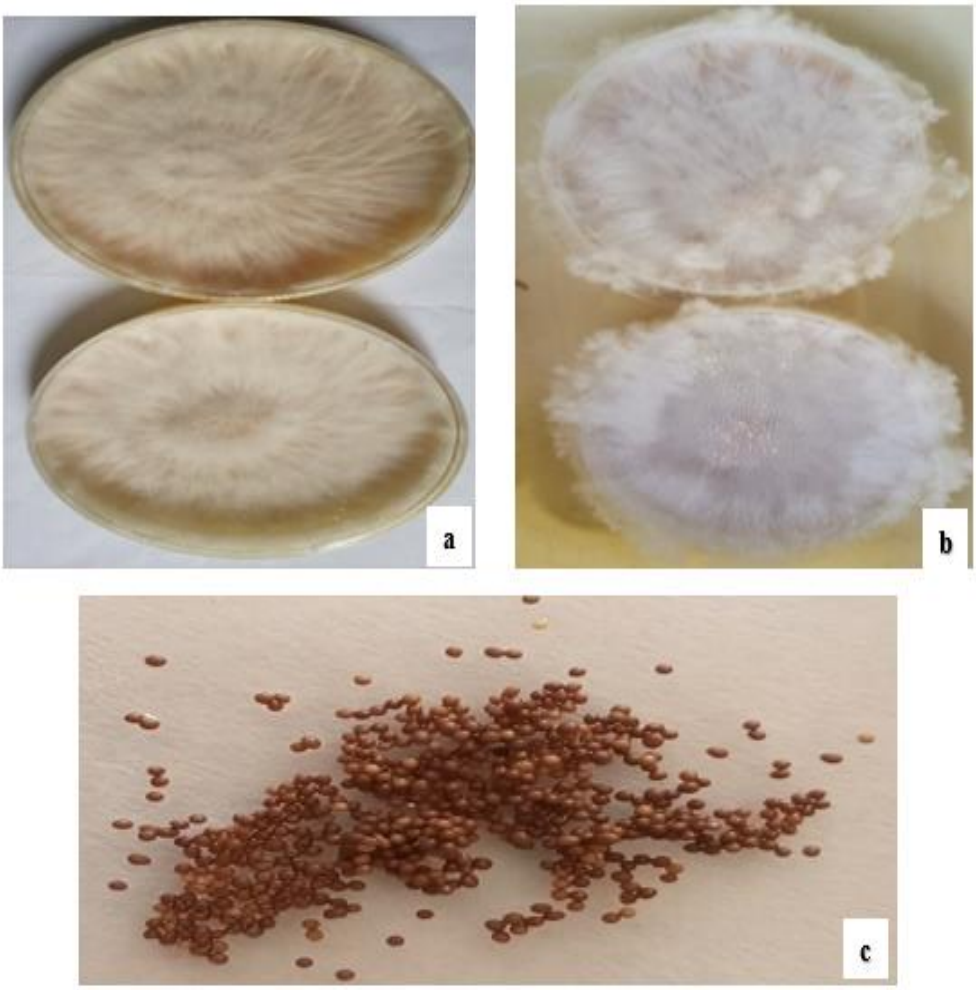

Fig. 4: a, b) Pure cultures of *A. rolfsii*: c) Sclerotia collected on old cultures of *A. rolfsii* isolate

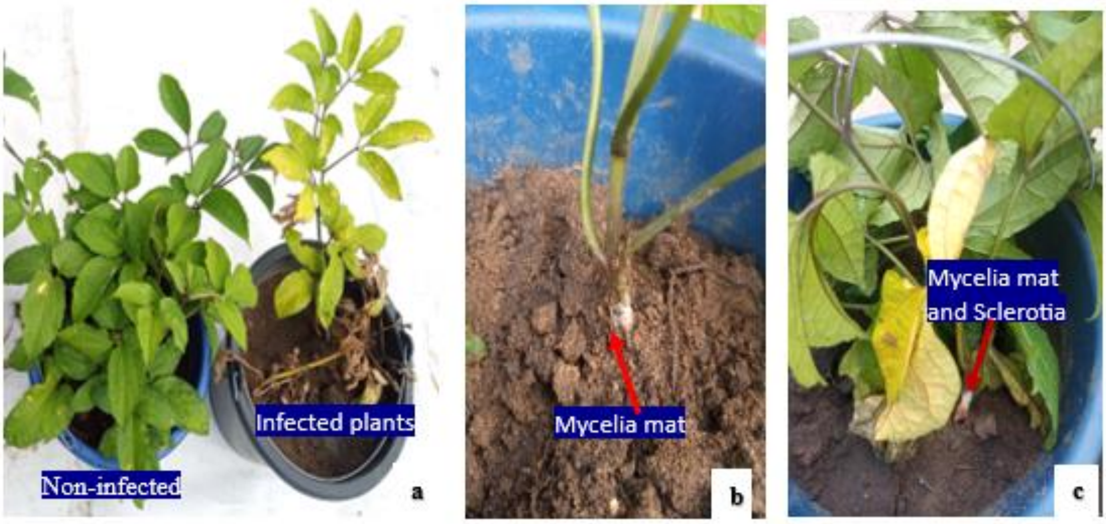

Fig. 5. a) T. occidentalis inoculated with A. rolfsii pathogen: b, c) T. occidentalis showing symptoms of A. rolfsii infection (mycelia mat and sclerotia)

3.3 Pathogenicity and Infection Process of *Athelia rolfsii* on *T. occidentalis* in the Study Area

Fig. 5: show disease symptoms and infection process by the rot pathogen *A. rolfsii*. All inoculated *T. occidentalis* plants showed symptoms of infection that were similar to field infected plants. Abundant whitish mycelia mats radiating from the inoculated soil around the base of the plant were seen at 3 - 4 days after inoculation. Other symptoms observed were; water-soaked regions on vines, patches on leaves and rapid wilting of vines and leaves. Infected vines became dried within 7 to 14 days post inoculations. Sclerotia were formed on dead vines and the fungus was reisolated from the infected tissue to confirm Koch postulates. Non-inoculated plants were healthy all through the period of observation.

3.4 Mean Incidence and Severity of *A. rolfsii* disease on *Telfairia occidentalis* in Abak, Akwa Ibom State

Analysis of variance showed high level of significance (P < 0.05) in the level of observed disease symptoms, sampling periods and locations (Table 4). The incidence and severity of *Athelial rolfsii* disease was higher in location B than A and C with mean value of (3.35 ±0.89 and 3.36 ± 0.08) in both sampling seasons. Consequently, total number of diseased *Telfairia occidentalis* plants were highest in sampling area (B), while locations A and C had varying level of disease severity in both sampling seasons. However, infected plants in location A and C per sampling period were statistically similar in both seasons (Table 5).

Table 4. Analysis of variance table showing level of significance between disease symptom level, sampling period and sampling location

Source			Sum of Squares	df	Mean Square	F	Sig.
Symptoms level * Location	Between Groups	(Combined)	32.868	2	16.434	7.683	.001**
	Within Groups		1276.965	597	2.139		
	Total		1309.833	599			
Sampling Period * Locations	Between Groups	(Combined)	33.333	2	16.667	85.286	.000***
	Within Groups		116.667	597	.195		
	Total		150.000	599			

Table 5. Show mean number of diseased plants during each sampling period and cropping seasons

Sampling parameters	Sampling locations	Number of diseased plants	Mean rate of incidence / Sampling seasons	
			July 2022	December 2021
Symptoms level	A	200	3.51±0.95a	2.95 ± 0.11c
	B	300	3.35 ±0.89a	3.36 ± 0.08a
	C	100	2.82 ±0.13c	3.15 ± 0.12b
	Total	600	3.31 ± 0.06	3.19 ± 0.06
Sampling period	A	200	1.50 ±.0.03a	1.50 ±0.03a
	B	300	1.66 ± 0.02a	1.66 ± 0.02a
	C	100	1.00 ± 0.00b	1.00 ± 0.00c
	Total	600	1.50 ± 0.20	1.50 ± 0.02

4. DISCUSSION

The study has shown that vine rot and wilt disease of *Telfairia occidentalis* that is prevailing in the study area is caused by *Athelia rolfsii*. The rate of incidence was significantly higher and symptoms of infection were similar to the reports of Mahadevakumar *et al*., [20] on *Cucurbita maxima*, [21] on *Phaseolus vulgaris*, [22] on *Justicia adhatoda* and [9] on field grown tomato in Northern Nigeria. On other economic crops such as cowpea and peanut, *Athelial rolfsii* disease has been reported and yield loss attributed to it reaches 50 and 80 % respectively [23,24]. Unfortunately, there are no reports of *A. rolfsii* incidence in Southern Nigeria. Hence, this study has presented the first report of *A. rolfsii* disease incidence and severity of infection on *T. occidentalis* in Abak Local Government Area of Akwa Ibom State.

The nature of symptoms expressed on diseased plants per sampling season were different. However, overall symptoms level of ~ 84 % was recorded in both sampling seasons respectively. Diseased symptoms observed during the sampling (rainy season) were majorly vine rot, whereas, severe wilting and patchy leaves were observed in the later (dry) season survey. The overall level of disease severity based on symptoms and rate of incidence were not statistically different in both sampling season and consistence to the reports by Rao *et al*., [25] and recent studies by Negesa *et al*., [26,27] in different regions in Africa, where incidence and severity level of blight disease caused by *A. rolfsii* on field grown tomato reaches 40 – 98 %.

The severity of infection observed in this study could be attributed to field humidity due to high rainfall (342.88mm average annual) and temperature regimes (23.7 - 32.33ºC average annual) in the study area, which coincide with the range of temperature required for optimal production of *T. occidentalis* and infectivity by *A. rolfsii* respectively. Generally, *T. occidentalis* grows well between 19 - 38 ° C (PFAF) with adequate environmental and field management practices such as staking and pruning. On the other hand, optimal temperature for the growth and pathogenicity of *A. rolfsii* on sweet potatoes in the United State lies between 27 - 35°C with sufficient soil water level and relative humidity [28]. Also, *T. occidentalis* production may require staking but in commercial production the vines are allow to creeps and interlace with each other on the surface of the soil resulting in highly dense populated and poor aerated conditions which predispose healthy vines to *A. rolfsii* inoculums in soils and facilitate infection process.

In addition to temperature range and field moisture conditions. Inadequate field management and agronomic practices adopted in the surveyed areas were also believed to influence the high level of vine rot and wilt disease incidence. During this survey, it was observed that cultural control practices that involves roguing, intercropping with non-host plants, field sanitation, adequate and timely implementation of preventive/control methods to prevent disease incidence were inadequately practiced. In many studies, poor agronomic practices have been reported to promote the level of pests and disease incidence on pepper [29,30].

The higher number of diseased plants and level of symptoms observed in sampling location B was not unconnected to the poor conditions of the field. Although another possible reason to this may be the practice of monocropping system that was predominant in this location B, which reflected in a denser population of *Telfairia occidentalis* plants. Unlike location A and C, where there were few other crops grown as inter and border crops in some of the surveyed plots in these locations. According to Tweneboah [31,32,30] monocropping was instrumental in the incidence and wide spread of fruit rot, mosaic disease and insect pests of pepper in Ghana. The result of this survey has provided additional information on the list of fungal pathogens earlier reported on *Telfairia occidentalis* in Nigeria and Cameroon [33-36].

5. CONCLUSION

The study has presented the first report on the prevailing fungal pathogen, that is responsible for vine rot and wilt disease of *Telfairia occidentalis* in the study area. *Athelial rolfsii* is associated with the diseases of many important crops including grasses and weeds. It is adapted to warm climate with high rainfall and relative humidity, hence selecting appropriate management methods may be challenging in many tropical areas of the world. From the result of this study, we recommended that available genotypes of *T. occidentalis* in the study area should be screened for natural resistant to *A. rolfsii* pathogen and to evaluate control methods that can be adopted during disease incidence in further studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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